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BUTACHLOR INDUCED OXIDATIVE AND HORMONAL IMBALANCE CORRELATED WITH OVARIAN ANOMALIES IN FRESH WATER INDIAN CAT FISH *CLARIAS BATRACHUS* (*LINN.*): SEM STUDY

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ABSTRACT

Fresh water Indian cat fish *Clarias batrachus* (Linn.) were exposed to Butachlor 2 chloro-N-(2-6 diphenyl) acetamide exposure at a dose of 1.5µl/L for 5, 10 and 15 days respectively. At the termination of each exposure blood samples were collected and analysed for serum Lipid peroxides (LPO), reduced glutathione (GSH), Thyroid stimulating hormone (TSH) and Estradiol level as per standard method of WHO. The ovarian tissues were fixed in chilled (4ºC) 2.5% gluteraldehyde followed by its post fixation in 1% OSO_4 in 0.1 M Phosphate Buffer at pH 7.2. The tisssues were further processed for SEM studies. The results showed a significant ($p<0.01$) increase in LPO level and simultaneous decrease in GSH level of fish. A significant decrease in serum TSH and significant ($p<0.05$) increase in serum estradiol were reported. Ultra structure of ovarian cells showed grooves, papillae and protuberances on the surface of the oocytes. Hence it can be concluded that butachlor sets in a wave of oxidative and hormonal imbalance leading to gonadal atrophy in fish.

KEYWORDS: Butachlor, *Clarias batrachus*, Estradiol, GSH, LPO, Ovarian tissues, SEM, TSH.

INTRODUCTION

Environmental contaminations due to natural and anthropogenic regions are inevitable since nature is uncontrollable and human development is an unending process. Pesticides differ from any other chemical substances because of their deliberate spread in the environment. The wide spread use of pesticides as one of the implications has created a lot of stress to the

aquatic world and threatened the survival of many species. Agricultural runoff and irrigational water introduces pesticides into aquatic environment Where they pose a significant toxicological risk to resident organism. Fishes get easily victimized to the harmful effects of these pesticides which diminish their health status and reproductive potentials. The impact of these pesticides on fish metabolism, biochemistry, histopathology and physiology have been vividly studied (Saxena.1999; Rawat *et al*., 2002; Ateeq *et al*., 2006).The deleterious impact of pesticide on gonads of fish have been studied by Lam (1983), Dutta (1994), Nolte et al. (1995) and Russel (1995). Pesticides have been considered as reproductive biomarker and potential reproductive endocrine disrupter (Hauser et al.,). They are known to interfere with the basal metabolism of fish and suppress reproduction, steroidogenesis (Singh & Canario, 2004) and gonadotropin level in fish by altering the secretory activities of hypothalamo- hypophyseal -/ thyroid- gonadal axis (Gorbman.1969, Volkoff.1999, Verma & Chand,2006; Chand et al.,2007; Berse et al.,2007). Butachlor is an organochlorine pesticide commonly used in India in agricultural fields and has been related to altered physiology and metabolism of fish. (Geng *et al*., 2005) It is one of the most widely used chloro acetanilide herbicide for the control of annual grasses and many broad leaf weeds in rice fields. It has been reported as a proven carcinogen (Ou *et al*., 2000). Adverse effect of butachlor on the biochemistry and metabolism of fish have been reported (Farombi et al.,2008). Ishizuka et al. (1998) have reported adverse effect of butachlor on rat's hepatic xenobiotic metabolizing potential. It is being studied extensively in relation to disease, their modulation by antioxidants and other context (Peixoto *et al.,* 2013**).** Lipid peroxidation (LPO) refers to the oxidative degeneration of lipid. It is the process whereby free radicals steal electron from the lipid in cell membrane resulting in cell damage. Peroxidation of lipids can disturb the assembly of membrane causing changes in fluidity and permeability, alteration of ion transport and inhibition of metabolic process. Alterations in serum level of LPO in fish due to different xenobiotic stress have been well documented (Rana, 1995; Kumar et al.,2008; Vinodhini and Narayanan, 2008; Sounderarajan *et al*, 2009). Reduced glutathione (GSH) is a dominant intracellular compound involved in cellular defense against oxidative injury (Numan, 1990). Any alteration in GSH level indicates the interference of pesticides on the metabolism of fish. Serum TSH is a regulatory neuro endocrine hormone responsible for regulating the concentration of free and bound T_3 and T_4. Any alteration in serum TSH marks the toxic status of the body. Serum estradiol is essential for the normal follicular development and ovarian physiology in fish. The reproductive toxicity of butachlor especially on gonad activities in female fish and its correlation with TSH, estradiol and

oxidative profile like LPO and GSH have been poorly understood. In view of above assumptions the present research work has been undertaken to assess the exact extent of correlation among oxidative profile, hormonal and ovarian cytoarchitechture *Clarias batrachus(Linn.)* in response to sub lethal oxidative exposure.

MATERIALS AND METHODS

Experimental animal: Air breathing fish, *Clarias batrachus* (commonly called Magur) were obtained from different wetlands of North Bihar, India. The standard length and weight of fish were in the range of 18±2 cm and 50±10 gm, respectively. They were brought to the laboratory, disinfected with 0.01% $KMnO_4$ solution and kept in different sized large plexi glass aquaria and plastic pools. Fishes were acclimated for 15 days in the laboratory condition. To maintain normal water temperature, cooler and exhaust were used around the aquarium. The aerated water was changed daily. During acclimation period they were fed pelleted feed prepared in laboratory (mixture of wheat flour + egg + starch as binder) @4% of their body weight and water was changed daily. After acclimation, fishes were divided into two groups. Each group contained 10 fishes. The experimental fishes were grouped into two- Group-I (Control) and Group- II (Butachlor treated), which is further subdivided into three sub groups-Group-II A (Butachlor treated for 5 days), Group-II B (Butachlor treated for 10 days) and Group-II C (Butachlor treated for 15 days).

Pesticide used: In the experimental protocol commercially brand "Butachlor (EC50%)" manufactured by SEGNOTECH Agro Pvt. Ltd. Lucknow (U.P.) was used. LC_{50} for 48 hours and 96 hours of butachlor for fish was determined as per standard protocol of APHA (2005) as 5.5µl/L and 4.2 µl/L respectively and accordingly a dose of 1.5µl/ L was selected in the present experiment for 5, 10 and 15 days exposure. Stock solution of the pesticide was prepared using distilled water, and then fishes were treated with 1.5µl/L of butachlor for consecutive 15 days. The solution was changed regularly.

Collection of blood samples: On the termination of exposure day, blood sample of both control as well as experimental groups were collected in a heparinized glass culture tube syringe from caudal vein. A small amount of blood was preserved for GSH estimation. Further blood in each group was processed for serum extraction by centrifuging blood at 5000 rev/min for 10 min. at 4ºC and stored at-20ºC for further hormonal and biochemical assessment.

Collection of ovarian tissues: Fishes were then anaesthetized with MS222 and the ovarian tissues were carefully removed, cut into small pieces with sharp surgical blades. For Electron microscope examination tissues were fixed in 2.5% gluteraldehyde in chilled (4°C) 0.2M phosphate buffer for 2-24 hrs. Post fixation was done in 1% OsO_4 in 0.1 M phosphate buffer at 4^{o}C. Then the tissues were dehydrated through graded series of alcohol up to amyl acetate. It was further Dehydrated up to CPD at 35^{o}C and 65-75 kgf/cm^2 for 30-40 min. in Hitachi HCP-2CP Drier. They were gold sputtered by EIB-2 gold coating machine and viewed and photographed under Scanning electron microscope "LEO-435" at SAIF-EM Facility Unit, Department of Anatomy, All India Institute of Medical Sciences, New Delhi.

Biochemical Analysis - All the reagents and kits were of analytical grade.

Method of LPO estimation: The level of serum LPO was estimated by the method of Ohkawa (1979) using UV spectrophotometer. Firstly 8% lauryl sulphate solution, 20% glacial acetic acid solution, working NaOH solution and 0.8% TBA solution (pH7.2) were prepared. Experimental serum sample, TDW (blank), 1,1',3,3'tetra methoxy propane(standard solution) were taken in three test tubes and 0.3 ml of 8% lauryl sulphate was added in each test tube. The test tubes were kept at boiling water for 60 min, cooled at room temperature, centrifuged at 4°C at 7000 rpm for 25 min. They were decanted and absorbance were read as test Vs blank at 532 nm in Spectronic 20 Bausch and Lamb spectrophotometer.

Method of glutathione estimation: The level of blood GSH was estimated by the method of Ellman (1959), later elaborated by Beutler (1963). For the biochemical analysis of GSH, 0.2 ml of EDTA blood in each case was added to 1.8ml lysine solution (disodium EDTA 1g/lit) and kept at 35-37^{o}c for 5 minute. 3ml of precipitating reagent (Metaphosphoric acid + EDTA + NaCl) was added to the solution, mixed well and kept at room temperature 35-37^{o}c for 5 min .This mixture was filtered through whatman filter paper to obtain clear filtrate. 1ml of clear filtrate was further mixed with 4 ml of freshly prepared disodium phosphate. This mixture was labeled as TA_1 and was observed in Spectrophotometer at 412nm. Another solution was prepared in the similar way same as $TA_1$and 0.5DTNB reagent (trisodium citrate + DTNB) was added to it, mixed well & kept at room temperature for 10 min. This was labeled as TA_2 .The TA_2 is observed in spectrophotometer at 412 nm. Then the standard was prepared by mixing 4 ml of sodium hydrogen phosphate to 1ml of filtrate. This solution was labeled as SA_1.Another solution was prepared same as SA_1 in which 0.5ml of DTNB reagent

was added, mixed well kept at room temperature for 10 min. This solution was labeled as SA_2. The reading was taken at 412nm. The activity of GSH was determined. All spectrophotometric observations were done on UV-VIS spectrophotometer.

Method of Thyroid Stimulating Hormone Assay by ELISA

The TSH kits (3rd generation micro well ELISA kit- Thyrophin MTY-TSH 048 manufactured by mono-bind Inc. California, USA) were purchased from Lilliac Medicare Pvt.Ltd., Mumbai. The sensitivity range of the kits was calculated as two times the SD from 5-10 pg for TSH, the sensitivity was 0.5µIU/ml. The hormone assessment of TSH were done on Merck 'mini mios' ELISA reader, as per standard protocol of WHO (2000).

Method of serum estradiol estimation

The estradiol kits (3rd generation micro well Free Estriol 48 ELISA kit, manufactured by Equipar diagnostics, Gaudenzio Ferrari, Saronno, Varese, Italy) were purchased from Matrix Health Care Pvt Ltd, New Delhi, India. The serum estradiol estimation in each case was done as per standard method of WHO (2000) by Merck mini mios ELISA reader.

Statistical analysis- Ten observations at random were taken in each case of biochemical assessment. They were subjected to statistical analysis including determination of Standard deviation, paired t test between control and different group of butachlor treated fish and two way Analysis of Variance (ANOVA) test. The data obtained at $p<0.005$, $P<0.01$and $p<0.001$were considered significant and mentioned in the text table I, II, III and IV.

For histopathological study at least ten micrographs were screened in each case and the micrographs of most frequently observed histological findings have been included in the text graph.

RESULTS AND DISCUSSIONS

Scanning Electron Microscopy of ovarian cells (control and butachlor treated)

Teleostean ovary is a hollow paired organ filled with numerous developing ovarian follicles. The internal lining of ovary is thrown into several folds called as Ovigerous lamellae enclosing ovocoel, the ovarian cavity. The Scanning Electron micrographs of control (Group-I) fish ovarian cells revealed normal three dimensional surface morphology of ovum. The mature oocytes with ovigerous lamella showed smooth and rounded surface. Surface epithelium was marked with interdigitate blebs (microvilli) and some densely scattered

electron dense macro granule over the surface (Fig.1).The oocyte of group II A fish showed amoeboid shape with prominent depression & raised portion with pits and grooves on the surface. This shows the initiation of formation of furrows. Magnified portion of pits showed hexagonal or triangular striation with prominent cracks (Fig.2). The oocyte of group II B fish showed marked increase in the number of groove and furrows at the follicular epithelial region with more depression and shriveled surface. (Fig.3).The oocyte of group IIC fish showed a number of grooves and depressions on the surface, eroded plasma membrane and blebs on the surface epithelium (Fig.4). Follicular epithelium has been seen completely loosened, ruptured and shrinking. Some materials from the oocyte were shown to be ooze out on the surface probably secretory granules and lysosomes on the oocyte surface. (Fig.4).

Biochemical observations: The biochemical findings of the present study have been elucidated in text tables I, II III and IV.

Table-1: Showing variation in serum LPO (µmole/ml) in different experimental group of fishes.

Serum LPO	Control (Group-I)	Butachlor treated (Group-II)		
		5 days (Group-IIA)	10days (Group-IIB)	15 days (Group-IIC)
(µmole/ml)	1.435±0.014	2.784±0.792[a]	2.848±0.047[b]	6.820±1.290[c]
		(+) 93.98%	(+) 98.49%	(+) 375.18%

Values are expressed in Mean ± SEM of ten replicates in each group. Paired t test were applied between control (group I) and butachlor treated (group IIA, IIB and IIC) fish. Significant responses are marked as a=p<0.05; b=0.01 and c=0.001.Figures in parenthesis show % increase (+) over control.

Table-2: Showing variation in blood GSH (mg/dl) in different experimental group of fishes.

Reduced GSH	Control (Group-I)	Butachlor treated (Group-II)		
		5 days (Group-IIA)	10days (Group-IIB)	15 days (Group-IIC)
(mg/dl)	4.59±0.367	3.6±0.316b[b]	2.7±0.615[a]	2.843±0.587[c]
		(-) 21.568%	(-) 41.17%	(-) 45.75%

Values are expressed in Mean ± SEM of ten replicates in each group. Paired t test were applied between control (group I) and butachlor treated (group IIA, IIB and IIC) fish. Significant responses are marked as a=p<0.05; b=0.01 and c=0.001.Figures in parenthesis show % decrease (-) over control.

Table-3: Showing variation in serum TSH (µIU/ml) in different experimental groups of fishes.

Serum TSH	Control (Group-I)	Butachlor treated (Group-II)		
		5 days (Group-IIA)	10days (Group-IIB)	15 days Group-IIC)
(µIU/ml)	0.65±0.207	1.188±0.084[a]	1.64±0.083[b]	3.20±0.406[c]
		(+) 82.76%	(+) 146.15%	(+) 392.30%

Values are expressed in Mean ± SEM of ten replicates in each group. Paired t test were applied between control (group I) and butachlor treated (group IIA, IIB and IIC). Significant responses are marked as a=p<0.05; b=0.01 and c=0.001.Figures in parenthesis show % increase (+) over control.

Table-4: Showing variation in serum estradiol (µIU/ml) in different experimental groups of fishes.

Serum estradiol	Control (Group-I)	Butachlor treated (Group-II)		
		5 days (Group-IIA)	10days (Group-IIB)	15 days (Group-IIC)
(µIU/ml)	1.193±0.086	1.883±0.075[c]	1.59±0.155[a]	1.46±0.157[a]
		(+) 57.83%	(+) 33.277%	(+)22.380%

Values are expressed in Mean ± SEM of ten replicates in each group. Paired t test were applied between control (group I) and butachlor treated (group IIA, IIB and IIC) fish. Significant responses are marked as a=p<0.05; b=0.01 and c=0.001.Figures in parenthesis show % increase (+) over control.

Lipid peroxides (LP) are products of oxidative damaged lipids resulting from lipid peroxidation reactions induced by thio barbuteric acid. It represents the extent of oxidative damages (Shedahel and Tappel, 1974). In the present study the normal serum LPO in *Clarias batrachus* have been recorded as 1.435µmole/ml. In group II A it shoot up significantly ($p<0.05$) by 93.98%. It showed an abrupt increasing trend in group II B and group IIC fishes where it has gone up significantly ($p<0.01$) by 98.49% and 375.18% over the control. Xenobiotics are known to increase LPO level in fish (Rana, 1995; Kumar *et al*, 2008; Vinodhini and Narayanan,2008 and Bhattacharya and Bhattacharya,2007).The products of lipid peroxidation such as malondialdehyde and 4-hydroxy novenal are highly toxic to the cell (Raleigh, 1985). Lipid peroxidation within membrane has devastating impact on the functional state of the membrane because it alters the fluidity and thereby allowing ions such as $ca^{+2}$to leak into the cell. The peroxyl radical formed from the lipid peroxidation can attack membrane protein and enzyme and also reinitiates the protein synthesis. The deformities in

the oocytes in the present investigation due to butachlor exposure seem to be due to excess production of LPO.

(Magnification of the SEM micrographs is represented by bar itself)

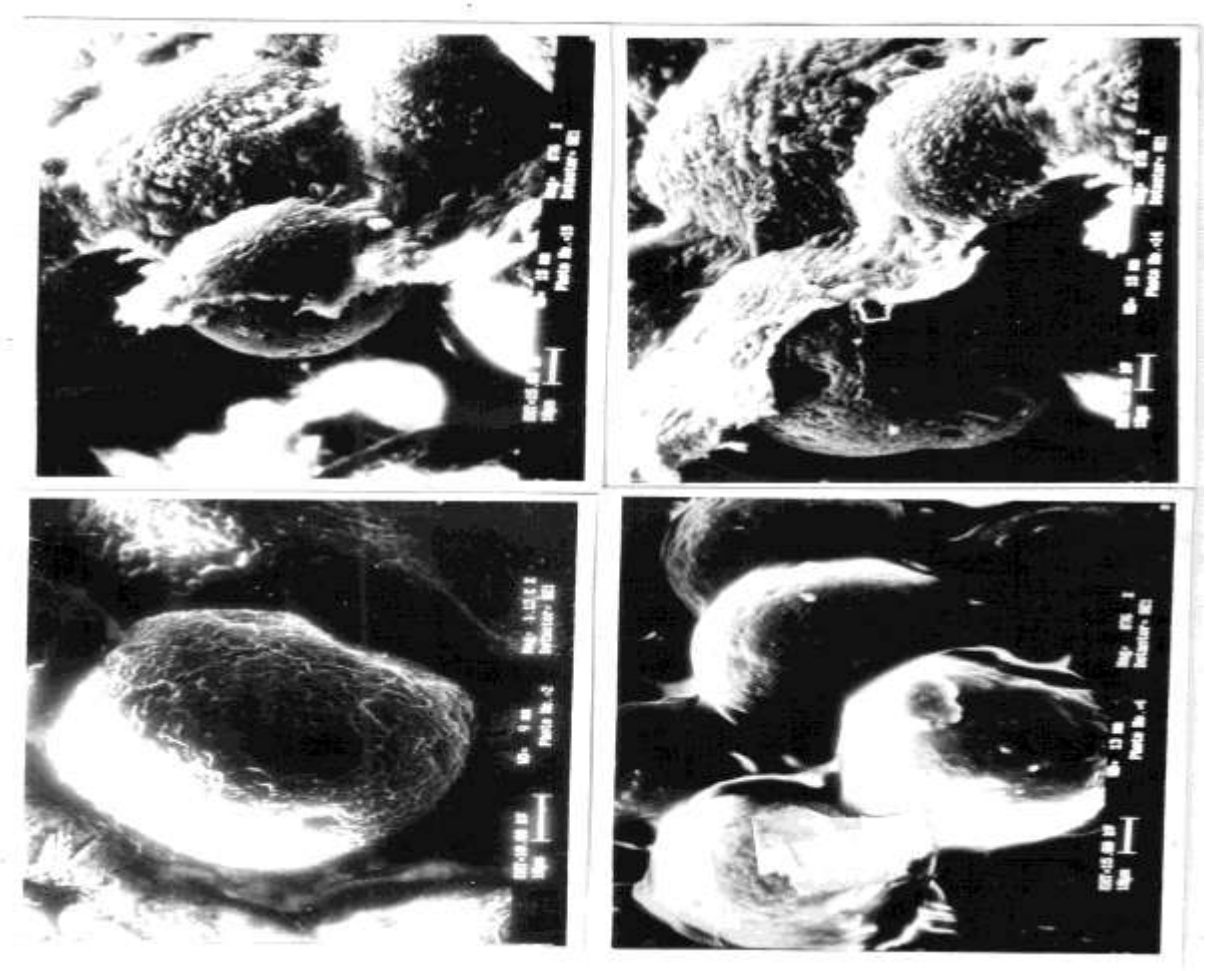

Fig.1: Scanning electron micrographs of ovary of control *Clarias batrachus* showing clusters of maturing oocytes on the ovigerous lamella (OL) with blebs (b) and dense macro granules scattered over the surface.

Fig.2: the ovarian cells of group IIA (1.5 µl/l Butachlor treated for 5 days) fish showing deformity with amoeboid shape of ovum forming depression, raised portion with pits and grooves on the surface.

Fig.3: The ovarian cells of group II B (1.5 µl/l Butachlor treated for 10 days) fish showing more groove and furrows at the follicular epithelial region with more depression and shriveled surface.

Fig.4: the ovarian cells of group II C (1.5 µl/l Butachlor treated for 15 days) fish showing a number of grooves and depressions on the surface, acellularity (AC) giving amoeboid appearance, eroded plasma membrane and blebs on the surface epithelium.

Glutathione is a major endogenous antioxidant produced by the cell. It participates directly in the neutralization of free radicals and reactive oxygen compounds (Scholtz et al., 1989).It is essential for regulation of nitric oxide cycle (Clement et al., 1999). It is very much essential for the immune system to work at its full potential. It also plays a fundamental role in majority of metabolic and biochemical reactions. In the present study the normal level of GSH was recorded as 4.59±0.367mg/dl. A sharp decreasing trend in blood GSH level has been observed in all the experimental groups. It showed significant ($p<0.001$) decline of 21.68%, 41.17% and 43.75% in group IIA, IIB and IIC respectively. As GSH is utilized in the detoxification mechanism, the GSH is converted into GSSG to neutralize the free radical. So the decreased level of GSH in group IIA fishes reveals that there is a great demand of GSH. Its further decline in group IIB and IIC clearly reveals the dominance of LPO and prolong exposure of butachlor lead to exhaustion of GSH pool of the body.

Organochlorines have been considered as reproductive biomarker and potential endocrine disruptor (Hauser *et al*, 2005).They are known to interfere with the basal metabolism of fish and suppress reproduction and steroidogenesis (Singh and Canario, 2004) and gonadotrophin level in fish by altering the secretory activities of hypothalmo-hypophyseal-thyroid/gonadal axis (Gorbman, 1969; Chand and Verma, 2006; Volkoff, 1999; Burse et al.,2007).In the present study butachlor exposure causes a significant ($p<0.01$) rise in serum TSH in all the experimental groups of fishes. The sharp increasing trend in serum TSH coincides with the increasing duration of butachlor exposure. A considerable increase of 82.76%, 146.15% and 392.30% in serum TSH have been recorded in experimental group IIA, IIB and IIC respectively. Similar kind of alterations in serum TSH and other thyroid function due to industrial wastes and chemicals have been reported by Pandey and Naralia (1998) and Brucker Davis (1998).

Normal serum estradiol is the index of good health of ovary. It leads to the proper growth, development and maturation of healthy follicles and minimize the number of atretic follicles, though atresia is a natural phenomenon. In the present study the normal estradiol level is 1.193ng/ml. However at shorter duration of butachlor exposure sharp increase in the level of estradiol have been found by 57.83% over the control while in group IIB and IIC a mild increase of 32.27% and 22.38% have been determined over control. The treatment of butachlor led to significant decline in the number of healthy follicles and increase in the number of atretic follicles leading to altered follicular kinetics. The decrease in the number of

healthy follicles in the ovary of butachlor treated fish might be due to imbalance in gonadal steroids which are essential for normal functioning of gonads (Sharpe, 1983).It may be correlated with the direct interference of butachlor to hypothalmo- hypophyseal axis and directly desensitizing ovary to gonadotrophin(Asch et al.,1990; Pasqualin et al.,1990; Smith et al, 1991)Induced serum TSH marks a case of thyrotoxicosis which impairs female gonadal function by desensitizing ovarian follicular cells to estradiol. Induced hypothyroidism causes delayed sexual maturation. The incubation of fish embryo with thyroid hormones is known to enhance the rate of embryonic development (Raine & Litherland, 2000).

Group IIA fish showed interdigitate furrow and depression on the surface of oocyte which seemed to be more aggravated in group IIB fishes. The amoeboid shape of follicular epithelial region with more depression and shriveled surface contributes to the follicular deformity. In fact any alteration in the cell surface reflect hormonal imbalance that interferes with the cellular architecture (Flickinger, 1979). In the present study the follicular epithelium has been seen completely loosened, ruptured and shrieked in group IIC fishes. The materials oozing out from the oocytes on to the surface seem to be secretary granules and liposomes. The abnormal cell shape and fragmented membrane occasionally buds from the surface and consequently reabsorbed following follicular atresia. The similar results have been reported by Darnell (2000) for the abnormally shaped erythrocytes.

CONCLUSION

The results of the present study clearly reveals that butachlor sets in a wave of hormonal imbalance causing sharp increase in serum TSH and estradiol leading to follicular atresia, formation of pits, clefts and furrows in maturing oocytes as evidenced in SEM observations. The oxidative stress generated due to butachlor exposure is marked by enhanced LPO level. Sharp decline in reduced GSH showed the non compliance of antioxidant system of fish body to combat the oxidative stress generated due to butachlor exposure. The present study provides first hand information regarding involvement of oxidative and hormonal stress in altered follicular dynamics in female fish.

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REFERENCES

1. APHA (2005): Standard methods for the examination of water and waste water (21st Ed.). A joint publication of American Public Health Association (APHA), The American Water Work Association (AWWA) and the water Environment Federation (WEF): 1368.
2. Asch, R. H., Smith, C. Y., Siler-Kroder, T. M. and Paurstein, C. J. effect of Δ^9 tetra hydrocannabinol during the follicular phase of the rhesus monkey, J. Clinical. Endocrinol, Metab, 1990; 52: 50-55.
3. Ateeq, B., Farah, M., Ali, M. & Ahmad, W. Induction of micronuclei and erythrocyte alteration in catfish Clarias batrachus by 2, 4-dichlorophenoxy acetic acid and Butachlor. Mutation Research, Vol. 2002; 518: 135– 144.
4. Barse, A.V., Chakrabarti, T., Ghosh, T.K., Pal Jadhao, S.B. Endocrine disruption and metabolic changes following exposure of Cyprinus carpio to dietyl phthalate. Pest. Biochem. And Physio. 2007; 88(1): 36-42
5. Battacharya, A. and Battacharya, S. Induction of oxidative stress by As in Clarias batrachus: involvement of peroxisomes, Ecotoxicol. Environ. Saf. 2007; 66: 178-187.
6. Beutler, E., Duron, O. and Kelly, B. Improved method for the determination of blood glutathione. Journ. of Laboratory and clinical Medicine, 1963; 61: 882.
7. Brucker Davis, F. Effect of environmental synthetic chemicals on thyroid function. Thyroid, 1998; 8(9): 27-56.
8. Chand, G.B., Verma, P. & Nath, A. Hormonal Imbalance and testicular atrophy in fresh water air breathing fish *Clarias batrachus*(Linn.) under sublethal doses of carbamate. In J. Ecophysiol. Occup. Hlth. 2006; 6: 19-24.
9. Clementi, E., Brown, G.C., Feelisch, M., Moncada, S., Bugg, S., O'Connell, M. J., Goldsbrough, P.B. and Cobbett, C.S. Phytochelatin synthase genes from Arabidopsis and the yeast Schizosaccharomyces pombe. The plant cell, 1999; 11(6): 1153-64.
10. Darnell, J., Lodish, H. Lodish and Baltimore (2000): Molecular cell biology, Sci. Am Books, New York, USA.
11. Dutta, H. M., Nath, A., Adhikari, S., Roy, P. K., Singh, N. K. and Dutta Munshi, J. Sub lethal malathione induced changes in the ovary of an air breathing fish H. fossilis: A histological study, Hydrobiologia, 1994; 294: 215-218.
12. Ellman, GL. (1959): Tissue sulfahydril groups. Archs. Biochem. Biophys. 82: 70-77.

13. Farombi. E.O., Ajimoko Y.R. and Adelewo O.A. Effect of Butachlor on antioxidant enzyme status and lipid peroxidation in fresh water catfish (Clarias garienpirues). 2008; 5(5): 423-427.
14. Flickinger, C. J., Brown, J. C., Kutchai, H. C. and Ogilvie (1979): Medical cell Biology, Hoft saunders,Philadelphia, USA.
15. Geng, B.R., Yao, D., Xue, Q.Q. Acute toxicity of the pesticide dichlorvos and herbicide butachlor to the tadpoles of four anuran species, Bull. Environ. Conta. Toxocol. 2005; 75: 343-9.
16. Gorbman, A. The thyroid function and its control in fishes. In "Fish Physiology" W. S. Hoar and D. S. Ranall eds. 1969; 2, Academic Press, New York and London.
17. Hausers, R. Williams, Altshul L.and Calafat, A.M. Evidence of interaction between polychlorinated biphenyls and phthalate in relation to human sperm motility. Environmental Health Perspectives, 2005; 113(4): 425-430.
18. Huxley, P.M. Mode of carcinogenic action of pesticide inducing thyroid follicular cell tumors in rodents". Environ. Health Perspect. 1998; 106 (8): 783-798.
19. Ishizuka, M., Iwata, H., Kazusaka, A., Hatakeyama, S. & Fujita, S. Effects of the agrochemicals butachlor, pretilachlor and isoprothiolane on rat liver xenobiotic-metabolizing enzymes.Xenobiotica, 1998; 28(11): 1029-1039.
20. Kumar, P., Prasad, Y., Ranjan, R., Swarup D., Pattanaik, A.K. and Patra, R.C. Accumulation patterns of cadmium in tissues of Indian catfish Clarias batrachus, Anim. Nutr. Feed Technol. 2008; 8: 115-119.
21. Lam, T. Z.:Environmental influence on gonadal activity in fish, In Hoar and Randall, Donaldson(Eds.)Fish Physiology IX, Reproduction Part- B, Academic Press New York , USA,1983.
22. Nolte, T.Honleman, J. H. and John, W. Histophysiology of chemically inducedtesticular atrophy in rat: Review. Exp.Ann. Toxicol., 1995; 47:247.
23. Numan, L., Hassan, M. and Stohs, S. Endosulfan induced depletion of glutathione and inhibition of glutathione peroxidase activity in rats. Gen. Pharmacol., 1990; 21(5): 625-628
24. Ohkawa, H. Ohisi, N. and Yagi, K. Assay of lipid peroxides in animal tissues by thiobarbituric reaction. Annal. Biochem. 1979; 95: 351.
25. Ou,Y. H., Chung, P. C., Chang, Y. C., Nago, F. Q., Hsu, K, Y.,& Chen, F.D. Butachlor, a suspected carcinogen, alters growth and transformation characteristics of mouse liver cells.Chem. Res. Toxicol. 2000; 13: 1321-5

26. Pandey and Niraliya. Impact of carbofuran on freshwater catfish H.fossilis.(Bloch.) Himalayan J. Environ. Zoo. 1998; 12: 249-254
27. Peixoto Francisco P., Carrola Joae, Coimbra Ana Maria, Fernandes conceicao.Trixeira Paulo , Coelho Luies, Conceicao Ivo, Oliveira Maria Manuel, and femandes Antonio Fontain.Oxidative stress responses and histological hepatic alterations in Barbel, Barbus bocagai, 2013; 29(1): 29-38.
28. Pasqualine, C. Sarrieau, A. D. and Assquillant, M. Estrogen like effects of12 dimethyl (a) anthranene on the female rat hypothalmo-pituitary axis, J. Steroid Biochem. 1990; 36: 485-491.
29. Raine, J.C. & Leatherland, J. F. Thyroid tissue in Rainbow trout embryos is not follicular and endogeneous thyroid follicle formation. 4th International Symposium of Fish Endocrinology,2000; Abst. No. 171.
30. Raleigh, J.E. Radioprotection of membranes Phamacol Ther, 1985; 39: 109-113.
31. Russel, L. D. The role of histopathology of testes in short term toxicology testing protocol. Inhalation Toxicology, 1995; 7: 961.
32. Rana, S.V.S., Singh, R. and Verma, S. Mercury - induced lipid peroxidation in the liver, Kidney, brain and gills of a freshwater fish, Channa punctatus. Japan. J. Ichthyol. 1995; 42(3/4): 255-259.
33. Rawat, D.K., Bias, V.S., Agrawal, N.C.A. Correlative study on liver glycogen and endosulfan toxicity in H. fossilis (Bloch) J. Environ. Biol. 2002; 23(2).
34. Saxsena, D.N. and Saxena, M. Events of biochemical integration during the reproductive cycle found in murrel Channa orientalis (Lin.). In Ichthyology Res. Advan (Editor, D.N. Saxena and Meena saxena,1999; 345-354. Oxford and IBH Publishing Com. Pvt. Ltd., New Delhi
35. Sharpe, R. M. Local control of testicular function, Quart. J. Expt. Physiol, 1983; 68: 265.
36. Smith, B. T., Plowchalk, D. R., Sipes, I. G. and Mattison, D. R. Comparison of random and serial in assessment of ovarian toxicity, Reprod. Toxicol. 1991; 5: 379-383.
37. Scholz, R.W., Graham, K.S., Gumpricht, E. and Reddy, C. Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation. Ann Ny Acad.Sci.,1989; 570: 514-7. Hughes RE., Reduction of dehydroascorbic acid by animal tissues. Nature 1964; 203: 1068-9.
38. Shedahl, J.A. and Tappel, A. L. Flourescent products from aging Drosophila melanogaster: an indicator of free radical lipid peroxidation damage. Exp. Gerontol. 1974; 9: 33-41.

39. Singh, P.B., Canario, A.V. M. Reproductive endocrine disruption in the freshwater catfish H. fossilis in response to pesticide γ-hexacholocyclohexane. Endocrinology and Environmental Safety, 2004; 58 (1): 77-83.
40. Soundararajan, M., Veeraiyan, G., Samipillai, S.S. Arsenic induced oxidative stress in fresh water catfish Tilapia mossambica. Journal of Phytology Section: General Science, 2009; 1(4): 267-276.
41. Verma, P. and Chand, G.B. Altered biochemical profile of carbohydrate, protein and triglycerides in Clarias batrachus and Heteropneustes fossilis due to rogor and endosulfan toxicity. Journal of Ecophysiology and Occupational Health. 2007; 7(1-2): 63-68.
42. Vinodhini, R. and Narayanan, M. Heavy metal induced histopathological alterations in selected organs of the Cyprinus carpio L. (common carp). Int. J. Environ. Res., 2009; 3(1): 95-100.
43. Volkoff, H. Worms, J.P., Amesbury, K. and Snelson, F.F. Structure of the thyroid gland, serum thyroid hormone and the reproductive cycle of Atlantic sting ray, Dasyatis sabina J. Exp. Zool. Oct. 1999; 1; 284 (5): 505-16.
44. WHO Reproductive Health Research at WHO, A new beginning, Biannual report1998-1999, Special Program of Research, Development and Research training in Human Reproduction, World Health Organization, Geneva,2000.
45. Yu, B.P. Cellular defenses against damage from reactive oxygen species, Physiol. Rev. 1994; 74: 139-162.