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HISTOPATHOLOGICAL CHANGES INDUCED IN THE GONADS OF FRESHWATER FISH CHANNA PUNCTATUS(BLOCH) ,UNDER CHRONIC STRESS OF HOUSEHOLD DETERGENTS

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ABSTRACT :

In the present study an attempt has been made to find the histopathlogical changes induced by two most commonly used detergents Ariel and Wheel in the gonads of fresh water fish Channapunctatus (Bloch).The test fish on exposure to the sublethal concentration of the detergents ,Ariel (5.69 mg/l) and Wheel (11.68 mg/l) for 30 days revealed several degenerative changes like arrest of spermatogenesis in case of testis and arrested vitellogenesis in case of ovary and delayed maturity of testis. The lesions however were more pronounced under Ariel toxicity. The G.S.I. underwent significant reduction ($p<0.01$) in case of Wheel and ($p<0.001$) in case of Ariel. The details have been discussed in this paper.

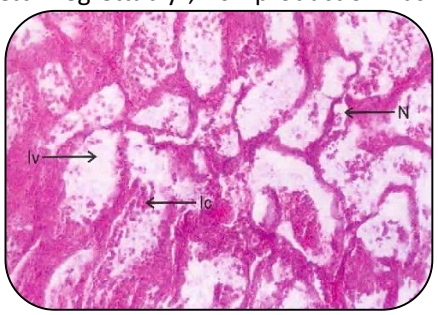
KEYWORDS : *histopathlogical, spermatogenesis, vitellogenesis.*

INTRODUCTION-

Present Indian market is flooded with a diverse range of detergents where a myriad brands compete with each other. However in this competition more and more harmful components are being continuously added by the manufacturers , which ultimately is bound to be further detrimental to the health of environment. Infect the virtually unregulated use of harmful chemicals in the Indian detergents industry is a situation that needs to be quickly reversed.

The necessity for studying detergents toxicity and its monitoring seeks top attention because of their voluminous discharge into aquatic system. Report indicate that almost all detergents or their components , especially Surfactants and builders ,are toxic to the aquatic organisms, including invertebrate and vertebrate fauna (Bindu and Philip,2001; Choudhary,P. and Jha,2014; Hodda and Rathee,2015). The worst aspect of surfactants is the fact that these are not completely mineralized in biological waste treatment plants and the poor waste water treatment facilities In our country coupled with significant cost increase in detergents rich sewage treatment have aggreveted the problems many fold.

Fishes are one of the most important aquatic fauna constituting staple food of high calorific value besides being the source of a number of beneficial by products, viz., liver oil, fish meal, fish manure, isinglass etc. Regrettably , fish production has been adversely affected due to non sustainable activities of human beings that exert both direct or indirect effects upon fish fauna. The polluted water destroys the suitable conditions needed for reproduction and also disrupts fish metabolism leading to large scale mortality for a number of times.(Abel ,1974; Pettersen et al; 2000,Trivedi et al;2001, Saxena et al.2005; Jawahar Ali et al;2015).Due to environmental contamination fishes face difficulty right from gametogenesis to maturity causing ultimate death of



embryos , juveniles and adults before they are capable of reproduction.

Keeping the above facts in view ,the present work entitled "HISTOPATHOLOGICAL CHANGES INDUCED IN THE GONADS OF FRESHWATER FISH CHANNA PUNCTATUS(BLOCH) ,UNDER CHRONIC STRESS OF HOUSEHOLD DETERGENTS. " was carried out with an objective to find out the lesions induced in key tissues ,viz, testis and ovary following exposure of the fish for 30 days to sublethal concentration of two branded and commonly used household detergents Ariel (5.69 mg/l) and Wheel (11.68mg/l) . Histopathological investigations appeared essential in view of the fact histology and histopathology are bio-monitoring tools in toxicity studies for assessing degree of pollution particularly for sublethal and chronic effects under stressful situations.(Bernet et al;1999, Vanderoost et al;2003).

MATERIALS AND METHODS

The fresh water fish ,Channapunctatus (Bl.) were selected as test animal for the present study. The fishes were procured live from the local non polluted ponds with the help of fisherman during early morning hours .They were brought to the lab. In wide mouthed large earthen pot half filled with water and covered with mosquito net. They were washed thoroughly in water and then rinsed in KMnO4 solution to get rid of dermal infection, if any. Thereafter healthy fish of an average weight (45-50 g) were transferred with the help of hand net to 60 litre rectangular glass aquaria,1/3rd filled with de-chlorinated tap water for acclimation in lab. Condition for about 15 days. No food was provided during the first four days of acclimation to enable the fish to accept the given food. They were fed with chopped goat liver ad libitum at 10:00 A.M.. Fishes were allowed to consume food up to one hour, after which unconsumed food was removed and water replenished to prevent decay and accumulation of wastes and depletion of dissolved oxygen content. No aeration was done and fishes were adjusted to natural photoperiod.

The test chemicals were Wheel (Hindustan Unilever Limited,Mumbai) and Ariel (Procter and Gamble Home Products Ltd; Mumbai). The procedures for preparation of test concentrations was performed following the methods of APHA et al.2005. The pH was recorded by a digital pH meter.Bioassays were performed to determine LC50 values (concentrations lethal to 50% of the test fish) of the detergents, Wheel and Ariel, for 24,48,72 and 96 hrs by adopting the most recent Arithmetic method of Dede and Kaglo (2001) and following all standard protocols and precautions as mentioned in standard methods (APHA et al.,2005).

One group of fish was exposed to 11.68 mg/l of Wheel and the second group to 5.69 mg/l of Ariel whereas the third group served as the control. The exposure media was renewed every 24 hr after the feeding schedules whereas only water was renewed in the control group. On day 30 of the exposure the fishes of each group was anaesthetized with MS222 (Tricane methane sulfonate Sandoz) for two minutes and weighed.Thereafter the fishes were dissected ,gonads taken out and embedded tissue were sectioned at 5-6 *µin thickness and then stained with Herrishaemotoxylin* and eosin stain (H & E) . The stined slides wereprocessed for routine histological examination. The selected slides were photographed . The mean and standard error (±SE) were calculated to determine Gonado somatic index (GSI) employing student 't' test (Fisher

RESULT AND DISCUSSION TESTIS:-

The testis of the control fish is composed of large number of semniferous tubules of varying size, closely bounded together by means of loose connective tissue stroma.Each tubule has a definite ,thin fibrous wall. Thetublar walls are lined internally with germinal epithelium. The interlobular space contain connective tissue ,blood capillaries and cells of leydig .Cells of various spermatogenic stages are present in each testicular lobule. The spermatogonium or sperm mother cell is spherical or roundedin shape and of largest size with central nucleus and comparatively clear cytoplasm. The primary spermatocytes are smaller in size but larger from secondary spermatocytes with darkly stained nuclei .The nuclei of secondary spermatocytes show thick aggregation or chromatin material. The spermatids are of further smaller size occupying a position just away from the centre. The sperm cells are centrally located having pin head and very few in number probably due to the fact that histological preparation were made during maturing phase.

The testes of detergent treated fish exhibited arrest of spermatogenesis or delayed maturity of the testis . The lesion however were more prounced under Ariel toxicity. 30 days exposure of the fish

*Channapunctatus*to a sublethal concentration (11.68mg/l) of wheel induced numerous degenerative changes like disorted shape of lobules and their vacuolization ,significant increase in thickness of lobular wall accompanied by decreased diameter of lobules and various spermatogenic cells, destruction of connective tissue and very sparse number of intrstitial cells of leydig as well as inflammation and distortion of semniferous epithelium. The G.S.I. underwent significant reduction from 0.262± 0.03 in control to 0.182± 0.016 (P<0.01).

Apart from these changes, Ariel exposure of the fish produced extensive cytotoxic damage and general inflammatory response in the testis. Further, stages upto spermatocytes could only be detected denoting almost complete retardation/arrest of spermatogenesis, extensive condensation of spermatogenic cells, autolysis of interstitial cells, vacuolationof tubular cells causing peculiar starry sky appearance of the testicular tissue and significantly reduced G.S.I.(0.163± 0.14 against the control value of 0.262 ± 0.03 (P< 0.001).

TABLE I: ALTERATIONS IN LOBULAR WALL THICKNESS AND DIAMETER OF LOBULE, SPERM MOTHER CELL, SPERMATOCYTES AND INTERSTITIAL CELLS IN THE TESTIS OF CHANNA PUNCTATUS EXPOSED TO DETERGENTS.

components	DIAMETER(µm)		
	Control	Wheel exposed	Ariel exposed
Lobular wall thickness	14.06± 0.32	16.32± 2.53a	17.78±1.47C
Lobule	137.30± 2.05	134.16± 2.74c	12.54±2.38C
Sperm mother cell	12.86± 0.42	12.57± 0.32NS	12.48±0.26NS
Primary spermatocytes	7.57± 0.38	6.98± 0.22b	6.74±0.25c
Secondary spermatocytes	4.86± 0.16	4.37±0.14b	3.82±0.16c
Interstitial Cell	3.21± 0.03	2.64±0.02c	ND

Each value represents Mean ± SE of 10 observations.

A= p<0.05; b=p<0.01; c=p< 0.001.

NS= Statistically not significant and ND= Not detected

Testes in fishes represent the most dynamic organs having a high cell turn over during the reproductive period and are vulnerable to a wide variety of chemical toxicants. Our findings listed above are in conformity with those of Jha et al;(1994); Yamazaki and Donaldson;(1968).The above adversely influenced testicular cycle reflecting degeneration and exhaustion under detergents stress have been associated with disturbed mitotic events. Similarly, other catastrophic changes,including condensation of spermatogenic cells, lobular vacuolization, loss of spermatids and sperm cells indicating spermatogenesis have been attributed to the lack of endogenous gonadotropins.

OVARY :-

The ovary of control fish on microscopic examination shows three usual layers, outer peritoneum ,middle tunica albuginea and innermost germinal epithelium which joins tunica albuginea at several place and projects into central lumen ovocoel in form offinger like projections called ovigerous lamellae. The ovary exhibitslarge number of ova in different developmental stage like stage I immature or pre vitellogenic oocytes, stage II vitellogenic or maturing oocytes, stage III oocytes or mature eggs. Upon exposure of the fish for 30 days to 11.68 mg/l Wheel and 5.69 mg/l Ariel detergents , a number of degenerative changes were induced in the ovary of the fish, all pointing towards arrested vitellogenesis. The ovary stressed for 30 days to 11.68 mg/l Wheel and 5.69 mg/l Ariel detergents , a number of degenerative changes were induced in the ovary of the fish, all pointing towards arrested vitellogenesis. The ovary stressed with Wheel detergent had large number of stage I and very few stage II oocytes and traces of pre-ovulatory degenerated oocytes. There was complete breakage and dissolution of ovigerous lamellae, separat± ion of ovarian follicles due to dissolution of interfollicular follicles and vacuolation of developing

oocytes. The diameter of oocytes and G.S.I. were significantly reduced (1.214 +_ 0.019 against the control value of 1.296+_ 0.18; p<0.01) .

TABLE II: RANGE AND MEAN VALUE OF THE DIAMETER OF DIFFERENT STAGES OF OOCYTES IN THE OVARY OF NORMAL AND DETERGENTS EXPOSED FISH CHANNA PUNCTATUS.

Oocyte stage	Range and mean value of diameter(µm)					
	CONTROL		WHEEL EXPOSED		ARIEL EXPOSED	
	Range	Mean value	Range	Mean value	Range	Mean value
Stage I	74.0-126.0±4.55	94.95±2.50	62.0-90.0±3.80	76.50±2.70***	48.0-70.0±3.0	56.65±2.40***
Stage II	182.0-290.0±2.85	219.75±5.35	122.0-136.0±2.30	126.50±2.50***	ND or Resorped	
Stage III	274.0-392.0±3.50	312.85±4.25	ND		ND	

Each value represents Mean ± SE of 10 Observations.

***=p<0.001.**

ND = Not detected

The ovary on Ariel exposure exhibited high degree of degenerative changes, all pointing towards arrest of vitellogenesis . The oocytes become deshaped ,follicular epithelial cells were disrupted and there was almost complete vacuolation of cytoplasm and the oocytes showed necrosis and clumping. The nuclei were displaced peripherally and the population of atretic oocytes increased considerably. Besides ,resorptionof stage II and III oocytes , stromal haemorrhage, significantly and greatly reduced diameter of oocytes and G.S.I. The G.S.I. of Ariel exposed ovary was 1.07 ± 0.008 against the control G.S.I. of 1.296 ± 0.18 (p< 0.01).

The results of the present study are in resemblance with those of Shrivastava et al.,(2008); Pathen et al; (2012): Makode (2012) and Kakde (2012) .The observed degenerative changes in the ovary have been attributed to direct action of detergents , lower level of gonadotropins and to the impaired /inhibited activities of alkaline phosphatases.

Thus the present gonadal histopathologies clearly reflect that input of detergents and their after wash into aquatic systems must be checked/ monitored on priority basis failing which there is every possibility of significant decline in ovarian weight and fecundity, delayed ovulation and insemination process ,all of which in turn may lead to less productivity of the fishes.

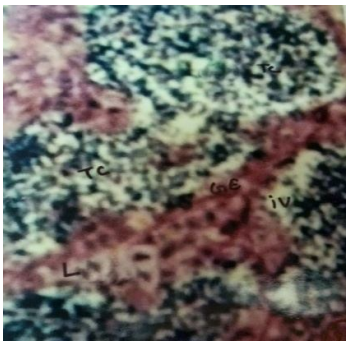


Fig.1.Control fish testis showing germinal epithelium (GE) Connective tissue (CT) and interstitial cells of leydig(L), inter lobular vacuolation(iv), and scattered tubular cells x60 H&E

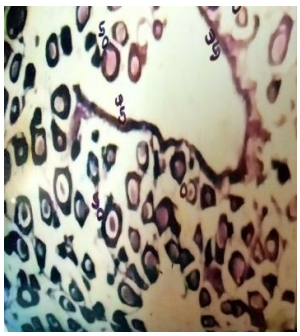


Fig.4. Immature control fish ovary showing germinal epithelium (GE), oogonium (OG) and oocytes (O).X60; (TC) H& E.

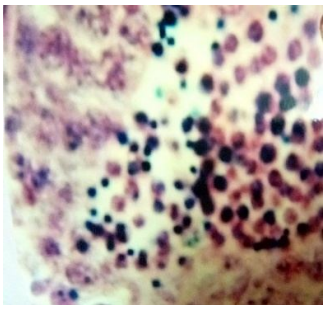


Fig.2.Breakage,degenration and dissolution of lobularwall and oedematous changes towards periphery of lumen in the testes of wheel exposed fish. X600; H& E Dissolution of ovigerous lamellae:separate

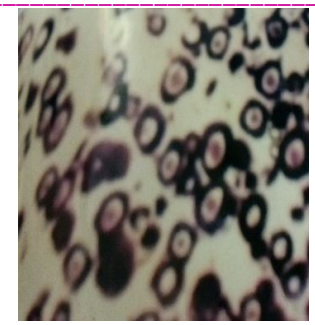


Fig.5.Wheel exposed fish ovary denoting arrested vitellogenesis as evident from large number of stage I oocyte ,complete Ovarian follicles due to autolysis of Connective tissue resulting into increased Inter follicular and vacuolation & Condensation of oocytes.x60; H&E

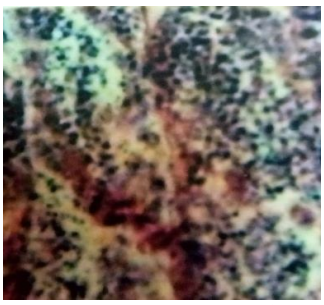


Fig.3. Extensive condensation of spermatogenic evident from inflammation and clump formation:vacuolation of tubular cells in the fish testis under Ariel stress. X600; H&E Towards of fish exposed to Ariel detergent detergent

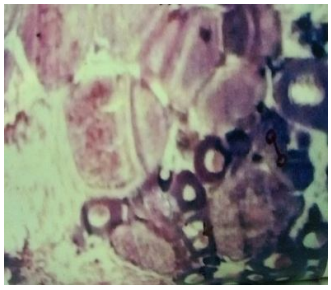


Fig.6. Degenerative changes in the ovary ofcells as the fish exposed to Ariel detergents showing necrosed oocytes (O-O), Nuclear displacement periphery (->) and large number number oocytes and stromalhaemorrhage.x60;H&E

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