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Research Article

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Effect of Manganese on Ovaries of *Garra gotyla gotyla*

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ABSTRACT

Presently an attempt has been made to study the effect of heavy metal manganese (0.64mg/l) on the ovaries of fish Garra gotyla gotyla for 9 weeks experimental duration. A reduction in number of oocytes as well as diameter of stage I, stage II and stage III has been observed in metal treated groups during the experimental period of 9 weeks. Necrosis, vacuolation, increase in interfollicular space and apperarence of atretic oocytes were other histological abnormalities observed in treated ovaries of Garra gotyla gotyla . GSI depicted a fall in its values compared to control groups (0.60) and was found to be 0.472 at the end of experimental period of 9 weeks.

Key words: *Garra gotyla gotyla, ovaries, necrosis, vacuolation and interfollicular space.*

INTRODUCTION

Evaluation of quality of oocytes is one of the useful parameter of reproductive health in fishes. Size of individual follicles, appearance of yolk and diameter of the vitelline envelope are some of the factors that can be evaluated histologically[1]. A number of studies have shown a direct relationship between the body burden of chemicals such as organochloride in gravid females and the concentration of these chemicals in eggs[18,23]. Workers like Sumpter[27], Rolland *et al*[24]., Jobling *et al*[8]., and Kime[11] recognized many environmental chemicals that act as endocrine disrupter and hence influence the reproductive health of wild aquatic animals including fish. Currie and Woo[4] also reported severe reproductive consequences in fishes exposed to a variety of pathogens and stressful conditions. It is also on record that even a slight change in the concentration of certain chemical compounds (metals, insecticides etc.) can negatively affect the histological properties of fish.

Manganese is one of the essential micronutrient involved in a wide range of biological processes (including enzymatic) and recognized as one of the most important component of fish diet[28] but its effects on reproduction of fishes appear to have received the least attention.

Presently, therefore a study has been undertaken to evaluate effects of 0.64mg/l manganese on the ovaries of *Garra gotyla gotyla* for an experimental period of 9 weeks.

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MATERIAL AND METHODS

G. gotyla gotyla were collected with the help of cast net from the Jhajjar stream of Jhajjar Kotli region of Jammu, J & K, India. The fishes were acclimatized in natural condition and then were divided into two groups control and treated. The control groups were not subjected to chemical treatment but treated groups were subjected to 0.64mg/l MnSO4. In control groups the fishes were maintained in normal, aerated tap water. The water in each plastic tub was replenished daily to keep the metal concentration unchanged. The experiment had three replicates and in each replicate 40L of water was added and 10 number of *Garra gotyla gotyla* was taken in each experimental tub. The fishes were dissected and their ovaries were carefully removed, excessive moisture was blotted and quickly weighed on an electronic balance. Ovaries were then fixed in bouin's fixative [freshly prepared from saturated solution of picric acid 75% to which formalin (20%) and acetic acid (5%) was added at the time of use]. After 24 hours treatment in bouin's fixative, ovaries were then washed, dehydrated and embedded in paraffin histo wax (54-56°C). 5-7 µm transverse sections of ovaries were cut with the help of microtome and were stained using haematoxylin-Eosin stain. Then prepared slides were observed under microscope. Gonado somatic index which is the percentage of gonads in body weight was calculated using following formula.GSI =weight of gonads (gm)/ weight of fish (gm) ×100.Percentage distribution of different stage oocytes was calculated by studying ovarian microslides at different focal points.

RESULTS

Control Groups

Histological sections of control ovaries showed the presence of oocytes of stage I (S-I, 61.5%), stage II (S-II, 30.8%) and stage III (S-III, 8.5%) respectively (Table 1). The oocytes of stage I were nearly spherical in shape with a distinct nucleus and had diameter ranging between 0.0230mm-0.0490mm while oocytes of stage II had a diameter of 0.0530mm to 0.1324mm (Table 1 and Figure 1) and showed many more nucleoli in their nucleus. Those of stage III were observed to be larger in size which ranged in diameter from 0.1160mm-0.3510mm. These stage III oocytes showed the presence of yolk vesicles in the periphery of ooplasm. Ovaries of control *Garra gotyla gotyla* did not show presence of any atretic oocyte. The gonado somatic index (GSI) at the time of start of experiment was recorded to be 0.60 in these control groups of fishes.

Ovaries exposed to 0.64mg/l sublethal concentration of manganese

The ovaries of fish *Garra gotyla gotyla* exposed to sublethal concentration of manganese showed a reduction in number of oocytes as well as diameter of stage I, stage II and stage III (Table 1) during the experimental period of 9 weeks (Table 1 and Figures 2-5). Reduction in diameter and number of different stage oocytes was observed to result in creation of large inter follicular spaces (Figure 4). Atretic oocytes were also observed and range between 0.5% to 18.2% (Table 1). Necrosis and vacuolation were other histological abnormalities observed in treated ovaries of *Garra gotyla gotyla* (Figures 2-3). GSI depicted a fall in its values compared to control groups (0.60) and was found to be 0.472 at the end of experimental period of 9 weeks.

Table 1: Effect of 20% sublethal concentration of Mn on the number and size of oocytes (mm) and GSI in fish *Garra gotyla gotyla*

Time Interval	S-I oocyte		S-II oocyte		S-III oocyte		Atretic Oocyte (%)	GSI
	Number	Diameter	Number	Diameter	Number	Diameter		
Control	61.5 ±0.84	0.0230±0.1420-0.0490±0.1523	30.2±0.24	0.0530±0.1324-0.2010±0.1362	8.3±0.55	0.1160±0.2355-0.3510±0.3622	Nil	0.60 ±0.65
1st wk	61.2±0.34	0.0230±0.1301-0.0490±0.3620	30.0±0.32	0.0525±0.1247-0. 2004±0.1240	8.3±0.34	0.1160±0.1520-0.3510±0.3264	0.5±0.02	0.595±0.24
2nd wk	60.5±0.25	0. 0230±0.0952-0.0490±0.1652	30.0±0.65	0.0525±0.3210-0.2000±0.3620	8.1±0.06	0.1160±0.1436-0.3510±0.2563	1.4±0.36	0.590±0.34
3rd wk	59.2±0.24	0.0228±0.1234 -0.0490 ±0.1523	29.8±0.80	0.0514±0.0260-0.1995±0.3246	8.0±0.49	0.1145±0.1436-0.3498±0.3479	3.0±0.01	0.550±0.21
4th wk	58.0±0.64	0.0220±0.1325-0.0450 ±0.1463	29.5±0.61	0.0513±0.4260-0. 1995±0.2340	7.8±0.45	0.1142±0.3362-0.3498±0.0324	4.7±0.04	0.510±0.56
5th wk	56.3±0.42	0.0215±0.1439-0.0420±0.1420	29.0±0.35	0.0510±0.1432-0.1990±0.3621	7.2±0.05	0.1140±0.1425-0.3490±0.3220	7.5±0.31	0.498±0.14
6th wk	55.7±0.31	0.0210±0.1630-0.0420±0.1742	28.5±0.20	0.0495±0.3601-0.1980 ±0.3620	6.5±0.28	0.1127±0.1434-0.3480±0.3300	9.3±0.21	0.490±0.34
7th wk	53.8±0.94	0.0195±0.0142-0.0390±0.1632	27.4±0.34	0.0486±0.0143-0.1972 ±0.3625	6.4±0.33	0.1114±0.2362-0.3475±0.2230	2.4±0.62	0.482±0.10
8th wk	52.5±0.27	0.0184±0.1520-0.0380±0.6322	25.0±0.05	0.0470±0.1436-0.1965±0.4263	6.2±0.49	0.1098±0.2100-0.3460±0.3216	16.3±0.10	0.479±0.62
9th wk	51.0±0.30	0.0170±0.6791-0.0350± 0.3140	24.8±0.41	0.0462±0.4261-0.1958±0.3620	6.0±0.73	0.1090±0.3220-0.3460±0.0140	18.2±0.04	0.472±0.14

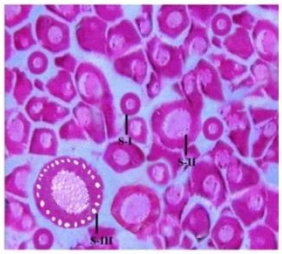

Fig. 1 Microphotorap of control ovary showing oocytes of stage I, II and III.

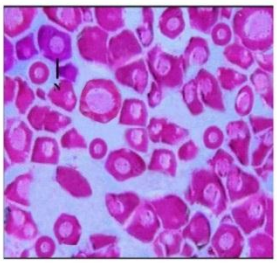

Fig. 2 showing necrotic oocytes after 3rd week of experimental period

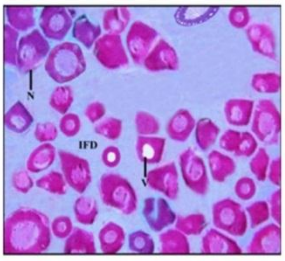

Fig. 3 showing vacuolated oocytes after 5th week of experimental period.

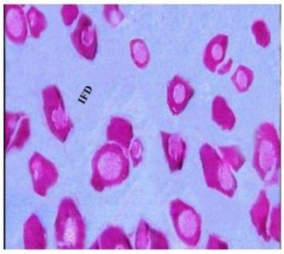

Fig. 4 showing increase in interfollicular space after 7th week of experimental period.

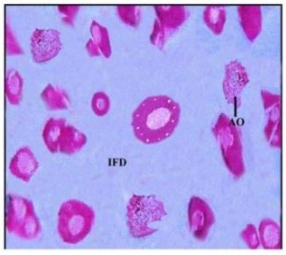

Fig. 5 showing atretic oocytes after 9th week of experimental period.

DISCUSSION

Presently various histopathological alterations were observed in the ovaries of fish *Garra gotyla gotyla* when exposed to 0.64mg/l of manganese. Reduction in number and size of oocytes of stage I, stage II and stage III was observed during the 1st week of experimental period (Tables 1 and Figures 2-5). Besides there was also observed a gradual decrement in number of oocytes (S-I, S-II and S-III) whose frequency increased with the advancement of experimental period (Table1). Similar to present findings, workers like Nath and Kumar[17], and Sioson and Herrera[26] also reported a reduction in number and size of oocytes in the ovaries of fishes *Colisa fasciatus*, *Oreochromis mossambicus* and *Heteropneustes fossilis* following exposure to metals like nickel and cadmium chloride.

Several authors in a bid to explain the observed retardation in the growth/ size of oocytes in fish treated with heavy metals have been stated this to be regulated through hypothalamo-hypophysial ovarian axis in fishes[2,3,19,21,22].

In this context, Ram and Sathyanesan[22] suggested suppression of activity of pituitary gonadotroph and somatotropin in fish *Channa punctatus* exposed to mercury. Nagahama *et al*[16]., states that pituitary gonadotroph secrete pituitary gonadotropin, an important element for the teleost oocyte to go for its long growth phase. Elaborating it further Herrera[6] expressed that pituitary gonadotrophs secrete estrogen by theca and granulosa cells. Low levels of gonadotropin secretion caused by exposure to mercury was reported by Saxena and Agarwal[25] to result in inhibition of proliferation and growth of oocytes and resorption of yolk in *Channa punctatus*. This low level of gonadotropin was held by them to be due to reduced estrogen synthesis necessary for vitellogenesis. Presently hypothalamo-gonadotrophic-gonadal steroidal axis though not studied but it seems that manganese by disrupting this very axis might be probable causative of alterations in number and size of oocytes in ovaries of fish *Garra gotyla gotyla*.

Based on above discussion, presently observed depression in ovaries can be attributed to exert either a direct action of manganese on ovary and/ or inhibitory action on pituitary gonadal axis.

Presently large interfollicular spaces were also observed which present author feels must be formed due to shrinkage/ reduction in size of oocytes of stage I, stage II and stage III besides decline of stromal tissue (Figures 2-5). Nath and Kumar[17], and Mishra and Mohanty[15] also reported large interfollicular spaces in ovaries due to shrinkage of oocytes under the effect of metal toxicity.

Atretic oocytes which have been observed to be absent in the ovaries of control fishes made their appearance during first week of experimental period though in very low percentage but their number kept on increasing with the advancement of experimental period (Table 1). Similar to present findings atretic follicles have also been reported by earlier workers like Kling[13] in fish *Tilapia leucostica* following an exposure to lebaycid, Nath and Kumar[17] in fish *Colisa fasciatus* following exposure to nickel, Sioson and Herrera[26] in ovaries of *Oreochromis mossambicus* under the nickel toxicity, Mishra and Mohanty[15] in fish *Channa punctatus* on exposure to chromium hexavalent.

Kapur *et al*[10]., and Pawar and Katdare[20] proposed decline in the level of ovarian 3-b-hydroxysteroid dehydrogenase (b-HSDH), a principle enzyme involved in the steroidogenesis, following heavy metal toxicity in the ovaries. Such decline in level of 3-b-HSDH during ovarian steroidogenin, they added may result consequently in an insufficient endogenous gonadotropin synthesis which according to them must be responsible for initiation of process of atresia in oocytes[20] and decrease in early and late vitellogenic oocytes[25]. Kirubagaran and Joy[12] attributed the atretic changes caused by mercury in *Clarias batrachus* as a direct action of mercury on the ovaries. Kumar and Pant[14] suggested a direct action of the heavy metals on the ovaries of *Puntius conchonius*.

Present author deriving support from findings of above workers is of opinion that reduction in synthesis of enzyme 3-b- HSDH though not studied presently as function of manganese toxicity seemingly by inhibiting the synthesis of gonadotropin, must have ultimately led to formation of atretic oocytes in the ovaries of *Garra gotyla gotyla*. Decline in number of stage I and stage II oocytes have been observed to be more rapid than stage III during present investigation on *Garra gotyla gotyla*. This simply implies their increased loss (stage II and III) from ovaries due to process of atresia. That it is so has already been advocated by Sioson and Herrera[26] who stated that heavy metal like zinc damages mainly younger oocytes. In this connection observations of Jobling *et al*[7]., on wild roach *Rutilus rutilus* living in river water receiving industrial effluents which contain large amount of heavy metals and Johnson *et al*[9]., in *Pleuronectes vetulus* inhabiting waterbodies receiving heavy chemical contaminants (including heavy metals) lend a strong support to present viewpoint that heavy metals (presently Mn) result in induction of atresia of oocytes.

Necrosis which is irreversible degenerative change can result in the death of tissue. Presently necrosis was observed from 3rd week onwards during experimental period (Figure 2). Necrosis further results in the vacuolation of oocytes of different stages (S-I, S-II and S-III). The number of necrotic and vacuolated oocytes increased with the advancement of experimental period. Similar to present observations workers like Mishra and Mohanty[15] also reported necrotic and vacuolated oocytes in the ovaries of fishes following an exposure to different metals and reported that such changes may be due to inhibitory action of metals on the pituitary-gonadal axis. Similar findings have earlier been documented by Dodd[5] and Nagahama *et al*[16].

Based on present observations and above discussion it can be stated that necrotic changes in oocytes in ovaries of fish *Garra gotyla gotyla* following manganese toxicity seemingly appear to be the result of toxic impact of manganese directly on ovaries by impairment synthesis and release of hormone from this axis. Gonado somatic index (GSI), a good index of gonads besides an indicator of onset of spawning season has been observed to witness loss during 1st week of experimental period and invariably remained low throughout the experimental period of 9 weeks (Table 1). The plausible reason for this decline in GSI has been attributed presently to increase in number of atretic oocytes and reduction in number of functional oocytes. Present observations also get strengthened from the findings of various other workers who also observed a decline in GSI in response to zinc treatment[19] in *Oreochromis niloticus*. That this heavy metal (Zn) is really responsible for decline in GSI has been proposed by them to be because of highly significant raised level of heavy metal Zn in ovaries of all treated fishes.

From the present discussion, it can be inferred that exposure of fish to manganese toxicity induce histological alterations in ovaries. It is apparent that the effect is time dependent. Manganese by altering the hypothalamo-hypophysial-ovarian axis and thereby suppressing the activity of gonadotrophs, present author proposes may induce degenerative changes in the ovaries of fish *Garra gotyla gotyla*.

CONCLUSION

Present results thus throw light that manganese when discharged into a water body adversely effects the inhabiting biota (presently *Garra gotyla gotyla*). At this juncture it may be added that such scenario if prevails on long term basis, may effect the reproductive potential (by reducing their fecundity) as evident by reduction in number and size of oocytes and increase in atretic oocytes. Such changes adversely effect the fish production potential of waterbodies for food on one hand and may lead to their ultimate extinction on the other.

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