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RESEARCH ARTICLE

EFFECT OF HEAVY METAL STRESS ON THE SEMINAL VESICLES OF *GLOSSOGOBIUSGIURIS* (HAM)

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ABSTRACT

Heavy metal pollution in aquatic ecosystems is a major environmental concern as these can accumulate in tissues and biomagnify the food chain interfering with the health and reproduction of fish. Fishes are sensitive indicators of pollutants present in the water. Heavy metal salts constitute a serious type of pollution of aquatic biota, because they are stable compounds. Tolerance to stress is likely to be lower in the reproductive tract than in any other organ system in fish. The fresh water gobiid fish *Glossogobius giuris* was exposed to sub lethal concentrations of heavy metals (Copper sulphate, 0.3 and 0.5ppm; Cadmium sulphate, 0.02 and 0.04ppm) for short term (24 to 96 hours) duration to understand the reproductive modulations. The effects of heavy metals on the seminal vesicles include vesicular regression, degenerating secretory cells and locules with minimal quantity of vesicular fluid and necrotic sperms. The study suggests that depletion of sperms in the seminal vesicle due to heavy metals alters the structure of the seminal vesicle, affects sperm motility and in turn the reproductive potential of the fish.

INTRODUCTION

Heavy metal contamination of aquatic system has attracted the attention of several investigators both in the developed and developing countries of the world. They are introduced into the environment by a wide range of natural and anthropogenic sources (Bineyet *al*., 1994 ; Wepener, *et al*.,2001) such as rapid population growth (Bineyet *al.,* 1994; Seymore, 1994), industrialization, urbanization and lack of environmental regulations (Pelgromet *al.,* 1994).Stress and its effects on fish have been the subject of numerous reviews (Barton and Iwama, 1991; Fagerlundet *al*., 1995). In fisheries management, the array of stressors that impinge on fish populations not only include those encountered in aquaculture, but also various methods of fish capture (Maule and Mesa,1994 ; Mittom and McDonald,1994), physical trauma(Gadomskiet *al*.,1994) and exposure of fish to environmental contaminants(Kime ,1995; Bonga,1997; Singh *et al*., 2006)). Amongst the pollutants contaminating water bodies metals play an important role(Witeskaet *al*., 1995) with high concentrations becoming toxic to fish (Wepener, *et al*., 2001). This release of heavy metals into the aquatic environment poses serious water pollution problems because of their toxicity (Evangelou,*et al.,* 2007) persistence and bioaccumulation in food chains (Rajathi and Sabhanayagam, 2011).

The structure and function of the seminal vesicles in male fishes have been observed and described by several workers (Fishelson 1991; Franceschini-Vinentini 2007).The changes in the morphology and the histology of the seminal vesicle during the reproductive cycles have been investigated by Sundararaj (1958) in *Heteropneustesfossilis*; Rastogi (1969 a) in *Mystusteengara*; Rao and Nadkarni (1979) in *Clariasbatrachus* and Murthy (1981) in *Glossogobiusgiuris*.Accessory reproductive organs in males are also known to be susceptible to toxic pollutants with Cadmiumcausing regression in the vas deferens of*Garramullya* (Wani and Latey 1982). Pandey and Shukla, (1980) have reported thickening of the muscle layer of both the sperm ducts and seminal vesicle in *Tilapia mossambicus* exposed to BHC and in *Tautogolabrusadspersus* exposed to ethinyl estradiol (Zaroogianet *al.,* 2001). In *G .giuris*, fenthionaffects the structure of the secretory epithelium of the seminal vesicles which is more pronounced during the non-breeding season (Murthy, 1995). Regression of seminal vesicle was observed after treatment with methallibure in *Clariasbatrachus* (Singh and Joy, 2000) and to thyroxine in *Clariasgariepinus* (Jacob *et al.,* 2005). Studies on the effect of malathion on the seminal vesicles of *G. giuris*revealed cytomorphological changes which includes thick interlocular connective tissue, clumped necrotic sperms and atrophied secretory cells (Sitavi *et al.*2010*).*

Studies on the reproductive biology of fishes have been made by a number of workers but there is paucity of information on the effect of heavy metals like Cadmium sulphate and Copper sulphate on the seminal vesicle of Gobiid fishes. Hence, in the present investigation attempts have been made to study the effect of heavy metals, on the histopathological aspects of the seminal vesicles of *G.giuris.*

MATERIALS AND METHODS

The Indian Gobiid fish, *Glossogobiusgiuris* (HAM) was collected at regular intervals from Kalavarpalli reservoir near Bangalore, India using cast and gill nets (mesh size : 10 mm). They were brought alive to the laboratory and acclimated for

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15 days in 50 litres glass aquarium (60"X30"X20") containing aerated tap water prior to experimentation. They were maintained at an ambient temperature of 27 °C ± 1°C and fed daily with earthworms. Only sexually mature male fishes weighing 10-15 g and measuring 10-12 cms in length were selected for toxicity studies.

Determination of LC $_{50}$ values

One hundred and twenty (120) fishes from the stock were used for the determination of LC $_{50}$.

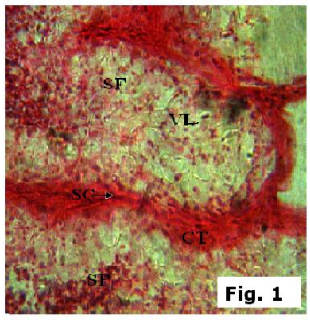
For the heavy metal toxicity study two sub lethal concentrations (0.02 and 0.04 ppm) were selected from the predetermined LC $_{50}$values of Cadmium sulphate to the test fish. Smiliarly two sub lethal concentrations (0.3 and 0.5 ppm) were selected from the predetermined LC_{50} values of Copper sulphate following the method of Finney, 1971.Fishes were divided into five groups of 10 each. Group I was used as control while group II and III were exposed to Copper sulphate (0.3 and 0.5ppm) and group IV and V were exposed to Cadmium sulphate (0.02 and 0.04ppm) for 24 and 96 hours.

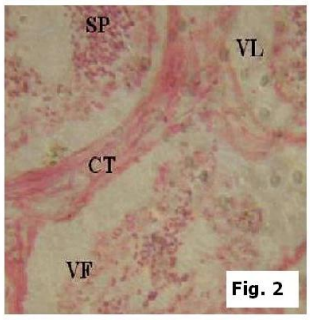
Histology of the Seminal vesicle

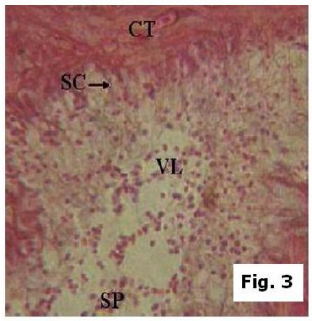
The fishes both treated and control was vivisected without anesthesia.Seminal vesicles were fixed in Bouin's fluid, dehydrated in ethanol and embedded in paraffin. Sections of 6 µ thickness were cut and stained in Delafield's haematoxylin and eosin for histological studies. The area of the six largest locules of the seminal vesicles of each fish was determined using calibrated ocular micrometer and an average was calculated.

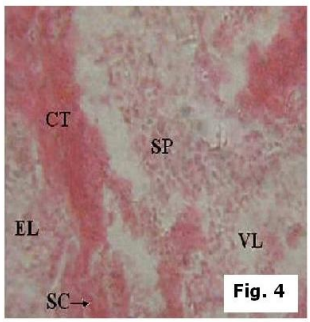
OBSERVATION AND RESULTS

Seminal vesicles are accessory glandular structures to the test is paired, flattened and fused posteriorly. The external surface of the vesicle is covered by a thin fibrous connective tissue sheath.The seminal vesicle consists of distinct vesicular locules of varying shape and size, separated by loose connective tissue sheath. The vesicular locules are lined by a single layer of secretory cells. The seminal vesicles exhibit seasonal changes that are closely related to that of the testis.In control fishes the seminal vesicle consists of locules filled with sperms and secretory fluid (Fig.1). The locules are of varying shape and size (188.83 ± 14.41µ), lined by a single layer of secretory cells and separated by loose connective tissue sheath.









Delafield's haematoxylin and eosin x 450 CT-Connective tissue; VL-Vesicular locule; VF-Vesicular fluid SC -Secretory cell; SP-Sperm; EL-Empty locule

Fig. 1 T.S. of seminal vesicle of control *G.giuris* showing locules lined by secretory cells and packed with fluid and sperms

Fig. 2 T. S of seminal vesicle of *G.giuris* exposed to 0.5 ppm Copper sulphate for 24 hours showing regressed locules , cysts of sperms and minimal vesicular fluid.

Fig. 3 T. S of seminal vesicle of *G.giuris* exposed to 0.3 ppm Copper sulphate for 96 hours showing disorganized necrotic secretory cells .

Fig. 4 T. S of seminal vesicle of *G.giuris* exposed to 0.5 ppm Copper sulphate for 96 hours showing indistinct interlocular connective tissue and clumped sperms.

In fishes exposed to 0.3ppm and 0.5 ppm of Copper sulphate for 24 to 96 hours, the seminal vesicles showed marked cytomorphological changes. The size of the locule decreased significantly when compared to control (Table 1). In fishes treated with 0.5ppm of Copper sulphate for 24 hours, the seminal vesicular locules showed regression in volume and contained cysts of secretory cells. Minimal vesicular fluid and sperms was observed in the locules (Fig 2). However, in fishes exposed to 0.3ppm Copper sulphate for 96 hours, the secretory cells became necrotic and highly disorganized.

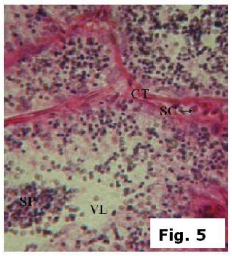
The locule had minimal vesicular fluid and sperms (Fig. 3). After exposure to 0.5ppm of Copper sulphate for 96 hours, the seminal locules appeared degenerate, merged with each other.The interlocular connective tissue is not clearly demarcated (Fig 4). The locules contained minimal vesicular fluid and sperms.

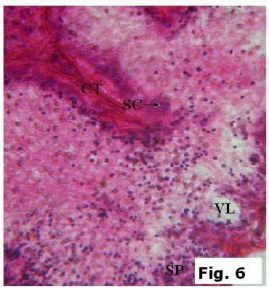
Table 1 Effect of Heavy metals on the Diameter (µ) of the seminal vesicular locules of *G.giuris* (Mean ± SE)

Time	Control	Copper Sulphate (ppm)		Cadmium Sulphate (ppm)	
Hours		0.3	0.5	0.02	0.04
	188.83±14.41				
24		156.25±26.52	160.00±14.25	186.25±22.55	163.12±16.05
96		150.62±15.32	173.10±13.70	176.87±24.01	173.75±9.86

In fishes exposed to 0.02ppm of Cadmium sulphate for 24 hours, the loculeswere smaller in size ((186.25 ± 22.55µ) when compared to control (188.83 ± 14.41µ) (Table 1). The loculeshad few pycnotic sperms withhighly disorganized secretory cells (Fig 6). The interlocular connective tissue was thick and merged with the adjacent membrane. On the other hand, in fishes exposed to 0.04ppm of Cadmium sulphate for 24 hours the locular diameter (163.12± 16.05µ) decreased further and showed minimal vesicular fluid withpycnotic sperms (Fig.7). Hypertrophied secretory cells with indistinct nuclei are present towards the periphery. In fishes treated with

0.02 ppm Cadmium sulphate for 96 hours, there was a significant reduction in the size of the loculeswith pycnotic sperms(176.87±24.01µ) when compared to control (188.83±14.41 µ) (Fig. 8). These changes are more significant in higher concentrations of Cadmium sulphate after 96 hours of exposure (Fig 9).





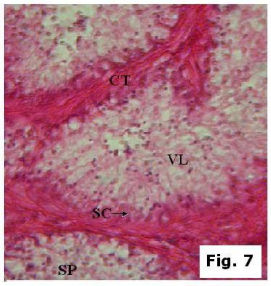


Fig.5 T. S of seminal vesicle of *G.giuris* exposed to 0.04 ppm Cadmium sulphate for 24 hours showing minimal vesicular fluid and pycnotic sperms.
Fig. 6 T. S of seminal vesicle of *G.giuris* exposed to 0.02 ppm Cadmium sulphate for 96 hours showing regressed locules and pycnotic sperms.
Fig.7 T. S of seminal vesicle of *G.giuris* exposed to 0.04 ppm Cadmium sulphate for 96 hours showing thickened interlocuolar connective tissue and few pycnotic sperms.

DISCUSSION

The structure and function of seminal vesicles in gobiid fishes have been observed and described by several workers (Weisel, 1949; Tavolga, 1955; Egami, 1960 and Murthy, 1981). Seminal vesicle and its role in the reproduction of teleosts have been reviewed by Rasotto and Rasotto (2001) and Chowdhury and Joy (2007).Disorganization of seminal vesicular locules, moderate quantity offluid in thelocules with necrotic sperms was previously reported by Sitavi*et al.,* (2010) on *G.giuris*exposed to malathion. Pandey and Shukla (1980) have reported the thickening of the muscle layer in the seminal vesicle of *Tilapia mossambica* after 20 days of treatment with BHC. Zaroogian*et al*., (2001) have shown ethinyl estradiol to thicken the interlocular connective tissue of the seminal vesicles in *Tautogolabrusadspersus.*Methallibure administration in *Clariasbatrachus*caused decrease in weight of the seminal vesicles (Singh and Joy,2000). Similar findings have been observed in the seminal vesicles of *G.giuris* after treatment with Copper sulphate and Cadmium sulphate. Further, the secretory cells are highly disorganized. A significant decline in locular diameter was also recorded. Murthy (1995) observed similar histopathological changes in the seminal vesicles of *G.giuris*after treatmentwith fenthion. At higher concentration of Copper sulphate and Cadmium sulphate the locules contained minimal vesicular fluid and cysts of sperms in atrophied condition. These findings corroborate the results obtained (Sitavi*et al.,* 2010) on the seminal vesicles of *G. giuris* subjected to malathion exposure. Similar changes have also been observed in other vertebrates (Kar and Kamboj1965; Niloufer and Shrobona 1997; Vanitakumari *et al.,* 1998; Nupurlal and Nath 1996; Takizawa and Horii 2002 and Narayana,*et al.,* 2006). Present finding are comparable with the histopathological anomalies in the seminal vesicles of fishes under the impact of various environmental pollutants.

The present study revealed that heavy metal stress induces histological alterations in the seminal vesicles of *G.giuris*. The studies infer that environmental stress alter the histoarchitecture of the seminal vesicle and affect the reproductive potential of *G.giuris.*

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