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Research Paper

Effect of cadmium chloride on testis of *Heteropneustes fossilis* and recovery by herbal compound Ashawagandha

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Abstract

The present study was carried out to evaluate the safe dose of cadmium chloride on the testis of *Heteropneustes fossilis* which were kept in aqueous solution of cadmium chloride of 0.5 ppm for 7,14 and 21 day. The histological effect of cadmium chloride in testis is characterized by regression after 7 day of exposure and spermatocytes in the seminiferous lobule showed cytololysis in 14 days duration. After 21 days, almost all the cells in seminiferous lobules were degenerated. The size of all spermatoganic cells significantly ($p< 0.001$) increased. Extensive damage in germinal epithelium, different spermeogenic cells and interstial cells were recovered after administration of Ashawagandha.

Keywords: Ashawagandha, Cadmium chloride, *Heteropneustus fossilis*, Seminiferous bules, Testis

Introduction

Heavy metals occur naturally in the environment and are found in varying levels in ground and surface water. Heavy metals are reported as pollutants which caused the metabolic, physiological and structural alterations in fish[1,2,3] . Among heavy metal cadmium has been shown to be responsible for a number of reproductive abnormalities in fish [4]. Cadmium obstructs numerous reproductive processes in fish such as sexual maturation, spermatogenesis, fertilization success and development of the fish [5,6]. The present study was taken to observe the abnormalities produced in testis by cadmium chloride and the damaged tissue was recovered by herbal compounds Ashawgandha.

Materials and Methods

Experimental animal: *Heteropneustus fossilis.*
H. fossilis measuring about length 12±5 cm and weight 25±5 gm.

Toxic agent: Cadmium Chloride
Cadmium was used for present study in the form of cadmium chloride ($CdCl_2$). The dose of cadmium chloride was decided after determination of LC_{50} value. It was found to be 0.5 ppm.

Recovery agent: The herbal compound Ashawagandha (*Withania somnifera*) is used for recovery of damaged tissue.

Experimental method: Fishes were acclimatized to laboratory condition for 7 days before the commencement of the experiment and were treated with 0.01 $KMnO_4$ solution to remove any dermal

infection. Fishes were fed with chopped prawn twice a day. The 72 hrs LC_{50} value of cadmium chloride was found to be 0.5 ppm in *H. fossilis.*

The fishes were divided in three group having36 fishes in each group.

Control group: Fish was maintained in normal water without any treatment for 21 days.

Treated group: Fish was exposed to the 0.5ppm solution of cadmium chloride for 21 days.

Recovery group: Fish was treated with cadmium chloride along-with Ashawagandha herbal compound for 21 days.

Histopathological study: Fishes of all experiment groups (control, treated and recovery)were sacrificed each after 7,14, and 21 days. Testis were fixed in aqueous Bouin's solution for 24 hours. The material was washed with water, dehydrated and cleared through graded alcohol and xyline respectively after filtration in paraffin blocks were prepared and section of 5 µ thickness were cut and stretched on albumenized slides. The slides were stained with Haematoxylin and Eosin[7] and mount in DPX for histological observation. All the data and results for final observation were processed in the form of microphotographs and table.

The diameter of spermatogenic stages were recorded and difference if any were compared by statistical analysis using student ' t' test [8].

Results and Discussion

Control group: Histologically, the control testis were composed of numerous seminiferous lobule and covered with thin tunica albuginea. The seminiferous lobules were round or oval shaped and contained spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa. The interlobular space contains connective tissue, blood capillaries and interstitial cells. Seminiferous lobule showing active spermatogenetic stages. Spermatogonia located along the periphery of the tubule. The spermatogonia are prominent with cell boundarie and have a large nucleus with prominent and darkly stained nucle. Primary spermatocytes cells are slightly smaller than spermatogonia. The nuclei are darkly stained with prominent nucleus and had eosinophic cytoplasm. Secondary spermatocytes was still smaller than primary spermatocytes and contains thick chromatin material and deeply stained nuclei. Spermatids were the predominant population followed by dividing spermatocytes and spermatogonia. The spermatids are smaller and spherical in shape. They look like dark spots and stain brightly. (Figure 1, 2 and 3)

Treated group
7 days: Showing insularism of tunica albuginea layer. Tunica albuginea was thick and seminiferous lobules become flattened showed regression. All spermatogenic stages were in degenerating process (Figure 2).

14 days: Treated testis showing insularism of tunica albuginea from its other layer due to this wide gap were visible. The degeneration in spermatogenic stages were visible. The spermatocytes were loosely packed and were surrounded by ill defined spaces. The areas occupied by spermatid and spermatozoa in the lobules showed cytolysis and many lobules had empty spaces due to degeneration of cells. (Figure 4)

21 days: The 21 days treated testis exhibited much more prounced changes in tunica albuginea. Complete insularism of tunica albuginea were exhibited. The lobular structure of the organ was completely lost. The big spaces among different spermatogenic cells due to vacuolization. The seminiferous lobules had deformed shape. The connective tissue stroma were completely lost and wide spaces were visible in the section (Figure 6). In all treated group the diameter of spermatogenic cells exhibited significant ($p<0.05$, 0.001) reduction in their size(Table 1).

Table 1: Diameter of spermatogenic stages of *Heteropneustes fossilis* in control and experimental group

S. No	Parameter	7 days (μ)		14 days (μ)		21 days (μ)	
		Control	Treated	Control	Treated	Control	Treated
1	Tunica albuginea (thickness)	0.072±0.006	*** 0.129 ±0.04	0.093±0.008	*** 0.12 ±0.02	0.10±0.005	*** 0.14 ±0.03
2	Seminiferous loubules	8.30±0.20	** 3.79 ±0.07	8.77 ±0.21	** 3.55 ±0.03	8.90 ±2.5	** 3.47± 0.07
3	Spermatogonia	0.326±0.018	*** 0.472±0.023	0.393±0.014	*** 0.528±0.029	0.40±0.016	** 0.60±0.132
4	Primary spermatocytes	0.148±0.014	*** 0.244±0.039	0.187±0.015	*** 0.228±0.018	0.21±0.028	*** 0.24± 0.01
5	Secodary spermatocytes	0.124±0.007	*** 0.216±0.021	0.172±0.015	*** 0.220±0.020	0.18±0.019	*** 0.22±0.016
6	Spermatid	0.031±0.003	*** 0.038±0.003	0.031±0.003	*** 0.033±0.003	0.03±0.004	NS 0.04±0.003
7	Interstitial cell	0.23±0.0160	*** 0.352±0.028	0.230±0.017	*** 0.367±0.047	244±0.015	*** 468±0.051

All values are expressed in Mean ± SEM
Total no. of samples for each observation: 10
Significant level (** p< 0.005, *** p< 0.001)
NS: Non significant.

In fish, the male reproductive system is especially sensitive towards adverse effects of heavy metals. Although heavy metal concentrations in water are rarely directly dangerous for fish, heavy metals are known to accumulate in fish tissues becoming extremely harmful[9]. Due to accumulation of heavy metals in gonads is related to decreased quality of gametes, including sperm motility have been reported by [10].

PLATE 1

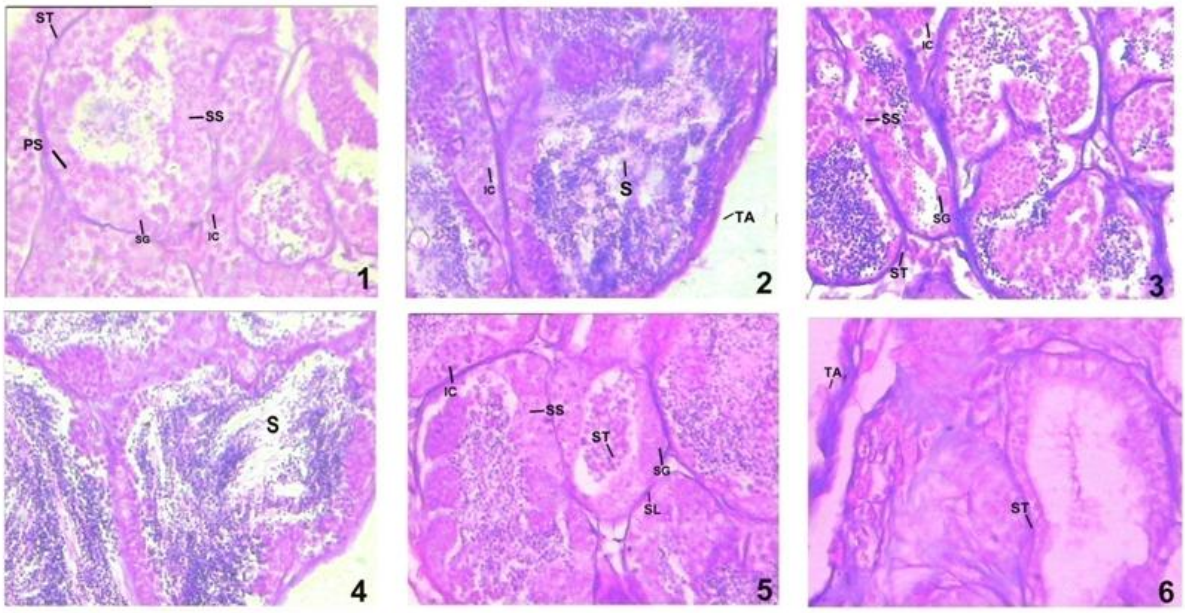

Plate 1: Microphotographs of testis of control and treated testis, *H. Fossilis* (H& E) x400

Figure 1 Control group (7 days): Showing normal structure of testis, seminiferous lobule, spermatogonia, primary and secondary spermatocytes were seen.

Figure 2 Treated group (7 days): Testis showing detachment of tunica albuginea and degenerating spermatogenic cells.

Figure 3 Control group (14 days): Showing all stages of spermatogenesis. Spermatide were found in majority of seminiferous lobule.

Figure 4 Treated group (14 days): Showing detachment of tunica albuginea form its other layer and degerating spermatocyte.

Figure 5 Control group (21 days): Showing normal structure, seminiferous tubules, spermatogonia, primary and secondary spermatocyte, spermatide were seen.

Figure 6 Treated group (21 days): Showing meny degenerating spermatogenic cells. The lobular become fibrous and without spermatogenic cells.

Cadmium exposure in fish exhibit degeneration of mature spermatocyte to spermatids, spermatozoa and leyding cells [11]. Heavy metals cadmium have been reported to inhibit gametogensis in the fish. The exposure of fish to cadmium caused testicular damage, disorganization and disintegration of the sertoli cells. Cadmium adversely affect the spermatogenic process, possible through primary injury to the vasculature of the testis[12,13]. Decreased spermatogenesis resulting from zinc toxicity has also been shown [14].

Abbreviations:
SL = Seminiferous Lobules
SG =Spermatogonia
PS =Primary spermatocytes
SS = Secondary spermatocytes
TA = Tunica albuginea
IC = Interstitial cell
ST = Spermatid
S = Sperm

Recovery group:
After 21 days treatment of cadmium chloride the fishes were administered with Ashawagandha.

In 7 days tunica albugenia become exhibited recovery. The wide gap was reduced due to regeneration of spermatogenic cells in seminiferous lobule. Many newly formed cells exhibited in the lumen of seminiferous lobules. (Figure 7)

In 14 days the testis of Ashawagandha group more pronounced recovery in their histological structure were recorded. Tunica albuginea loser its thickness. The tubules were recovered in their shape and almost become oval in shape. The primary and secondary spermatocytes recovered and many dividing stages were seen in growing cells. (Figure 8)

In 21 days the tunica albuginea become thin and normal in appearance. The seminiferous lobule exhibited normal in size and shape with comparison to control . Spermatogenic cells were recovered and regenerated. The tubules contains almost all spermatogenic stages which were lost due to strees. All the cells occupy full space in seminiferous lobules and due to this wide spaces were fully packed with regenerating cells (Figure 9). In all recovery group the diameter of the spermatogenic cells reveal significant ($p<0.01$ and 0.001) improvement in shape and configuration (Table 2).

The effect of cadmium chloride, on testes were shown to be reversible. In the case of *p. sarana*, when the cadmium chloride treated fishes are transferred to clean water, the lobules exhibit a gradual recovery, the spermatogenesis resumes the normal level and the germinal epithelium is again intact by 30 days to a large extent [15].

Table 2: Diameter of spermatogenic stages of *Heteropneustes fossilis* in experimental and recovery group

S. no	Parameter	7 days (µ)		14 days (µ)		21 days (µ)	
		Treated	Recovery	Treated	Recovery	Treated	Recovery
1	Tunica albuginea (thickness)	*** 0.129±0.03	** 0.117±0.01	*** 0.128±0.02	*** 0.110±0.011	*** 0.146±0.030	*** 0.103±0.00
2	Seminiferous lobules	** 3.79±0.07	** 4.71± 0.13	** 3.55±0.03	*** 4.05± 0.087	** 3.47±0.07	*** 4.65±0.08
3	Spermatogonia	*** 0.472±0.02	*** 0.367±0.019	*** 0.528±0.02	*** 0.452±0.024	** 0.609±0.13	** 0.448±0.02
4	Primary spermatocytes	*** 0.244±0.03	*** 0.158±0.016	*** 0.228±0.01	*** 0.189±0.015	*** 0.24 ±0.01	*** 0.223±0.02
5	Secodary spermatocytes	*** 0.216±0.02	*** 0.168±0.010	*** 0.220±0.02	*** 0.177±0.012	*** 0.213±0.01	*** 0.201±0.01
6	Spermatid	*** 0.038±0.003	NS 0.033±0.003	*** 0.033±0.003	*** 0.031±0.003	NS 0.040±0.003	NS 0.036±0.003
7	Interstitial cell	*** 0.35± 0.02	*** 0.278±0.023	*** 0.367±0.047	*** 0.271±0.024	*** 0.468±0.051	*** 0.266±0.018

All values are expressed in Mean± SEM
Total no. of samples for each observation: 10
Significant level (** p< 0.01, p< 0.005, *** p< 0.001)
NS: Non significant.

PLATE-2

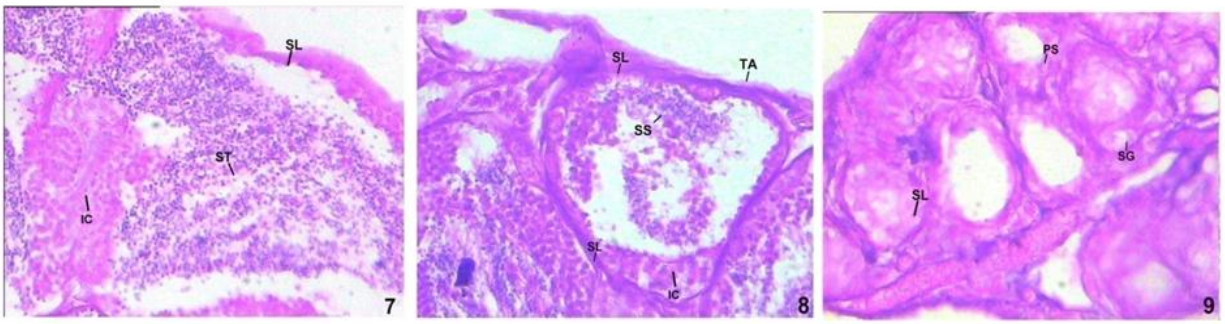

Plate 2: Microphotographs of testis of recovery of *H. fossilis* (H& E) x 400

Figure 7: 7 days Ashawgandha : Showing many regenerating spermatogenic cells normal appearance of spermatogonia, primary spermatocytes in the seminiferous lobule were arranged accordingly.

Figure 8: 14 days Ashawgandha: Showing regeneration of primary and secondary spermatids and interstitial cells.

Figure 9: 21 days Ashawgandha: Showing reduced spaces, spermatogonia and spermatogenic cell population increased.

Abbreviations:
SL = Seminiferous lobules
SG =Spermatogonia
PS =Primary spermatocytes
SS = Secondary spermatocytes
TA = Tunica albuginea
IC = Interstitial cell
ST = Spermatid

Conclusion

The present experimental condition exhibited that the cadmium chloride produced deleterious effects on testicular activity and recovery by herbal compound reveals that the fish gradually regain its damaged tissues. Further studies are required to know the exact cause of the damages produced by metal and recovery of tissues by herbal compounds.

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References

1. Jiraungkoorakul W., Sahaphong S., Kangwanrangsan N. and Kim M.H., Histopathologcal study, The effect of ascorbic acid on cadmium exposure in fish *(Puntius altus),* J. Fish. Aquat. Sci., 1, 191-199 **(2006)**

2. Jiraungkoorakul W., Sahaphong S., Kangwanrangsan N. and Kim M.H., Histopathologcal study, The effect of ascorbic acid on cadmium exposure in gill of *Puntius altus,* Int. J. Zool. Res*.,* 3, 77-85 **(2007)**

3. Jiraungkoorakul W., Sahaphong S., Kangwanrangsan N. and Zakaria S., The protective influence of ascorbic acid against the genotoxicity of waterborne lead exposure in Nile tilapia oreochromis niloticus (L.), J. Fish Biol., 73, 355-366 **(2008)**

4. Sharma S., Vyas V., Tamot S., and Manhor S., Histological changes in the testis of air–breathing fish, *Heteropnuestes fossilis* (Bloch) following Cadmium chloride exposure, 3(2),1216-1221**(2013)**

5. Jezierska B., Witeska M., Chojnacki, A., Kurzak, K., Mitrus, C., Skrzyp, J., Socha, S., Skrzyczyńska, J., Szczerba, L.W., Tchórzewski, J., Wojtasik, J. (Eds.), University of Podlasie, Poland**.** Metal toxicity to fish in Spermatozoa against toxic effect of cadmium ion, Comp. Biochem. Physiol., C 153, 422-429 **(2001)**

6. Dietrich M.A., Dietrich G.J., Hliwa P., Ciereszko A., Carp transferrin can protect spermatozoa against toxic effects of cadmium ions, Comp. Biochem. Physiol. C, 153, 422-429 **(2011)**

7. McManus J.E.A., Histological demonstration of mucin after periodic acid, Nature, 158, 201 **(1946)**

8. Ribelin W.E., Migak G.I., The Pathology of Fishes, Wiscon. P.Madi. Wiscon., 537**(1975)**

9. Kime D.E., Ebrahimi M., Nysten K., Roelants I., Moore H.D.M. and Ollevier F., Use of computer assisted sperm analysis (casa) for monitoring the effects of pollution on sperm quality of fish; application to effects of heavy metals, Aquatic Toxicology*,* 36(1)**,** 223-237**(1996)**

10. Shen H.M., Sangiah S., Na^+,K^+-ATPase, glutathione and hydroxyl free radicals in cadmium chloride-induced testicular toxicity in mice, Archives of Environmental Contamination and Toxicology, 29, 174-179**(1995)**

11. Das R.C., Cadmium toxicity to gonads in freshwater fish, *Labeo bata* (Hamilton), Arch. Hydrobiol., 112, 467-474**(1988)**

12. Kar A.B. and Das R.P., Testicular changes in rats after treatment with cadmium chloride, Acta Biol. Med. Ger., 5, 153-17**(1960)**

13. Katti S.R. and Sathyanesan A.G., Changes in the hypothalamo-neurohypophysial complex to lead treated fish, *Clarias batrachus* (L), Z Mikrosk-anat. Forsch. Leipzig. 100, 347 **(1986)**

14. Sheweita S.A. and Tilmisany A.M., Mechanisms of male infertility: Role of antioxidant, Curr. Drug Metab., 6, 495-501 **(2005)**

15. Kumari M. and Gopal Dutt N.H., Cyclical changes in the pituitary gonadotrophs in relation to testicular cycle in *Puntiussarana* (Hamilton). Folia Morphol **(1989).**