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RESEARCH ARTICLE

HORMONAL AFFECTS OF SILVER NANOPARTICLES IN FRESHWATER FISH "*CYPRINUS CARPIO*"

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ABSTRACT

Selecting In the present study silver nanoparticles were exposed to fish Cyprinus carpio and the changes in the hormonal level were observed. Silver nanoparticles and silver-containing compounds cause adverse effects in human and animals. Environmental pollutants cause an impairment of fish internal function and a characteristic elevation of plasma thyroid hormones. In fish, hormones are critical towards maintaining proper physiological function and amongst the hormones found in fish the thyroid hormones, thyroxine (T4) and triiodothyronine (T3) are known to play an important role in fish growth. Disruption of thyroid axis due to silver nanoparticles may seriously affect normal development, differentiation, growth and reproduction in many fishes. Silver nanoparticles in triiodothyronine (T_3) and Thyroxine (T_4) level exhibited a significant decrease throughout the study period. Thyroid hormones regulate a wide range of biological processes associated with development, somatic growth, metabolism, energy provision. Thyroid hormone axis poses a significant hazard to human and wildlife health from interference of exogenous salts. The need for development and valid, ion of an in vivo assay for detection of thyroid system disrupting nanoparticles arises from concern that a considerable number of compounds have the potential to interact with different species of thyroid function and thyroid hormone action. The majority of studies on endocrine control functions have concentrated on the suppression of immune response by decreased secretion of thyroid hormone in response to environmental pollution.

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INTRODUCTION

Pollution is the introduction of contaminants into the natural environment that causes adverse affect. Pollution can take the form of chemical substances and nanoparticles in aquatic environment. Water pollution by industrial effluents containing organics and heavy metals pose a serious hazard to the aquatic biota and public health (Velma *et al*., 2009). Silver is present in different forms in the environment especially in the living organisms. The most common is metallic silver, silver salts (ionic silver), silver complexes and colloidal silver. Metallic silver is dissolves in acids and salts like nitrate and silver nitrate is formed. Aqueous solution of soluble silver nitrate contains silver in the form of hydrated silver cations $Ag(H_2O)_n^+$ which is typical "ionic silver". However, silver cation can be complexed with various organic ligands and silver with 100µm size particles of small sized particle is the silver nanoparticles, the overall charge of the toxicant is toxic in the aquatic environment. Furthermore, highly stable complexes are known which are not dissociated in the solution or biological liquids. Silver nanoparticles and silver-containing compounds cause adverse effects in human and animals. Most studies explain the various toxic effects of silver on the human body and the environment following intensive ingestion and inhalation of silver compounds. A number of studies describe the effects of occupational exposure to metallic and soluble silver compounds. Silver has a potential antimicrobial activity towards many pathogens (Hill and Pillsbury, 1999) and it has been used in the pas for medication purposes. Environmental pollutants cause an impairment of fish internal function and a characteristic elevation of plasma thyroid hormones may not be able to compensate overall fitness for the survival (Buckman *et al*., 2007). Thyroid plays an important role in ion regulation, energy metabolism, growth and reproduction (Vander Geyten *et al*., 2001). Fish are sensitive to nutritional state due to stress of thyroid hormones. In fish, hormones are critical towards maintaining proper physiological function and amongst the hormones found in fish the thyroid hormones,

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thyroxine (T4) and triiodothyronine (T3) are known to play an important role in fish growth (Higgs *et al*., 1982) and early development (Lam, 1980). The activation to T3 is carried out almost entirely in peripheral tissues by denomination of the outer ring (ORD) of T4 Deiodination of the inner ring (IRD) result in the production of the inactive metabolite 3, 3', 5, '-T3 (Rt3) (Vasser, *et al*., 1996). IRD also inactivates T3 by converting it to 3, 3'-diiodothyronine. Denomination controls plasma and tissue thyroid hormone levels and thyroid hormone biological activity. The enzymes responsible for thyroid hormone denomination are called Iodothyronine (Vander Guyton *et al*., 2001). Thyroid hormones (TH_s) are known to play a role in many metabolic processes and are essential for normal growth, differentiation and development of vertebrates in silver nanoparticles (Morgado *et al*., 2007).

Alterations of plasma thyroid of fish were observed in fish *Cyprinus carpio* to environmental nanoparticles (Power *et al*., 2011). nitrite in *Oreochromis mossambicous* (Subash peter *et al*., 2007), copper (Anderson, 1996), Cadmium (Hoole, 1997), Cadmium in *Peralichty dentatus* (Anderson, 1986, Hoole, 1997). Environmental salinity in Teleosts (Shepherd *et al*., 1997). Nitrite in sea bream and teleost *Sparus saeba*. (Deane *et al*., 2007). *Hoplias malabaricus* exposed to methyl mercury showed decreased hormonal status (OliveraReiberio *et al*., 2006).Exposure of silver nanoparticles showed a decrease in plasma T_4 and T_3 in *Juvenile rainbow trout*, (Bleau *et al*., 1996). Hormones have been measurable in blood and circulating levels and are exposed to nanoparticles. Silver nanopartcles in species hormones usually act on several target tissues, and osmoregulatory hormone may regulate ion or water change across gills, kidney, intestine and skin in a fish and its secretion may be subjected to complex feedback (Folmar *et al*., 1993) reported that aquatic toxicologist, measurement of circulating levels hormones can provide additional information on the sub lethal effects of many chemicals and nanoparticles. The author stated that significant changes in circulating hormone levels may be observed. The main function of thyroid gland is to synthesis the Iodothyronine, 5, 3', 5' tetraiodothyronine, (T4) Thyroxine and 3, 5, 3' Triiodothyronine (T3). These are peptides containing iodine. The two most important hormones are T3 and T4 and these hormones are essential for life and have effects on body metabolizing growth and development. Disruption of thyroid axis due to silver nanoparticles may seriously affect normal development, differentiation, growth and reproduction in many fishes.

According to Griffith (2008) silver and silver nanoparticles exposure in a teleost fish shows a decrease in T_4 by 40% and 60% in fish that were exposed to 25 and 50 mg/ 1 nanoparticles, respectively, whereas T_3 remain unchanged. There is a decrease in T_3 and T_4 in fresh water fish when exposed to nitrite (Jensen. 2003), *Cyprinus carpio* to acidic water and nanoparticles (Nagae *et al*., 2001). In the present study decrease of hormone T4 and T3 occurs due to the exposure of silver nanoparticles. In contrast to a decrease in T_3 and T_4, a sublethal levels results in increased hormone levels in freshwater fish (Anderson, 1996). For example aluminium exposure showed an increased in hormone in trout (Brown and Sadler, 1989). Cadmium is also known to increase the hormone levels in Trout (Olsson *et al*., 1995). Heavy metals like mercury, copper and cadmium showed an increase in hormone levels in trout (Marc *et al*., 1995). When Atlantic salmon (Salmon salar) induced to acid and limed river waters the T_4 hormone is increased and T_3 Triiodothyronine is gradually decreased (Brown *et al*., 1993). Silver nanoparticles have been reported to affect thyroid hormone status in many fish species (Van der Ven *et al*., 2006) but the effect of silver and silver nanoparticles on this group of hormones, in fish has yet to be reported. Considering the huge importance of thyroid hormone in fish physiology (reproduction, growth and metamorphosis regulation). In the present study the effects of silver nanoparticles on T_4 and T_3 levels were decreased in an Indian major carp, *Cyprinus carpio.*

MATERIALS AND METHODS

Hormone Analysis

Thyroxine (T_4) and Triiodothyronine (T_3) were estimated using Enzyme linked immunoabsorbent assay (ELISA of hormones) using kits.

RESULTS

Table 1 and Table 2 represents the data on changes in the Thyroid hormone (T_3 and T_4) of fish *Cyprinus carpio* exposed to concentration of silver nanoparticles to sublethal toxicity. Silver nanoparticles in triiodothyronine (T_3) and Thyroxine (T_4) level exhibited a significant decrease throughout the study period. The significant decrease in triiodothyronine (T_3) of -9.87,-9.67, -9.49, -9.09, -7.28 at the end of 7th , 14th, 21st, 28th, 35th, days and Thyroxine (T_4) level was directly proportional to the exposure period showing a percent decrease – 11.76, -11.00, -10.00, -9.09, -6.50 at the end of 7th , 14th, 21st, 28th, 35th, days respectively. These were significant ($P < 0.05$) variations among the treatments.

Table 1. Plasma Thyroxine (T_4) (ng/ml)

Exposure period (in days)	Control	Experiment	Percent change
7	1.62 ± 0.007d	1.46 ± 0.004e	-9.87
14	1.55 ± 0.007c	1.40 ± 0.004c	-9.67
21	1.58 ± 0.007d	1.43 ± 0.004e	-9.49
28	1.65 ± 0.007c	1.50 ± 0.004d	-9.09
35	1.51 ± 0.007d	1.40 ± 0.004e	-7.28

Table 2. Plasma Triiodothyronine (T_3) (ng/ml)

Exposure period (in days)	Control	Experiment	Percent change
7	0.17 ± 0.004a	0.15 ± 0.004a	-11.76
14	0.18 ± 0.007c	0.16 ± 0.001a	-11.00
21	0.20 ± 0.007c	0.18 ± 0.007c	-10.00
28	0.22 ± 0.007c	0.20 ± 0.007c	-9.09
35	0.16 ± 0.001a	0.15 ± 0.004a	-6.50

DISCUSSION

The endocrine systems of teleost fish *Cyprinus carpio* produces a variety of hormones like growth hormones, steroid hormones, gastrointestinal hormones, pancreatic hormones, and thyroid hormones (T_3 and T_4). Among various hormones the thyroid hormone T_3 and T_4 plays an important role. These hormones were found to be present in eggs and embryos of a high variety of fish species showing their importance even at these early life stages (Leatherland, 1994; Power *et al*., 2011). High concentrations are present in fish eggs and increased levels are reported during metamorphosis or during the larval-juvenile transition (Leatherland, 1994). In light of this evidence, disruption of the thyroid axis may seriously compromise normal development, differentiation, growth or reproduction in many vertebrates (Brown *et al*., 2004; Boas *et al*., 2006). The regulation of hormone secretion by the hypothalamus and pituitary has been established as the hypothalamus – pituitary – Inter renal (HPI) axis ion fish (Verburg – Van kemenade *et al*., 2001). The thyroid systems of fish and mammals are similar in many respects, with one major difference. The mammalian system is driven primarily through the central brain – pituitary – thyroid axis that regulates thyroid secretion of both T_4 and T_3. In fish, the thyroid system does not appear to be driven primarily by the central brain- pituitary- thyroid axis.

The main function of thyroid gland is to synthesis the lodothyronine, hormone 3,5,3',5' tetraiodothyronine, (T_4, Thyronine) and 3,5,3'Triiodothyronine (T_3). 3,5,3-Triiodothyronine (T_3) is the most active hormone and binds with high affinity to nuclear receptor (TL_s) while L- Thyronine (T_4) which is precursor of T_3 binds with low affinity and has few dircect actions (Darras *et al*., 1998). Yen and Chin (1994) reported that thyroid hormones have a small hydrophobic thyronine nucleus that medicates their action by binding to specific nuclear receptors, which act directly on target genes bringing about a cellular response. There are peptides containing iodine. The two most important hormones are T_3 and T_4 and these hormones are essential for life and have many effects on body metabolizing growth and development. Thyroxine (T_4) is the most commonly measured thyroid hormone for diagnosis of thyroid function. T_4 assay provides a rapid and sensitive method for serum using highly specific T_4 monoclonal antibody and a T_4 enzyme labeled conjugate solution. Silver nanoparticles pollutants have been reported to detrimentally affect thyroidal hormone status in a number of fish species (Brown *et al*., 2004; Van der Ven *et al*., 2006). Slight increased in plasma T_4 concentrations were also reported in mink exposed to 1 mg Aroclor 1242 per day relative to control mink. Earlier studies that examined the effects of PCBs on the hypothalamus – pituitary – thyroid axis in mink similarly revealed little or no PHAH- induced toxicity. Perchlorate salts are well documented as thyroid disruptors, and the effect can be manifested as thyroid histopathology as well as alteration of thyroid hormone status (Brown *et al*., 2004). Rainbow trout fed Aroclor 1254 showed no changes but rainbow trout fed a mixture of Aroclor 1242 and 1254 had lower plasma T_4 and T_3 concentrations. However, no effects were observed in fish from the CYPIA and CYP2 treatments (Buckman *et al*., 2007).

Silver nanoparticles induce a hyperactivity of the thyroid follicles which results in a decrease of the T_4 concentrations the T_3 concentration, which is regulated by the deiodination in peripheral tissues, is not affected. Studies have demonstrated that massive experimental increase of T_4 in fish did not increase T_3 levels, concluding that increase in T_4 do not drive T_3 production (Brown *et al*., 2004). Histopathological changes of the thyroid indicative were found after exposure. They also reported that one of the most consistent findings is that PCB exposure disturbs the levels of circulating thyroid hormones, especially T_4. Changes in thyroid histological appearance and plasma thyroid hormone levels were reported in coho salmon, *Oncorhynchus kisutch*, and Chinook salmon, Oncorhynchus tshawytscha collected in Great Lakes. Subchornic sures to waterborne Se increase plasma T_3 and T_4 levels in juvenile rainbow trout, *Oncorhynchus mykiss* (Miller *et al*., 2007). Exposure to Se increased plasma cortisol and it has been documented that cortisol influences thyroid hormone metabolism (Brown *et al*., 1991). Moreover, Se is an integral part of the deiodoinase enzymes involved in thyroid hormone sysnthesis. When eels were exposed for a longer period of time (7 days), Oliveira *et al*., (2008) noted an unaltered (T_4) in plasma and a (T_3) decrease.

Variety of mechanisms can results in a changes of the thyroid status, such as an alternation in the hypothalamus and/or pituitary status, biosysnthesis and secrection steps of T_3 and T_4, uptake by peripheral tissue, or hormone catabolism and clearance rates (Hontela *et al*., 1995. Oilveria *et al*., 2008 suggested that the plasma concentration of T_3 is not influenced (short-term) by plasma concentrations of T_4. Rather, thyroid stimulating hormone stimulates T_4 production, and T_3 concentration is regulated in peripheral tissue by T_3 – producing and-degrading deiodinases (Van der Geyten *et al*., 2001b). Since T_3 and T_4 are identical in all vertebrates, it might be that the peak of plasma T_4 concentration can cause an initial augmentation in metabolism, in order to handle the first stress response (Eyckmans *et al*., 2010). They also reported that the altered T_3 concentrations in plasma can be product of a deprived transformation of T_4 into T_3. Results from mammalian toxicological studies suggest at least four classical pathways of EDC action on the thyroid system including inhibition of thyroid iodine uptake, inhibition of thyroid peroxidase, displacement of TH from plasma transport proteins, and induction of hepatic T_4 glucuronidation leading to enhanced T_4 excretion. Almost all studies have reported some influence on thyroid cascade that is, therefore, a sensitive biomarker of exposure. However, the interpretation of the thyroid changes and the assessment of effect are more complex because it is difficult to distinguish between direct and indirect nanoparticles action on the thyroid cascade, which has a considerable capacity to compensate for abuses that otherwise would disrupt thyroid hormones homeostasis. Indeed, silver nanoparticles induced change in fish thyroid function has yet to be causally linked conclusively to decreased fitness or survival. However literature on the impact of toxicity on primary stress response like hormonal levels in Indian major carps are scanty. Thyroid hormones regulate a wide range of biological processes associated with development, somatic growth, metabolism, energy provision. Thyroid hormone axis poses a significant hazard to human and wildlife health from interference of exogenous salts. The need for development and valid, ion of an in vivo assay for detection of thyroid system disrupting nanoparticles arises from concern that a considerable number of compounds have the potential to interact with different species of thyroid function and thyroid hormone action. The majority of studies on endocrine control functions have concentrated on the suppression of immune response by decreased secretion of thyroid hormone in response to environmental pollution.

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