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Hormonal Response of Freshwater Fish *Cyprinus carpio* (Linnaeus) to Dimethoate Exposure

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ABSTRACT

Hormonal response in Cyprinus carpio to sub-lethal Dimethoate toxicity was investigated by estimating the serum levels of T_3 (triiodothyronine), T_4 (thyroxine), cortisol, prolactin, and insulin in control and sub-lethal (0.50 mg/l) dose for the periods of 1h, 6h, 12h, 24h, and 3 days. In control Cyprinus carpio, the serum level of T_3 ranged from 0.81 ± 0.01 to 0.85 ± 0.02 ng/ml; T_4 from 1.95 ± 0.01 to 1.99 ± 0.01 ng/ml; cortisol from 12.21 ± 0.01 to 12.26 ± 0.01 µg/dl; prolactin from 0.54 ± 0.01 to 0.59 ± 0.01 ng/dl; and insulin from 1.69 ± 0.01 to 1.75 ± 0.01 µU/ml up to maximum period of 3 days maintained in a pollution-free tap water. Exposure to the sub-lethal level of dimethoate cause stress in the fish as is indicated by a change in the serum hormone levels. From the results, it can be concluded that Dimethoate causes a decrease in metabolic rate and indirectly reduces the toxic effect of pesticide as is evident from the reduced levels of T_3 and cortisol. Increased level of insulin is known to favor adaptive tissue glycogenesis, besides its role in increasing the lipogenesis that may be advantageous for the fish in sequester pesticide residues, thereby increasing pesticide tolerance in the fish. Increased prolactin level indicates a possible hydromineral regulatory effect on the kidney and gills of fish under pesticide toxicity. The fish appears to adaptively recover its hormonal profile following a prolonged exposure period of 24 h to 3 days.

Keywords: *Cyprinus carpio, Dimethoate, Hormonal response, Triiodothyronine, Thyroxine, Cortisol, Prolactin, Insulin .*

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1. INTRODUCTION

Intensified use of agrochemicals causes massive destruction of nontarget animals such as fish by surface runoff, urban drainage, and leaching. Pesticides not only cause physiological changes but also sometimes become lethal to the fish. Increased pesticide contamination of aquatic environments gained the attention of researchers all over the world, with much concern in the last few decades to collect the data regarding the impact of different pesticides on different fish species. Dimethoate (O, O-dimethyl-S-[2-(methylamino)-2-oxoethyl] phosphorodithioate) is a broad range organophosphorus insecticide introduced in 1956 used globally to control a broad range of insects in the agriculture and other household insects (Adoni *et al*., 1985). In India, it was used in an increasing trend with 368.86 M.T during 2016-17 (Indiastat, 2017).

Many studies have investigated the effect of pesticides on different aspects of various fish species including change in behavior (Rao *et al*., 2003; Halappa and David 2009), tissue protein content (Venkataramana *et al*., 2006; Lakshmanan and Rajendran, 2013), blood glucose level (Das and Mukharjee 2003; Jee *et al*., 2005), enzyme activity (Jyothi and Narayan, 2000; Sivaperumal and Sankar 2013), Hematology (Yekeen and Fawole 2011; Qayoom *et al*., 2018), Histology (Deka and Mahanta 2012; Mohamed, F. A., 2009), chromosomal aberration and carcinogenic effect (Rishi and Grewal 1995; Saxena and Chaudhari 2010).

However, a few studies carried out on the changes in different levels of serum hormones in response to pesticide showed that exposing Labeo rohita to dimethoate and lambda-cyhalothrin decreases the level of T_3 and T_4 and increases TSH level (Dey and Saha 2014). *Oreochromis mossambicus* leads to a reduction in the serum cortisol level under exposure of different agrochemicals as reported by Karthikeyan *et al*., 2004 and Pandya *et al*., 2018, and *Channa striata* and *Labeo rohita* also show a reduction in cortisol level as reported by Sumathiral, 2006 and Akhtar *et al*., 2011. Based on the available information, the present work was carried out to evaluate the response of serum hormones under dimethoate exposure of *Cyprinus carpio* (Linnaeus) for 3 days by measuring T_3 (triiodothyronine), T_4 (thyroxine), cortisol, prolactin, and insulin in the control and dimethoate exposed fish.

2. MATERIAL AND METHODS:

Test animals:

Common carp (*Cyprinus carpio*) is a widespread freshwater omnivorous fish of eutrophic water in lakes and large rivers of Asia and Europe and is the third most frequently introduced species to every part of the world. It prefers large bodies of

slow or standing water with vegetative sediments and feeds on crustaceans, algae, plants, and invertebrates. In this study, fish were brought from the local fish market of Aligarh and were acclimatized in large tanks. Fish were fed with fish feed two times in the 24-hour cycles. Water in each tank was renewed on alternate days in the evening and regular check of infection was carried out. A randomly selected batch of healthy fish (100-120 g) was transferred to the tank and maintained under laboratory conditions (25 ± 2℃) and allowed to acclimatize. No feed was given prior to 1 day of exposure to pesticide.

1. Control:

Control group was maintained in pesticide-free tap water for a maximum period of 3 days with the temperature of (25 ± 2 ℃); pH (7.4 – 7.5); dissolved oxygen 7.8 mg/l; salinity 0.6355 ppt, total hardness 466 mg/l; and electrical conductivity 767µS/cm.

2. Pesticide exposure:

Fish were exposed to 0.50 mg/l of commercial dimethoate (EC 35 %) based on LC_{50} 1.6 mg/l value (Singh *et al*., 2009). Both tanks were covered with mesh. In the experimental tank equal proportion per liter of pesticide was added after water renewal.

3. Blood sample collection:

An equal number of fish from both control and exposed tank were selected for blood sample collection at 1h, 6h, 12h, 24 h and 3 days. 1 ml of blood from each fish was collected in vials and allowed to clot for 1 h at room temperature and for 2 h at 14 ℃. Serum was separated from each vial after clotting and stored at -80 ℃ until the hormonal assay was performed.

4. Estimation of serum T_3 (triiodothyronine)

Serum T_3 and T_4 level estimation of both control and exposed samples was done by the enzyme immunoassay (EIA) method of Walker (1977) and Wistom (1976). OD of both control and exposed samples was taken at 450 nm filter within 10 minutes using microplate reader and serum T_3 and T_4 concentration was represented in ng/ml.

5. Estimation of serum cortisol

Using the ELISA method of Zilva and Pannall (1984) serum cortisol level was quantitatively measured. OD of both control and exposed samples was taken at 450 nm and the level of serum cortisol was represented in mg/l.

6. Estimation of serum prolactin

Serum prolactin quantitative determination was carried out by the EIA method of Uotila *et al.,* (1981). OD of both control and exposed samples was taken at 450 nm filter using microplate reader and the level of serum prolactin was represented in ng/dl.

7. Estimation of serum insulin

Serum insulin level was estimated by ELISA method of Andersen *et al*., (1993). OD of both control and exposed samples was read at 405 nm filter using photometer and the level of serum insulin was represented in µU/ml.

3. RESULTS AND DISCUSSION:

Figure 1-5 represents hormones level in control and dimethoate exposed fish during 1h, 6h, 12h, 24h and 3 days. Fish responded to environmental pollutants by perturbed serum hormone level. A significant decrease in serum T_3 level and cortisol level was reported in pesticide exposed fish with a maximum decrease of T_3 occurred at 12 h and maximum cortisol reduction at 24 h (Fig. 1). Unlike T_3, the elevation of T_4 occurred at 1 h, 6 h and 3 days. Prolactin level showed a significant increase of +41% at 12 h. Serum insulin level also elevated with a maximum increase of +57% at 12 h (Fig. 5).

Triiodothyronine (T_3) and thyroxine (T_4) profoundly increase the metabolic rate of the body. The major circulating hormone out of the two is T_4. Approximately 33-40 percent of it is converted to T_3 by 5'- deiodinase, and this deiodination is important in the mechanism of action for thyroid hormone (Sterling *et al*., 1973). Reduction or complete absence of T_3 production decreases basal metabolic rate by 40 – 50 % and cause abnormalities in growth, development, reproduction, and behavior (Guyton and Hall, 1991). Thyroid hormones are unique in affecting almost every tissue of the body through life (Hadley and Levine, 2005).

Dimethoate caused a significant reduction in the serum T_3 level (table 1) in *Cyprinus carpio* indicating a reduction in basal metabolism thereby indirectly reducing the toxic effect. On the other hand, increasing level of T_4 occurs by a decrease in the conversion rate of T_4 into T_3. Thangavel *et al*., 2005; 2004 also reported a similar change in T_3 and T_4 level in *Sarotherodon mossambicus* after dimecron and endosulfan exposure. Studies by other investigators (Brown *et al*., 1978; Himick and Eales, 1990; Waring *et al*., 1996; Waring and Moore, 2004) also showed an increase in T_4 during different stresses.

Cortisol is a glucocorticoid with a regulatory effect on metabolism by stimulating gluconeogenesis of the liver. It also causes a moderate decrease in the rate of glucose utilization by depressing the oxidation of nicotinamide adenine dinucleotide (NADH) to form NAD^+. Both factors are responsible for the increase in blood glucose level. Cortisol production is regulated by a negative feedback loop by altering CRH and ACTH secretions of hypothalamus and pituitary (Hontela, 2006). Acute or subchronic stresses cause an immediate and marked increase in ACTH secretion by the anterior pituitary gland followed within minutes by greatly increased adrenocortical secretion of cortisol. While reduced cortisol level indicates no stress, impairment of HPI axis or interrenal exhaustion (Miller, 2006; Miller and Hontela, 2011). In the present study on *Cyprinus carpio,* a significant reduction was observed in cortisol level in comparison to the control indicating impairment in HPI axis or interrenal exhaustion as supported by early findings of (Pandya *et al*., 2018) in *Oreochromis mossambicus.* Similar results of impairment of interrenal axis under toxicity were reported by Dorval *et al.,* (2003), and Leblond and Hontela, (1999) on exposure to endosulfan and heavy metals. Reduced cortisol level could be considered as an adaptive stress response under toxicity to maintain low metabolic rate as supported by findings of other investigators in *Oncorhynchus mykiss* (Gagnon *et al*., 2007), *Oreochromis mossambicus* exposed to thiodon and dimecron (Parvatham *et al*., 2004; Thangavel *et al*., 2005), *Channa striata* exposed to sevin (Sumathiral, 2006), *Labeo rohita* exposed to endosulfan and dietary pyridoxine (Akhtar *et al*., 2011), *Clarias gariepinus* exposed to endosulfan (Ezemonye and Ikpesu, 2010), and *mystus vittatus* exposed to quinalphos (Swarnalatha, 2015). Significant recovery (- 16%) on 3-day indicates an increase in metabolic rate.

The serum prolactin level in control fish does not show any significant variation. However, dimethoate exposed fish showed significant elevation, 1 h, 12 h, and 3 days after reaching the control level. Prolactin has been shown to

produce a variety of distinctive actions in animals, including effects associated with reproduction, growth, osmoregulation and the integument (Bole-Feysot *et al*., 1998). In addition, prolactin may produce synergistic action with ovarian, testicular, thyroid, and adrenal hormones. Environmental contaminants are known to disrupt the osmotic and ionic balance of the fish (Kumaraguru, 1995).

Molecular and biochemical studies carried out in several teleosts fish demonstrated the presence of receptors for prolactin in skin, gills, kidney, and intestine of Nile tilapia, rainbow trout, goldfish and sea bream (Auperin *et al*., 1994; Le Rouzic *et al*., 2001; Sandra *et al*., 2000; Santos *et al*., 1999), indicating that these are the principal osmoregulatory organs in the fish. Prolactin is known to be hypercalcaemic, which is difficult to separate this function from osmoregulation (Flik *et al*., 1994; Kaneko and Hirano, 1993) as the epithelia are important for calcium uptake, they are known to have a complementary function of osmoregulation. The elevated prolactin level in *Cyprinus carpio* indicates that fish tries to overcome the pesticide stress by regulating water and mineral balance in gills and kidney. Similar results were obtained by Janick and Merkens (2001), Larsson and Haux (2002), Karthikeyan *et al*., (2004) and Thangavel *et al*., (2005).

Insulin is the major hypoglycemic hormone besides its important role in lowering and storage of blood glucose level (Von Mering and Minkowski, 1889) is also important for fatty acid synthesis, protein metabolism, and growth (Guyton and Hall, 1991). In the present study, *Cyprinus carpio* fish showed a significant increase in insulin level with (+ 57%) at 12 h under dimethoate exposure compared to control. Increased insulin level causes decrease in blood glucose level due to pesticide toxicity and reduction in the metabolic rate by decreasing levels of T_3 and cortisol as is evident from the studies done by Sharma (1999); Srivastava and Singh (1981); Thangavel *et al*., (2005) on different fishes. Both T_3 and cortisol are known to increase blood glucose level by the degradation of insulin and by increasing hepatic glucose 6–phosphatase activity. All these actions have a hyperglycemic effect and if the pancreatic reserve is low, it may, in fact, lead to B cell exhaustion. Pesticide toxicity is known to reduce cortisol level by interrenal exhaustion or impairment of HPI axis thereby reduction of blood glucose level occurs as supported by early findings of Pandya *et al*., (2018).

A similar reduction in blood glucose level was observed in carbofuran-exposed *Clarias batrachus* (Mukhopadhyay *et al*., 1982), endosulfan- and quinolphos-exposed *Channa punctate*, (Sastry and Siddiqui 1983; 1984), and ziram-exposed *Sarotherodon mossambicus* (Nivedhitha *et al*., 1998). The observed hypoglycemia could be due to the increased rate of conversion of glucose into lactate under anaerobiosis to meet the energy demand by fish for its survival during pesticide toxicity (Singh and Sharma, 1998).

Increased insulin level is known to favor adaptive tissue lipogenesis to make pyruvate availability for fatty acid synthesis, indicating fish adaptation 'insulation mechanism' to sequester pesticide residues as tolerance to pesticide toxicity (Ramaswami, 1990; Fabacher and Chambers, 1971; Reinert, 1970;). Further increased insulin also inhibits the action of hormone-sensitive lipase, thus, promotes the storage of fatty acids in the adipose tissues. Therefore, from the present study, it could be stated that increased insulin level in *Cyprinus carpio* under dimethoate toxicity favors increased lipogenesis to confiscate pesticide residues as a tolerance.

4. CONCLUSION:

As an adaptive response under pesticide toxicity, fish alleviates its metabolic rate as is evidenced by the reduced level of T_3 and cortisol. Dimethoate causes impairment of HPI axis by decreasing the metabolic rate due to reducing cortisol level in the fish. The increased level of prolactin plays a significant role in the acclimation process as fish enhances its hydromineral regulatory effect to meet the demand of the new environment. Insulin is known to reduce blood glucose level besides its role in increasing lipogenic activity to sequester pesticide residues under pesticide toxicity. Following 3rd day of exposure, the fish adaptive adjustment occurred in the sub-lethal dimethoate exposure as evidenced by the recovery of hormonal profile in comparison to the control.

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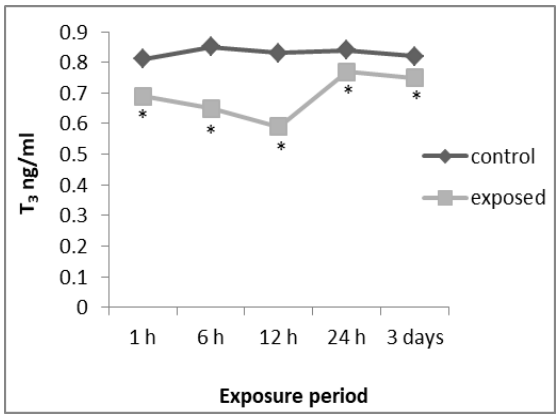

Fig. 1. Serum Triiodothyronine (T3) levels (ng/ml) in control and dimethoate exposed *Cyprinus carpio*. (significant *p<0.05)

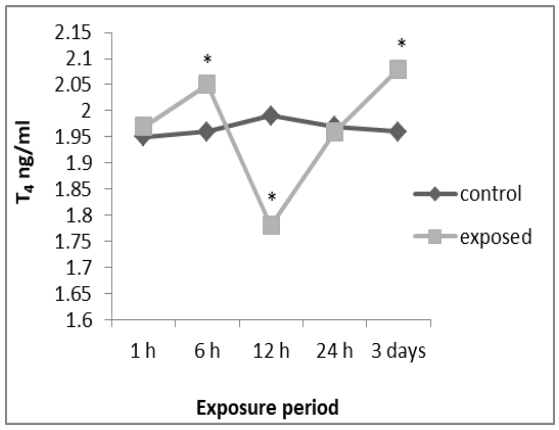

Fig. 2. Serum thyroxine (T4) levels (ng/ml) in control and dimethoate exposed *Cyprinus carpio*. (significant *p<0.05)

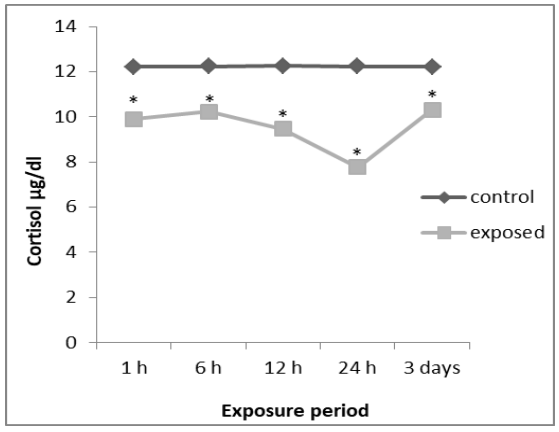

Fig.3: Serum Cortisol levels (µg/dl) in control and dimethoate exposed *Cyprinus carpio*. (significant *p<0.05)

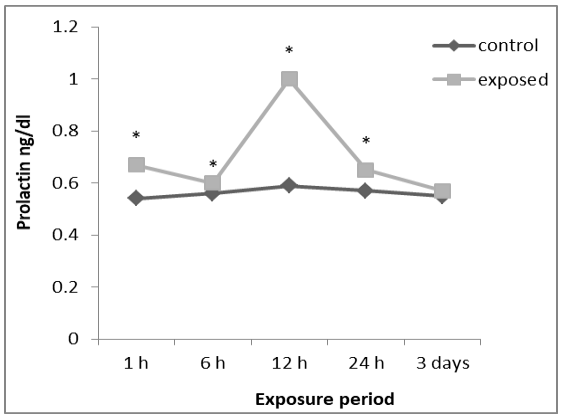

Fig. 4: Serum Prolactin levels (ng/dl) in control and dimethoate exposed *Cyprinus carpio*. (significant *p<0.05)

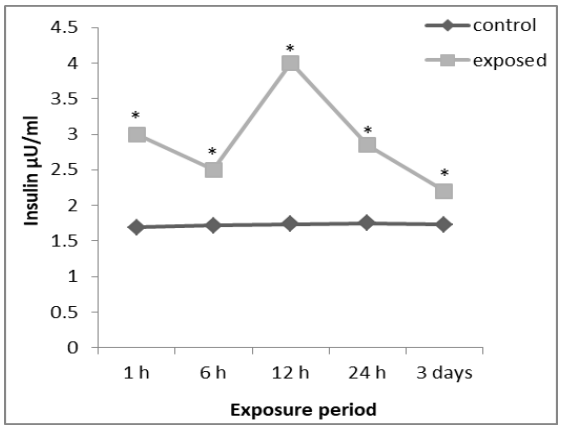

Fig. 5: Serum Insulin levels (µU/ml) in control and dimethoate exposed *Cyprinus carpio.* (significant *p<0.05)