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A Preliminary Study on the Effects of Hyperosmotic Stress on Plasma Levels of Thyroid Hormones in Male Catfish, *Clarias gariepinus*

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Abstract

Male air-breathing catfish, *Clarias gariepinus* were exposed to hyperosmolar mannitol solution (250 mM) to determine its effect on the plasma levels of thyroxine (T_4) and triiodothyronine (T_3) during the winter, spring, summer and autumn seasons. During the winter season, hyperosmolar mannitol solution significantly increased the plasma levels of T_4 throughout the duration of exposure, whereas the plasma T_3 levels significantly increased only at 12-48 h. During the spring season, significant decreases in the plasma levels of T_4 were observed only at 12 and 48 h, and significant increases in the plasma levels of T_3 were observed only at 6-24 h. During the summer season, the plasma levels of T_4 significantly increased at 6 h and decreased at 24-48 h, while the plasma levels of T_3 significantly increased only at 6 and 24 h. During the autumn season, the plasma levels of T_4 significantly increased at 6, 12 and 48 h and the plasma levels of T_3 significantly decreased at 6, 24 and 48 h. These results indicate the season-dependent and differential effects of the hyperosmolar mannitol solution on the plasma levels of thyroid hormones in *Clarias gariepinus*.

Keywords: Thyroid hormone, Thyroxine, Triiodothyronine, *Clarias gariepinus*, Catfish, Hyperosmotic stress; Mannitol

1. Introduction

Osmotic conditions in an aquatic environment have a profound influence on various metabolic processes in the aquatic vertebrates. Changes in the osmolarity of the aquatic environment act as a stress and induce increase in the corticosteroid hormones that are commonly called stress hormones (Eddy F.B., 1981; Hegab S.A., 1984; Patino R.., 1988; Takei Y., 2006; Arjona F.J., 2008). Osmotic stress, depending on the variation in osmolarity, induces a number of adaptive responses. Aquatic vertebrates have developed several adaptive strategies including osmotic regulation for their successful survival (Davenport J., 1985; Hanke W., 1985; Marshall W.S., 2006; Norris D.O., 2006). Short-term adaptive strategies for overcoming osmotic stress seem to involve dynamic regulation of electrolytes, water content, metabolic rate, activity patterns etc., while long-term adaptations against osmotic stress may influence the neuroendocrine system, reproductive behavior and even reproduction (Leloup J., 1985; Fineman-Kalio A.S., 1988; McCormick S.D., 1990; Haddy J.A., 2000; Swanson C., 1998; Orozco A., 2002; Takei Y., 2006). The immediate responses shown by the fish exposed to osmotic stress have been reported to affect their subsequent adaptations and survival (Eddy F.B, 1981; Wedemeyer G.A, 1981).

A number of hormones have been reported to be involved in the osmotic and ionic regulations in fishes (Eddy F.B., 1981; Mancera J.M., 2002; Carrera E.P., 2006; Sakamoto T., 2006; Sangiao-Alvarellos S., 2006; Arjona F.J., 2008). Cortisol reportedly plays an active role in the process of osmoregulation in the fish species (Hegab S.A., 1984; Madsen S.S., 1990; Redding J.M., 1991; Mancera J.M., 1999; Mommsen T.P., 1999; McCormick S.D., 2001; Mancera J.M., 2002; Takei Y., 2006). An increase in the medium osmolality with mannitol from 206 to 290 or 353 mosmol caused a significant increase in the spontaneous secretion of cortisol in the Coho salmon, *Oncorhynchus kisutch* (Walbaum) (Patino R., 1988). Further, corticosteroids have been reported to affect thyroid activity and levels of thyroid hormones in fishes (Brown S.B., 1991; Redding J.M., 1991; Walpita C.N., 2007). Therefore, there is a possibility that osmotic stress-induced increase in corticosteroids may influence the thyroid activity as well. Since thyroid hormones are involved in the regulation of a wide spectrum of fish physiology including metabolic pathways, osmoregulation, oxidative metabolism, and water and electrolyte metabolism (Knoeppel S.J., 1982; Matty A.J., 1985; Peter M.C.S., 1987; Gupta B.B.P., 1991; Schreiber A.M., 1999; Leena S., 2000; Lynshiang D.S., 2000; Peter M.C., 2000; Eales J.G., 2006; Klaren P.H., 2005, 2007), there is also a strong possibility that the circulating levels of the thyroid hormones may be influenced by the hyperosmotic stress. However, there is a scarcity of information on the effects of hyperosmotic stress on the circulating levels of thyroid hormones in fish species in the tropical/subtropical regions. It is also not known whether the effects of osmotic stress on the thyroid hormones, if any, depend on the seasons in aquatic vertebrates in general and in fishes in particular. Therefore, keeping in view the lack of information and the importance of thyroid hormones in fish physiology, it was thought worthwhile to investigate the effects of hyperosmotic stress on the plasma levels of thyroid hormones in male catfish, *Clarias gariepinus* during the different seasons of the year.

2. Materials and Methods

All experiments were conducted on adult males of air-breathing catfish, *Clarias gariepinus*. *C. gariepinus* is a teleost that breed during monsoon (June-August), and lives in shallow rivers, ponds and muddy places. *C. gariepinus* can survive in low water levels with little oxygen. *C. gariepinus* (body weight: 90-100g; body length: 23-27 cm) were purchased from the local fish suppliers. The fish were maintained in clear plastic tubs and acclimatized at least for 15 days in the laboratory under natural climatic conditions at Shillong (25°30′ N, 91°52′ E; 1450 ASL; minimum water temperature varies from 4 to 6° C; maximum water temperature varies from 23 to 25° C). During acclimatization, the fish were fed daily with minced earthworms and commercial fish food (Tokyu) *ad libitum*. Water was changed everyday.

In order to study the influence of hyperosmotic stress, the acclimatized fish were divided in to 2 groups. One group of the fish were maintained in normal water and served as the control group. In order to expose the fish to hyperosmotic stress, the fish of the second group were exposed to 250 mM mannitol solution. After exposure to the hyperosmolar mannitol solution, 4 fishes from each group were removed by hand net and killed by a blow to the head. Blood samples from each group were then collected from the post-caudal region at 6, 12, 24 and 48 h. Also, blood samples from the control group were collected at 0 h and served as the 0-h control group. For obtaining plasma, blood samples were centrifuged at 3000 xg using a centrifuge (REMI: Model R23), plasma separated and stored at -10^{o}C in a refrigerator for the measurement of the total thyroxine (T_4) and triiodothyronine (T_3) concentrations. The experiments were conducted during the winter, spring, summer and autumn seasons. Radioimmunoassay (RIA) kits were obtained from the Division of Radiopharmaceutical Operations, Board of Radiation and Isotope Technology (BRIT), Mumbai. Mannitol was purchased from SISCO Research Laboratories Pvt. Ltd, Mumbai.

2.1 Measurement of plasma levels of total thyroxine (T_4) and triidothyronine (T_3)

The plasma levels of T_4 and total T_3 were measured with the help of RIA kits, RIAK5/5A for T_4 (sensitivity 0.625 ng/ml based on 93.67% *B/Bo* intercept) and RIAK4/4A for T_3 (sensitivity 0.0375 ng/ml based on 90.77% *B/Bo* intercept). The RIAs of T_4 and T_3 were conducted following the manufacturer's protocols with slight modifications where the hormone-free serum was replaced by hormone-free fish plasma (Gupta B.B.P., 2002). RIA was validated using hormone-free fish plasma and different concentrations of standard solutions of T_3 (range: 0.0375-2.4 ng/ml) and T_4 (range: 0.625-20ng/ml). The hormone-free fish plasma used in the assay was prepared by 2 cycles of addition of dextran-coated charcoal to pooled fish plasma, continuous stirring for 6 hours and centrifugation. Polyethylene glycol (PEG) solutions (12% for T_3 and 22% for T_4) were used to separate the bound and free fractions of T_4 and T_3 in the respective RIA. The intra- and inter-assay variations were found to be on an average less than 3.5% and 6.5% for T_3 and T_4, respectively. The radioactivity in the bound fraction was counted with the help of a well-type gamma counter (Electronic Corporation of India, Hyderabad). The concentrations of total T_4 and total T_3 were expressed as ng/ml of plasma.

The data were statistically analyzed with the help of analysis of variance (ANOVA). A probability of $P < 0.05$ was considered as significant.

3. Results

During the winter season, the plasma levels of T_4 were significantly increased after 6 hours of exposure of the fish to hyperosmolar solution of mannitol, and the elevated levels were maintained up to 48 hours of exposure to the hyperosmolar solution (Figure 1). However, exposure to the hyperosmolar mannitol solution significantly increased the plasma levels of T_3 only after 12 hours, and the T_3 levels remained significantly elevated up to 48 hours under exposure to the hyperosmolar solution (Figure 1).

During the spring season, in general, the plasma levels of T_4 were found to be lower as compared to the control groups following exposure to the hyperosmolar solution of mannitol, however, significant decreased in T_4 levels were recorded only at 12 and 48 hours of the exposure to the hyperosmolar mannitol solution (Figure 2). Unlike T_4 levels, the plasma levels of T_3 increased significantly only after 6 hours and remained significantly increased for up to 24 hours of exposure to the hyperosmolar solution of mannitol (Figure 2).

During the summer season, the plasma levels of T_4 were found to increase after 6 hours of exposure to the hyperosmolar mannitol solution, to return to control levels after 12 hours of the exposure, and thereafter, to decline significantly after 24 hours, and to remain significantly lower than that of the control group for up to 48 hours of the exposure to the hyperosmolar solution (Figure 3). In general, the plasma levels of T_3 were found to be higher following exposure to the hyperosmolar solution of mannitol, however, significant increases in plasma levels of T_3 were recorded only at 6 and 24 hours of the exposure to the hyperosmolar solution of mannitol (Figure 3).

During the autumn season, exposure to the hyperosmolar mannitol solution significantly increased the plasma level of T_4 only at 6, 12 and 48 hours (Figure 4). However, the plasma levels of T_3 were significantly decreased following exposure to the hyperosmolar solution of mannitol only at 6, 24 and 48 hours (Figure 4).

4. Discussion

Thyroid hormone concentrations of fishes increased during seawater (SW) acclimation (Redding J.M., 1984; Leloup J., 1985). However exposure of fish to SW during smoltification generally reduces the thyroid hormone concentration (Dickhoff W.W., 1982; Specker J.L., 1984). In contrast, in the Coho salmon retained in freshwater (FW) after smoltification, the sudden transfer to SW increases the T_4 titer (Folmer L.C., 1981; Redding J.M., 1984). Recently, it has been reported that the renal and hepatic outer-ring deiodination (ORD) activities increased concomitantly with the decrease in plasma-free T_4 levels in the fish transferred to extreme salinities (5 per thousand and 55 per thousand) (Arjona F.J., 2008). It has also has been reported that, hyperosmotic challenge resulted in significant and sustained decreases in kidney type I deiodinase (D1) and liver type II deiodinase (D2) activities suggesting an alteration in the levels of thyroid hormones in trout (Orozco A., 2002). In addition, hypoosmotic stress increased liver iodothyronine deiodinase type 2 (D2)

activities in euryhaline teleost, *Fundulus heteroclitus* L. (Orozco A., 1998). But it is important to mention that these studies were not conducted during different seasons of the year and hence could not delineate the seasonal responses of the thyroid hormones to the hyperosmolar stress. Therefore, this might be the first study of its kind in which the study was conducted during different seasons of the year to determine whether exposure to different duration of hyperosmolar mannitol solution affects the plasma levels of thyroid hormones in a season-dependent manner in male *Clarias gariepinus*.

In the present study on male *Clarias gariepinus*, we found that hyperosmolar mannitol solution differentially increased or decreased the plasma levels of T_4 and T_3 in a season-dependent manner. Hyperosmolar mannitol solution was found to elicit significant and consistent increase in the plasma levels of T_4 throughout the duration of the experiment during the winter season, and a general decrease in the T_4 levels was observed only during the spring season. However, during the summer season, hyperosmolar mannitol solution transiently increased and decreased the plasma T_4 levels, with a significant increase observed at 6 hours and significant decrease at 24 and 48 hours. Further, exposure to the hyperosmolar mannitol solution generally increased the plasma T_3 levels during all the seasons, except during the autumn season when the hyperosmolar mannitol solution was found to reduce the plasma T_3 levels throughout the duration of the exposure. It, thus, seems that hyperosmolar stress, depending on the season, differentially increases or decreases the plasma levels of T_4 and T_3 in *C. gariepinus*. Since thyroid hormones are involved in osmoregulation in fishes (Lebel J.M., 1992; Leena S., 2000; Peter M.C., 2000; Klaren P.H., 2005, 2007), the observed seasonal-dependent increase or decrease in the T_4 and T_3 levels following exposure to the hyperosmolar stress might be related to the increased or decreased energy-dependent osmoregulatory activities of the fish, and this remains to be investigated. The increase in the circulating thyroid hormones following a hyperosmotic stress in juvenile salmon has been reported to play an important role in their adaptability to SW (Folmar L.C., 1981). Other endocrine hormones that are affected by environmental salinity may influence thyroid activity as well (Brown C.L., 1983; Redding J.M., 1984; Weisbart M., 1987; Young G., 1989).

Exposure of the juvenile Coho salmon, *Oncorhynchus kisutch* to the osmotic stress (SW containing 345 mM NaCl) has been shown to decrease plasma T_4 concentrations after 24 h, while addition of low concentration of NaCl (12 to 24 mM) to FW increases the plasma levels of T_4 (Specker J.L, 1984) suggesting that the inhibitory and stimulatory effects of the osmotic stress depends on the osmolarity of the aquatic environment. In the rainbow trout, exposure to hyperosmotic stress was reported to have a quick (within 2-4 h) transient stimulatory effect on the plasma T_4 levels and delayed stimulatory effect (after 24-48 h) on the T_3 levels (Orozco A., 2002). Orozco A. (2002) further reported that transfer of FW rainbow trout to 5 parts per thousand SW significantly increased the circulating levels of T_4 at 2, 4 and 48 hours, and circulating levels of the T_3 rose sharply only at 24 and 48 hours post-transfer. Further, recently, it has been reported that the transfer of stenohaline catfish, *Heteropneustes fossilis* (Bloch) from FW to 30% SW significantly increased plasma levels of T_4 (Sherwani F.A., 2008). These reports taken together with present findings seem to suggest

that the levels of thyroid hormones are rapidly but differentially affected by the osmotic (hyperosmolar/hypoosmolar) stress in a species- and season-dependent manners.

On the basis of the present findings, it may be concluded that hyperosmolar stress, depending on the season, differentially increases or decreases the plasma levels of T_4 and T_3, which might be related to the increased or decreased energy-dependent osmoregulatory activities of the fish. Thus, the observed season-dependent different and differential responses in the levels of the thyroid hormones to the hyperosmotic stress might be an adaptive strategy to meet the energy demands associated with the osmoregulatory activities of the fish, *Clarias gariepinus*

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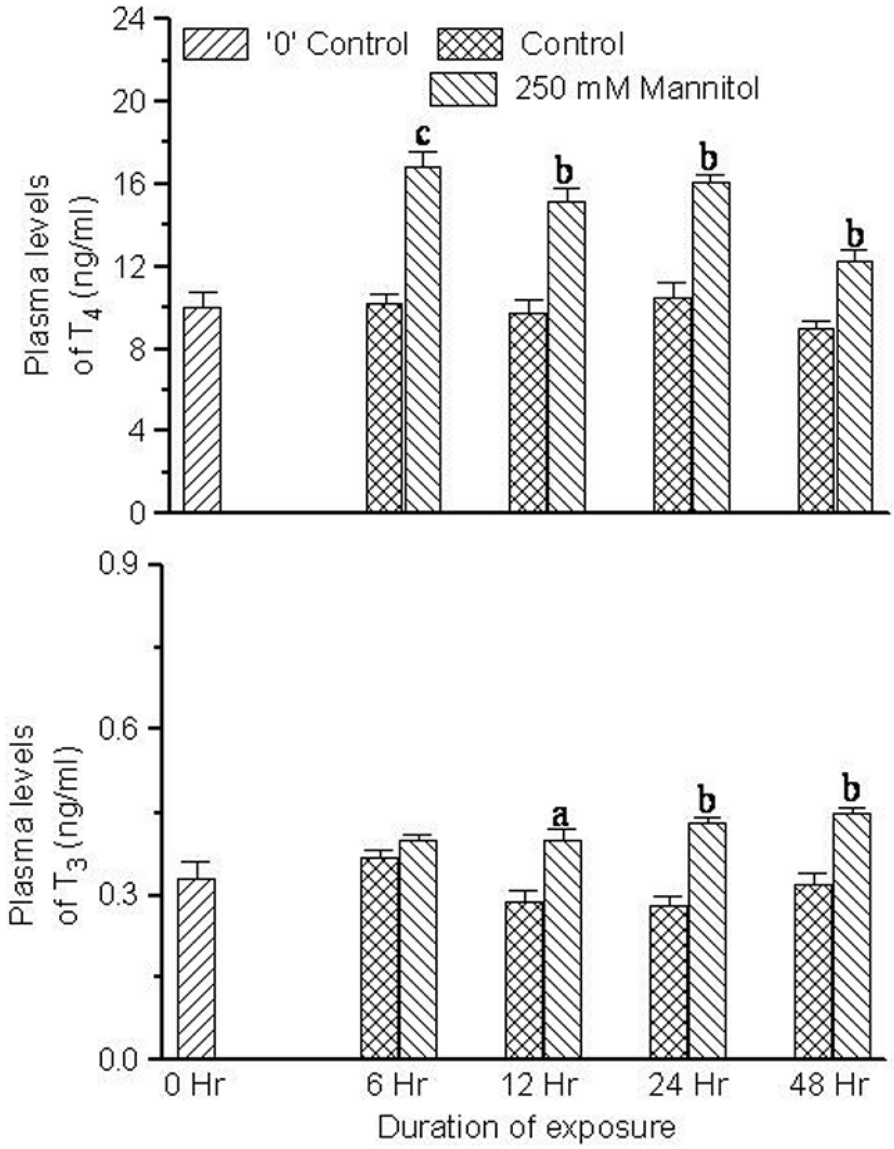

Figure 1. Changes in the plasma levels of the thyroid hormones (T_4 and T_3) following exposure to the hyperosmolar mannitol solution in male *Clarias gariepinus* during the winter season.

Vertical bars indicate standard error of mean (SEM); N = 4.

[a, b, c] Differ significantly from the respective Control group: $P < 0.05$, 0.01 and 0.001, respectively.

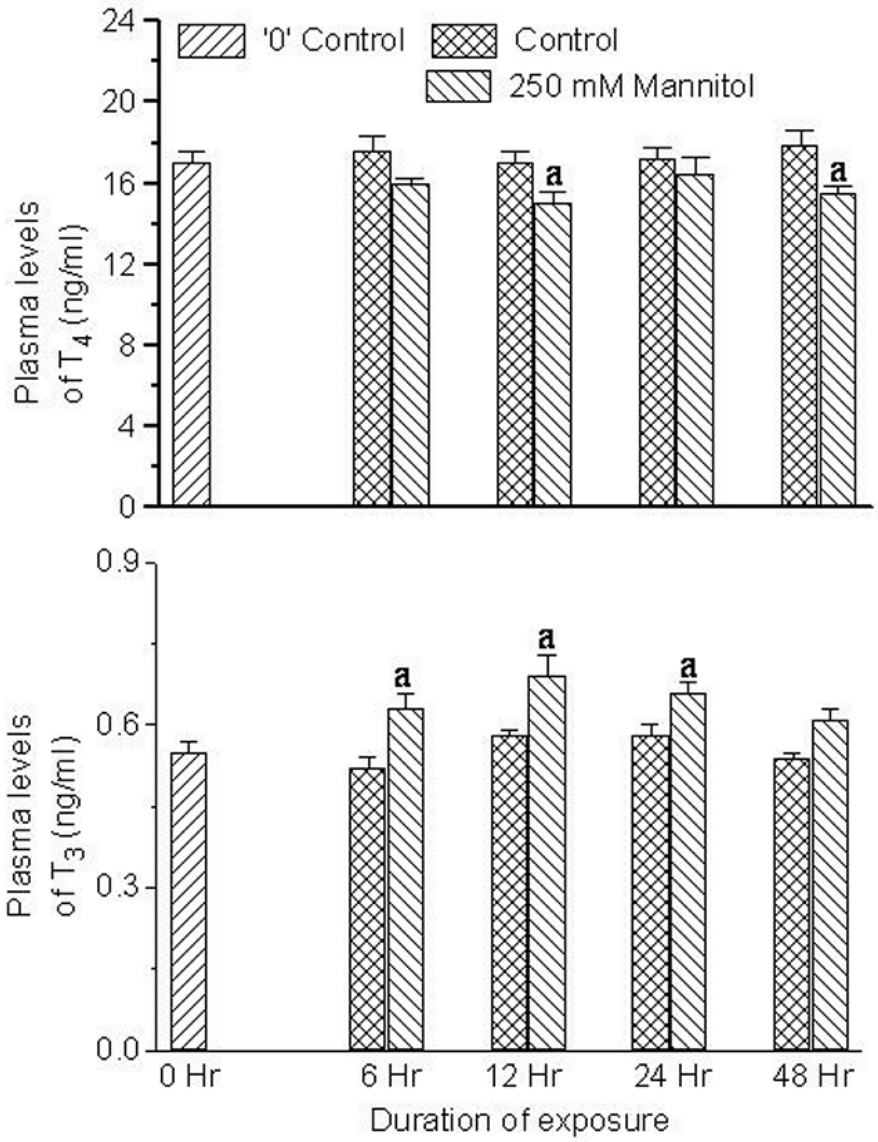

Figure 2. Changes in the plasma levels of the thyroid hormones (T_4 and T_3) following exposure to the hyperosmolar mannitol solution in male *Clarias gariepinus* during the spring season.

Vertical bars indicate standard error of mean (SEM); N = 4.

[a, b] Differ significantly from the respective Control group: $P < 0.05$ and 0.01, respectively.

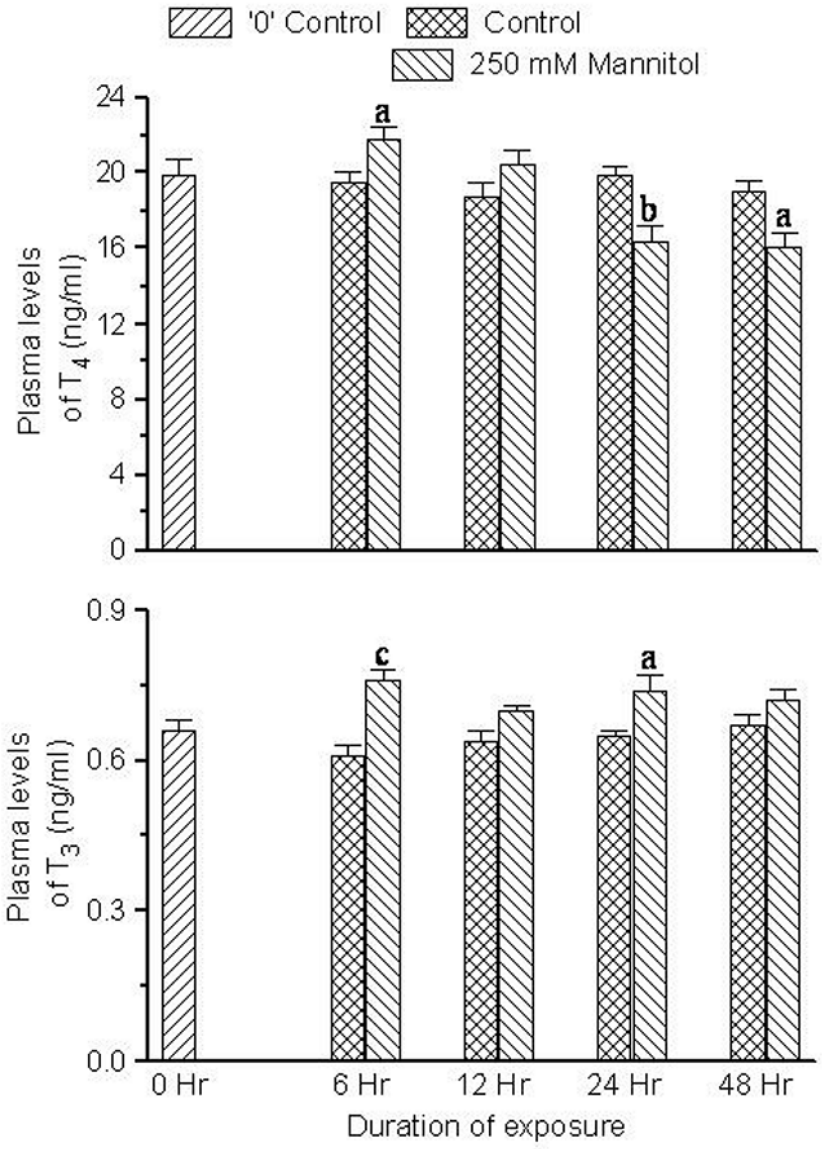

Figure 3. Changes in the plasma levels of the thyroid hormones (T_4 and T_3) following exposure to the hyperosmolar mannitol solution in male *Clarias gariepinus* during the summer season.

Vertical bars indicate standard error of mean (SEM); N = 4.

[a, b, c, d] Differ significantly from the respective Control group: $P < 0.05$, 0.02, 0.01 and 0.001, respectively.

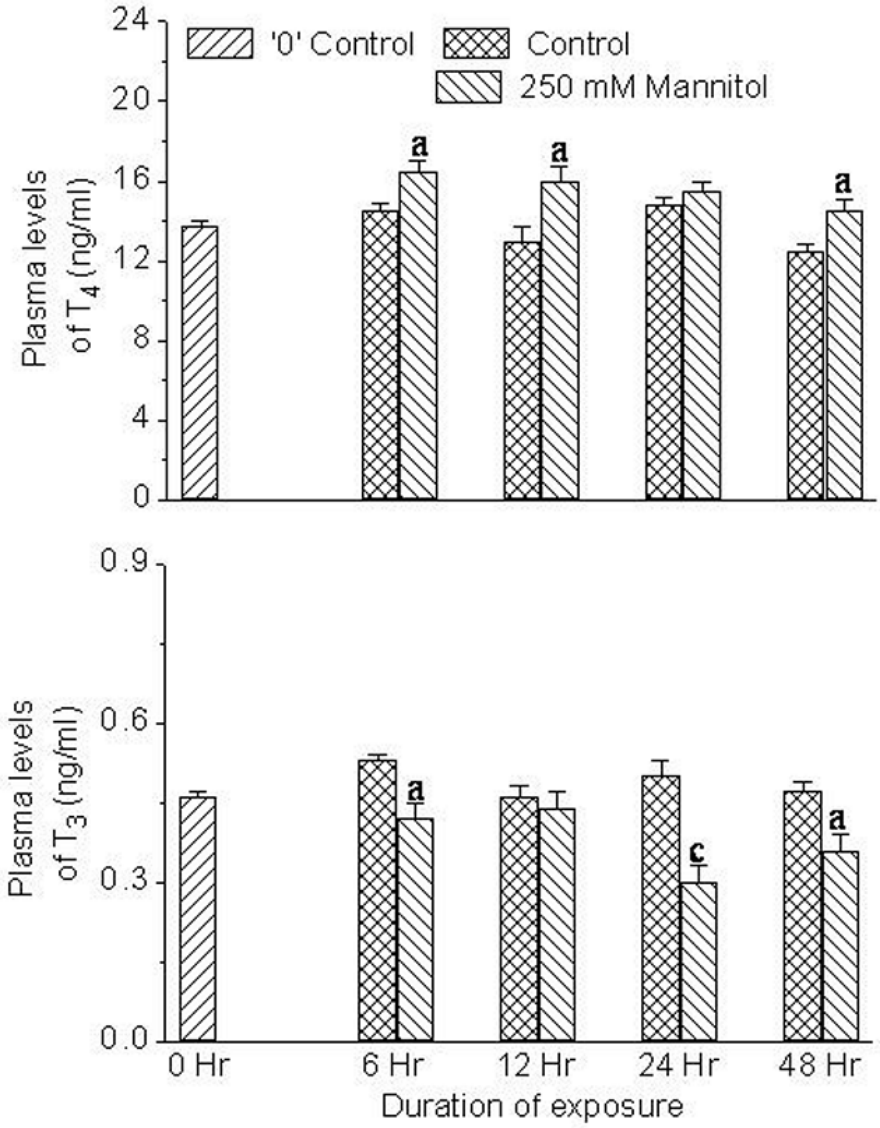

Figure 4. Changes in the plasma levels of the thyroid hormones (T_4 and T_3) following exposure to the hyperosmolar mannitol solution in male *Clarias gariepinus* during the autumn season.

Vertical bars indicate standard error of mean (SEM); N = 4.

[a, b, c] Differ significantly from the respective Control group: $P < 0.05$, 0.02 and 0.01, respectively.