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Comparative study of the effect of calcitonin hormone on some biochemical parameters in serum for two fish species Coptodon zillii, Astronotus ocellatus.

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Abstract

The role of calcitonin and its work mechanism is still under argument in fish. This study is trying to determine its role and factors affecting the mechanism of its work in two species of fish from different environments. The study investigates the effect of this hormone on parameters: Calcium, urea, creatinine, alkaline phosphatase, FSH and LH hormones. Coptodon zillii fish were brought from the local river environment and Astronotus ocellatus from ornamental fish shops, and were acclimatized in the laboratory. The fish samples were divided into two groups. The first group was injected with 40 microliters of calcitonin for 15 consecutive days. Then they were injected every two days until the end of the experiment. Blood was pulled from the fish after three treatments, time 15 days, 30 days and 45 days, respectively. The results showed no significant differences in the calcium levels in serum for C. zillii treated with calcitonin. whereas the calcium levels in serum for A. ocellatus decreased significantly at a probability level of $P \leq 0.05$. There was no significant difference in urea and creatinine levels in C. zillii. Whereas calcitonin reduced the urea levels in A. ocellatus gradually, creatinine was not affected in this species as well. alkaline phosphatase decreased in C. zillii with significant differences at the probability level $P \leq 0.05$. However, its levels in A. ocellatus gradually increased with significant differences at the probability level $P \leq 0.05$. FSH decreased in C. zillii with significant differences at the probability level $P \leq 0.05$, was not affected in A. ocellatus. LH was not affected by calcitonin in both species. It is concluded that calcitonin has a role in regulating calcium in fish blood, in addition to other functions, but its physiology varies according to the species.

Keywords: calcitonin, calcium, fish, Coptodon zillii, Astronotus ocellatus.

1- Introduction

Calcitonin (CT) is a peptide hormone secreted from the thyroid gland. It reduces the levels of Calcium (Ca^{+2}) in the plasma, produced from C cells adjacent to the thyroid follicle in mammals, and in lower vertebrates these cells are concentrated in a specialized gland known as ultimobranchial (1).

The normal function of calcitonin in mammals is the balance of blood calcium (2) It leads to hypocalcemia its action is antagonistic with parathyroid hormone (PTH) in regulating the concentration of Ca^{+2} (1). However, studies differed on its effect on blood calcium in fish depending on species and protocol (3,4). Some studies have confirmed that its role in fish is similar to that in mammals as mentioned in the study on carp *Cyprinus carpio* (5), *Danio Rerio* (6), and *Poecilia latipinna* (7). Whereas hormone action has not been confirmed on hypocalcemia in some species (8-11). This opened the way for more research to determine the actual role of this hormone in fish, studies suggested that it has a role in the regulation of reproduction (12-14). Studies have indicated a relationship between the level of calcitonin and some sex hormones in the plasma, but they have not been able to explain that relationship (5,15,16). Other functions of CT in fish have been reported, suggesting that topically produced CT in the small intestine may inhibit calcium absorption during feeding (17). The CT in the serum keeps the balance of calcium in the bone to prevent over-resorption bone (18). The mRNA encoding of calcitonin receptor has also been detected in some of flounder fish tissues, indicating the diverse functions of CT in adult fish (19,20). Calcitonin gene CGRP is

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also widely found in *Fugu rubripes*, but most of its gene expression is in the UBG and brain (21). No specific function in fish has been known yet in which it requires further studies on its relationship with different body tissues to clarify its role (11). One potential task of this distribution is that it acts as a Neuropeptide (21).

Therefore, the present study considers the effect of calcitonin on some biochemical parameters in two different fish species. Tilapia *Coptodon zillii* that living in the local river environment, Oscars *Astronotus ocellatus* that living in ornamental fish aquariums. These parameters are represented with: Ca^{+2}, alkaline phosphatase (ALP), urea (Ure), creatinine (Crea), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Trialing to answer questions about it, and to verify that one of the determinants of its work is the concentration of Ca^{+2} in the aquatic environment in which the fish live. Attention to fish came to its characteristics and importance, which have recently been proven in various scientific fields (22-24).

2- Materials and Methods

Tilapia *C. zillii* were brought from the local river environment and Oscars *A. ocellatus* were brought from ornamental fish shops. The fish were transferred to the Fish Breeding Laboratory at the College of Education for Pure Sciences / Basra University. The average weight of the fish used in the experiment was 50 g, and the aquariums under the same laboratory conditions were temperature, salinity, and pH. After he adaptation period, the fish samples were divided into two groups, each group included about 20 fish. Group I fish were injected for both species with 40 microliters calcitonin per 50 g fish weight. The equivalent of one IU per 100 grams of fish weight (11). Group II fish were injected with normal saline of 0.6% and used as a control group. The fish were injected 15 consecutive days, and then they injected every two days until the end experiment that lasted 45-day, it was injected at below the dorsal fin. Blood samples were obtained from both control and treatment groups for the two studied species. Blood withdraw was after three treatments with hormone; they are 15 days, 30 days and 45 days respectively, with three replicates from each group. Blood withdraw was done by cutting the tail and withdraw the blood according to the capillary action by special small capillary tubes (Hematocrit) and collecting them into small tubes (Eppendorf). Then, blood samples were put in the centrifuge (Centrifuge 5424 Eppendorf) to obtain the serum. The ratio of the studied blood parameters was measured using the Chemistry Analyzer (Mindray BS-200). After obtaining the data, they were statistically analyzed by the statistical program SPSS and the ANOVA test was adopted to show the statistical differences of the studied parameters between the control group and treated groups.

3- Results

The results showed that the level of Ca^{+2} in the blood of *C. zillii* was gradually increased during CT treatments (Table 1, Figure 1). However, this rise was not significant (Table 2). whereas the CT caused a decrease in blood Ca^{+2} level for *A. ocellatus* during the first treatment and continued to decrease to the second treatment (Table 3, Figure 1). There were significant differences between groups at the probability level $P \leq 0.05$ (Table 4).

Table 1: Rates of the biochemical parameters studied for serum in control group and treated groups for fish *C. zillii*.

Parameters	Cont. (Mean±SD)	Treat.1 (Mean±SD)	Treat.2 (Mean±SD)	Treat.3 (Mean±SD)
Ca^{+2} mg/dl	10.3 ± 0.91	11.96 ± 3.08	16.6 ± 8.85	20.9 ± 10.64
Ure mg/dl	7.16 ± 0.76	5.33 ± 2.08	6.5 ± 0.5	9 ± 1
Crea mg/dl	0.53 ± 0.38	0.95 ± 0.05	0.7 ± 0.2	0.96 ± 0.20
ALP U/l	29.9 ± 14.8	2.7 ± 0.7	13.86 ± 10.92	30.4 ± 9.30
FSH mU/ml	0.27 ± 0.01	0.14 ± 0.03	0.10 ± 0.08	0.28 ± 0.07
LH mU/ml	0.33 ± 0.18	0.31 ± 0.25	0.27 ± 0.15	0.49 ± 0.48

Table 2: Statistical differences of the studied biochemical parameters rates of serum between the control group and treated groups in fish *C. zillii* at probability level $P \leq 0.05$.

Parameters	P- value between groups					
	cont. & treat.1	cont. & treat.2	cont. & treat.3	treat.1 & treat.2	treat.1 & treat.3	treat.2 & treat.3
Ca^{+2} mg/dl	0.99	0.70	0.32	0.85	0.46	0.87
Ure mg/dl	0.33	0.91	0.33	0.67	0.02*	0.14
Crea mg/dl	0.22	0.82	0.19	0.60	1.00	0.55
ALP U/l	0.04*	0.29	1.00	0.57	0.04*	0.27
FSH mU/ml	0.08	0.03*	0.98	0.91	0.04*	0.01**
LH mU/ml	1.00	0.99	0.91	0.99	0.87	0.80

Note: Star marker indicates significant statistical differences.

Table 3: Rates of the biochemical parameters studied for serum in control group and treated groups for fish *A. ocellatus*.

Parameters	Cont. (Mean±SD)	Treat.1 (Mean±SD)	Treat.2 (Mean±SD)	Treat.3 (Mean±SD)
Ca^{+2} mg/dl	17.85 ± 1.15	11.56 ± 2.87	8.3 ± 2.53	10.16 ± 1.81
Ure mg/dl	11 ± 2.64	10 ± 2	8 ± 1	5.5 ± 0.5
Crea mg/dl	1.4 ± 0.68	1.06 ± 0.15	0.83 ± 0.15	1 ± 0.3
ALP U/l	4.06 ± 3.93	4.63 ± 1.22	17.63 ± 6.74	32.83 ± 6.5
FSH mU/ml	0.13 ± 0.1	0.05 ± 0.02	0.093 ± 0.08	0.066 ± 0.02
LH mU/ml	0.025 ± 0.005	0.015 ± 0.005	0.075 ± 0.055	0.015 ± 0.005

Table 4: Statistical differences of the studied biochemical parameters rates of serum between the control group and treated groups in fish *A. ocellatus*. at probability level P ≤ 0.05.

Parameters	P- value between groups					
	cont. & treat.1	cont. & treat.2	cont. & treat.3	treat.1 & treat.2	treat.1 & treat.3	treat.2 & treat.3
Ca^{+2} mg/dl	0.03*	0.003**	0.01**	0.33	0.86	0.73
Ure mg/dl	0.89	0.23	0.02*	0.53	0.05	0.36
Crea mg/dl	0.66	0.30	0.54	0.87	0.99	0.95
ALP U/l	0.99	0.04*	0.001***	0.057	0.001***	0.02*
FSH mU/ml	0.53	0.91	0.69	0.87	0.99	0.96
LH mU/ml	0.97	0.20	0.97	0.11	1.00	1.00

Note: Star marker indicates significant statistical differences.

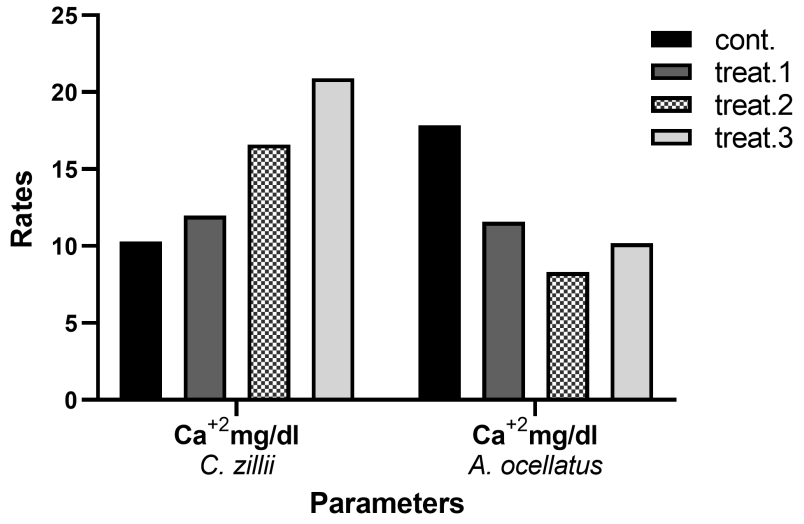

Figure 1: Serum Ca^{+2} rates for control and treated groups of both fish species.

The results of the measurement of Ure and Crea in the blood showed that the injected CT in fish was not a clear effect on the rate of Ure in *C. zillii*. Their rates did not show a clear difference between the control group and treated groups (Table 1, Figure 2). There were no significant differences between these groups, but their rate increased in the third treatment from the first with a significant difference at the probability level P ≤ 0.05 (Table 2). In *A. ocellatus*, CT appears to have caused a gradual decrease in blood Ure level during the three treatments (Table 3, Figure 2). This decrease was significant in the third treatment at the probability level of P ≤ 0.05 (Table 4).

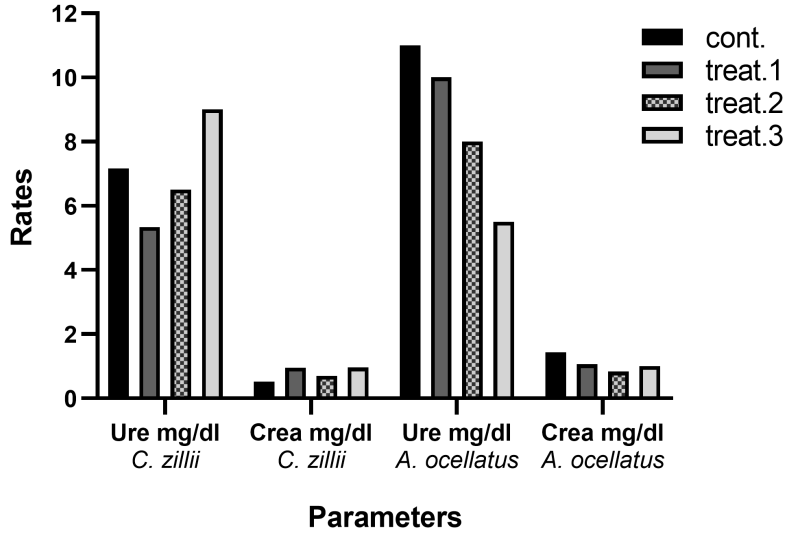

Figure 2: Serum Ure and Crea rates for control and treated groups of both fish species.

The results showed that the levels of Crea in the blood of both species *C. zillii* and *A. ocellatus* were not affected by CT injected in those fish. Their rates did not show a clear difference between the control group and treated groups (Tables 1,3, Figure 1 and 2), and there were no significant differences between these groups (Tables 2,4). CT had a significant effect on ALP in *C. zillii* fish. The level of this enzyme showed a very significant decrease during the first treatment, and then its rate began to rise relatively in the second and third treatment (Table 1, Figure 3). The difference was significant between rates of the control and the first treatment groups, as well as between the first and third treated groups at the probability level $P \leq 0.05$ (Table 2). Whereas CT increased the level of ALP in the blood of *A. ocellatus* gradually, particularly during the second and third treatments (Table 3, Figure 3), which showed significant differences at the probability level $P \leq 0.05$ (Table 4).

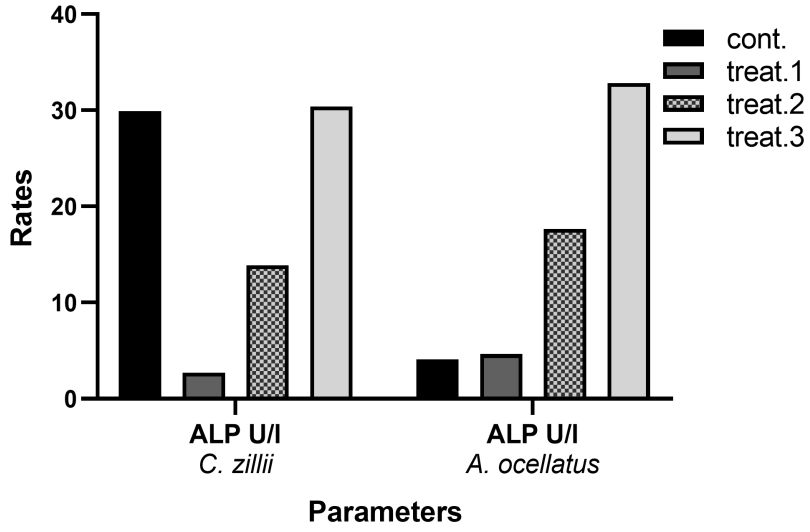

Figure 3: Serum ALP rates for control and treated groups of both fish species.

For sex hormones FSH and LH, CT caused a disorder in the levels of FSH in the blood of *C. zillii*. It decreased its levels in the first and second treatments, and it returned to its levels in the third treatment (Table 1, Figure 4). There were significant differences between the control and the second treatment groups, the first and second treatment groups, and the second and third treatment groups at the probability level $P \leq 0.05$ (Table 2). In *A. ocellatus*, the effect of CT on FSH was less pronounced on the rates of treated groups (Table 3, Figure 4). There were no significant differences between them (Table 4). There was no significant effect of CT on the LH level in both species *C. zillii* and *A. ocellatus*, and there were no significant differences between the control group and treated groups (Table 2,4).

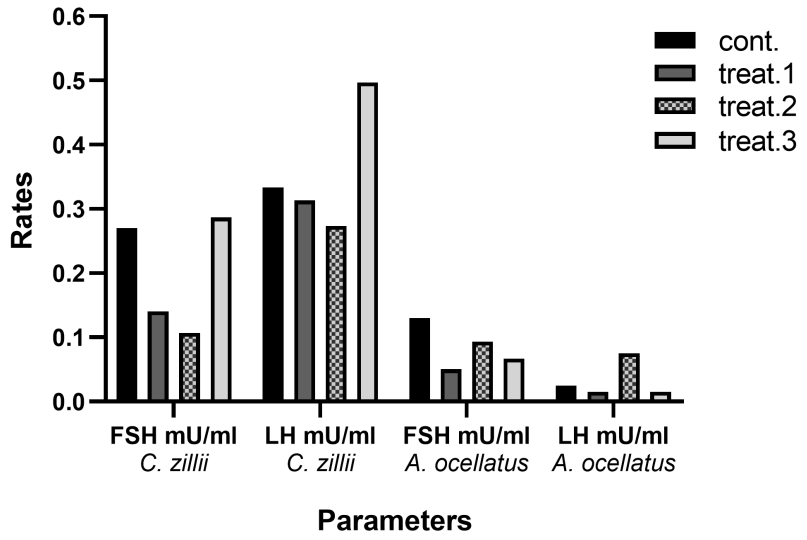

Figure 4: Serum FSH and LH rates for control and treated groups of both fish species.

4- Discussion

Calcitonin is a hormone involved in the regulation of blood calcium in different animal host (1). Although some studies have not proved this role in some fish species (4,10,11). However, several studies have shown that CT induces Hypocalcemia in the blood of some other fish such as eel (26), *Cyprinus carpio* (5, 27), and *Poecilia latipinna* (7). The results showed that CT did not cause the same action on blood calcium in the two studied species, as it caused excessive blood calcium for *C. zillii*. The rise was gradual through the three treatments but it was not a significant difference between the control group and treated groups. Whereas the effect of CT was evident in the low blood calcium level in *A. ocellatus*. The difference was significant between the control group and the treated groups at the probability level $P \leq 0.05$. The difference in the effect on the two species as if it remains questions about this hormone in fish.

The involvement of CT in the regulation of Ca^{+2} levels in fish blood cannot be ruled out, and this has been pointed out by numerous researches directly or indirectly through multiple mechanisms, including that it inhibits the activity of Osteoclasts cells (5,18). Or accompanied by an enhancement of the activity of Osteoblasts cells at the same time, as is its role in mammals (1,28). Okuda et al.(17) found out that the hormone is produced topically in the intestinal lining of Goldfish and inhibits the absorption of Ca^{+2} during feeding. Some studies have also indicated that CT affects the osmotic regulation of the epithelial lining of the gills and inhibits transport process of Ca^{+2} through which it occurs (6,29,30). An expression of the hormone receptor gene CGRPR has been found in both intestines and gills in the flatfish (19). The results of the measurement of Ca^{+2} in the blood of *A. ocellatus* were correspond with those studies from where the effect of CT on calcium level reduction, the other conflicting experiments (4,10,11), which correspond to the results of measuring Ca^{+2} in the blood of *C. zillii*, may not be due to the different role of CT in species, but rather for other reasons. One of the reasons suggested is the high levels of CT in the blood plasma that makes the blood saturated with endogenous CT. Therefore, there is no response by exogenous administration CT (11). Srivastava et al. (11) has deduced in his experiments on fish *Heteropneustes fossilis* that the determinant of the response is the medium in which the fish live. In other words, the amount of endogenous CT in the plasma is determined by the concentration of Ca^{+2} in the aquatic environment in which the fish live, and the response occurs when the level of Ca^{+2} in the water medium is moderate and maintains moderate levels for endogenous CT to be able to respond to the CT. This is not consistent with the results of the current study, as the two species studied *C. zillii* and *A. Ocellatus* were in the same aqueous medium but their response was different. The study suggests that this difference is due to species-specific physiological aspects such as the amount of dose capable of effecting. The dose used in the experiment may be sufficient to affect *A. ocellatus*, but it was not for *C. zillii*. It should be noted that Fouchereau-Peron et al.(31) stated that the high doses of CT cause Hypocalcemia, but it is counterproductive if it is in low doses, causing hypercalcemia. This was observed in the results of the measurement of Ca^{+2} in the blood of *C. zillii*, which rates gradually increased during the three treatments but did not reach significantly. It also CT interferes with other hormones in the regulation of Ca^{+2} levels in fish blood, such as Parathyroid, Prolactin, Stanniocalcin (4,10). There may be a variation in the mechanism of the hormone action and its function between those hormones regulating Ca^{+2} from one species to another.

Ure and Crea blood tests are usually an indicator of the health of animals, including fish. Increased or decreased secretion is usually caused by impaired kidney and liver function. The results show that the effect of CT on Ure levels was not similar on both species. This increases the likelihood that hormone physiology varies between species. Ure levels in the blood of *C. zillii* treated were not significantly affected, whereas levels of Ure in the blood gradually decreased for *A. ocellatus* during the three treatments, which may indicate the cumulative action of the hormone in reducing Level of body metabolism. The other possibility of CT action on the Ure level in the blood may be due to a malfunction of the kidney due to the hormone. This varies according to the function of the hormone and mechanism of its action in the species. The level of Crea in the blood was more stable than Ure in both *C. zillii* and *A. Ocellatus*. The natural limits of Crea may indicate the safety of creatine metabolism in these fish, and therefore the stability of the muscular activity of fish, particularly not observed inactivity or high activity on the fish behavior during the experiment. It is worth to mention no previous study dealt with the effect of CT on the levels of Ure and Crea in the blood to our knowledge.

ALP is produced by several organs in the body, and its rise or decrease in blood above normal limits is a criterion of damage to those organs alone or in combination (32). Numerous studies have indicated that there is a relationship between CT and ALP; therefore, a relationship with the tissue producing it, regardless of the type of relationship whether negative or positive. It was found that increased immune CT level in patients with cirrhosis was related to increased ALP activity in plasma (33). Miyamoto et al.(34,35) found out that CT regulates and increases ALP activity in the LLC-PK1 cell line derived from pig-kidney, and the hormone modifies enzyme levels in patients with Paget's disease of bone (36). An increased ALP was observed in rats when administered calcitonin subcutaneously (37). CT was negatively associated with ALP in cats with chronic kidney disease (38). The effect of CT on the ALP enzyme varied in the two studied species, as with Ca^{+2} and Ure, this confirms the different in hormone physiology in fish depending on the species.

The level of ALP in the blood decreased during the first treatment in *C. zillii* with a significant moral difference, then returned to normal during the second and third treatments. This means that the CT effect only during the first treatment and then the effect gradually removed and returned to be normal without cumulative effect. Whereas the levels of ALP in the blood of *A. ocellatus* have gradually increased among the three treatments, this is evidence of the cumulative effect for CT. Based on the results of the measurement of ALP in the blood, the present study does not exclude the possibility affected any the organs producing ALP enzyme in the body of both types *C. zillii* and *A. ocellatus*. The variation in the influence and its intensity between the two species reinforces the likelihood of difference in the physiology of CT between species. However, they attribute the strongest probability of rising of the ALP in *A. ocellatus* to the affected bone tissue in this fish. Based on the results of the measurement of Ca^{+2} in the blood, which showed that the hormone worked to reduce Ca^{+2} clearly in contrast to its level in *C. zillii* that did not appear, which puts a high probability that the hormone effect reached to bone cells. References have indicated that increased activity of Osteoblasts increases the release of ALP (1,39). Studies have shown that CT at certain concentrations increases ALP activity and levels through its activity on bone cells (40,41). One study also reported that CT increases the ALP level in Cartilaginous cell cultures of the rabbit (42). This also adds the possibility that cartilage cells in fish have been involved in increasing the ALP level due to the hormone effect. This also adds the possibility that cartilage cells in fish have been involved in increasing the ALP level due to the hormone effect, particularly that the fish from species that contain large amounts of cartilage forms the bone core (43,44).

Sexual hormones FSH and LH are of great importance. The high or low levels of any of them in the blood causes many problems in the body. The results of the measurement of FSH in the blood varied in the two studied species. In *C. zillii*, FSH decreased significantly during the second treatment and then, it returned to its level in the third treatment. This is observed with ALP in the same species. CT did not show significant effect on FSH level in *A. ocellatus* during the three treatments. As for LH, the results did not show a clear effect of calcitonin on its levels in the blood for both species *C. zillii* and *A. ocellatus* during treatments. There is no previous study dealt the relationship between CT and these two hormones, however, there have been studies of the CT effect on other sex hormones in fish in particular, after it was observed that high levels of CT in the plasma during the peak of the breeding season in different species (5,12-14). This may indicate the relationship of calcitonin with the reproduction in fish. The present study supports this possibility, based on the results of FSH low in *C. zillii*. The lack of CT effect on FSH in *A. ocellatus* may be due to The dose amount or hormone physiology differences among fish species.

5- Conclusions

The current study showed that calcitonin affects the level of calcium in fish blood. It is also believed through the results that this hormone plays other roles in fish. Related to metabolism and reproduction. But its physiology varies according to the species. The calcium concentration in the aqueous medium in which fish live is not the only factor in determining its role in the fish.

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