Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(1): 119 – 129 (2020)
www.ejabf.journals.ekb.eg

Effect of Gonadotropin Releasing Hormone Injection on Physiological Changes and Reproductive Hormones in *Clarias gariepinus*

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ARTICLE INFO

Article History:
Received: Dec. 6, 2019
Accepted: Dec. 29, 2019
Online: Jan. 2020

Keywords:
Gonadotropin
Releasing hormone
Clarias gariepinus
hematology
Reproductive hormones

ABSTRACT

Evaluation of gonadotropin releasing hormone (GnRH) to physiological changes, spawning and reproductive parameters of African catfish, *Clarias gariepinus* in the current study has been conducted to determine the effectiveness of GnRH in stimulation the maturation and ovulation of African catfish. GnRH at 4, 8 and 12 µg/kg body weight dissolved in 0.6% NaCl under a natural temperature regime. The fish from Abbasa farm were fed with 30 % protein diet during experiment. Fish 550 - 600 g body weight were injected with a doses of (4, 8 and 12 µg/kg body weight) three times a week. Result showed multiple ovulations and spawns within a period of approximately 30 days. Levels of 17β- estradiol (E2), testosterone (T) and follicular stimulating hormone (FSH) were elevated at 30 days' post treatment, preceding the spawns with the highest fecundity. These results show that administration of GnRH induces multiple spawns in fish where, approximately 100% increase in red blood cells (RBCs), white blood cells (WBCs), hematocrit value (Hc) and hemoglobin content (Hb) respectively. Also, there are increased in plasma glucose level, total protein, aspartate and alanine amino transferase activities (AST and ALT) and increased in plasma creatinine and uric acid concentration after administration of GnRH. These data suggest that fish respond to administration of GnRH by increasing their hematology, enzymes of liver functions and kidney functions. Injection of GnRH at 4, 8 and 12 µg/kg was found to be effective to induce ovulation in African catfish. 100% ovulation was also observed for the fish treated with GnRH. Also, plasma level of E2, FSH and T increased significantly with the association of ovulation. The present study indicates that administration of GnRH is effective for sexual maturation and ovulation in African catfish.

INTRODUCTION

Gonadotropin releasing hormone has been used to induce ovulation and spawning in African catfish, *Clarias gariepinus* to stimulate maturation and ovulation. Gonadotropin hormone releasing hormone is a peptide hormone that is responsible for stimulating the release of gonadotropins from pituitary and consequently influence the steroid hormones production level in the ovary (Peter and Yu, 1997). Carp pituitary extract (CPE) and Human Chorionic Gonadotropin (HCG). While most commonly used GnRHs are originally from mammalian was used for manipulation of the reproductive cycle of fish, still there appears to be many other forms of GnRH present in the fish species. Among other forms of GnRH are salmon GnRH (sGnRH), mammalian GnRH (mGnRH), catfish GnRH (cfGnRH), seabass GnRH (sbGnRH), chicken GnRH-II (cGnRH-II), herring GnRH (hrGnRH), medaka

GnRH (mdGnRH) and pulsatile GnRH (pGnRH) have been identified in the brain of different teleosts (Montaner *et al.,* 2001). Fish share two forms of GnRH namely (mGnRH) and (cGnRH-II) with other vertebrates, but nine variants have been identified in fish (Philippa and Sherwood, 2005). One of the reasons for lack of ovulation in cultured fish is the failure of pituitary to release gonadotropin (GtH-II) to stimulate maturation and ovulation (Zohar and Mylonas, 2001 and Coccia, *et al.* (2010) Synthesis of vitellogenin and increase in ovarian size during final oocyte maturation is controlled by 17ß-estradiol. Spawning of *Ictalurus catus*, and *Ameiurus nebulosus* using carp pituitary extract and LHRHa (Fobes, 2013). Carp pituitary extract (CPE) and luteinizing hormone-releasing hormone analogue (LHRHa) are two well-known hormones for controlling ovulation in channel catfish. Ramy, *et al.* (2014) reported that the egg mass and the level of 17β-estradiol (E2) increased at 6 h in all treated groups with GRH in African catfish. Shokr (2015) indicated that administration of follicular stimulating hormone and luetinizing hormone (10, 20 and 40 μg kg− 1 body weight) increase blood constituents, plasma glucose level, total protein and AST, ALT activities, creatinine and uric acid level and increasing their WBCs, enzymes of liver functions and kidney functions. Maac. *et al*. (2020) investigated that GnRH injection increased serum LH and GH levels only in fish at the regressed stage but exerted both stimulatory and inhibitory effects on GnRH-induced LH responses depending on season. T3 treatment mainly had stimulatory effects on circulating LH levels and inhibitory effects on serum GH concentrations. The hypothesis that GnRH and GnIH are important components of multifactorial mechanisms that work in concert with T3 to regulate reciprocal control of reproduction and growth in goldfish. Sedigheh, *et al.* (2020) reported that GnRH is a neuropeptide known to regulate reproduction in vertebrates. Gonadotropin-releasing hormone associated peptide (rGnRH/GAP) as an alternative of the previous GnRHs and native extracted hormone from tissue, to induce final maturation in fish.

The desired outcome of this respective work is to determine the effectiveness of GnRH as an inducing agent for maturation and ovulation in African catfish, *C. gariepinus*, a commercially important food fish. To achieve this outcome, it requires the understanding of the potent dosage needed to bring ovulation. Following that, determined the effectiveness of GnRH on plasma sex steroid levels of 17ß-estradiol, FSH and testosterone also, changes T3 in plasma and liver and kidney functions were measured to obtain in vivo steroidogenic activity responses following hormone administration.

MATERIALS AND METHODS

Clarias gariepinus body weight 550 to 600 g was obtained from Abbasa farm. The fish were held in large tanks contained water from their farm. The fish were fed with 30 % protein diet during experiment. The fish were divided into four groups, control group and three groups injected intramuscularly with GnRH at 4, 8 and 12 μg/kg body weight dissolved in 0.6% NaCl under a natural temperature regime (Fobes, 2013). The GnRH used was (Bachem Bioscience, King of Prussia, PA). Control fish were treated with 0.6% NaCl. During experiment fish were injected 3 times per week with GnRH. The Gonado Somatic Index (GSI) was calculated on monthly basis by following standard method (Le Cren, 1951). The GSI was calculated using the formula: GSI = (GW/ (BW- GW) x 100, Where GSI is Gonado Somatic Index, GW is gonad weight and BW is total body weight with intact gonad. Fecundity was calculated according to Armando, *et al.,* 2009. Fecundity was

calculated using the formula: fecundity: En=z/W, where En=number of eggs per weight (g) of fish; z=number of eggs; W=total weight of fish. Spermiating males were identified by the release of sperm after application of gentle abdominal pressure approximately every week.

Blood analysis:

The blood samples were collected after 30 days from arterial caudal with heparinized syringes from control group and injected groups, respectively according to Soivio, *et al.* (1972). Blood samples were placed into microtubes (2.0 mL). All samples were collected in the early morning hours and were processed for hematological analysis according to Soivio and Oikari (1976). The total cell count (erythrocytes and leukocytes) were performed by the diluent/dye direct method outlined by Natt and Herrick (1952) in a Neubauer chamber at a dilution of 1:100. Following the total cell count of nucleated cells (leukocytes) in the Neubauer chamber. Blood was used for erythrocyte (RBCs) count (Dacie and Lewis 1984), hemoglobin (Hb) content (Vankampen, 1961) and hematocrit (Hct) value (Britton, 1963) determination. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) were calculated. The remaining samples of blood were centrifugation at 4000 rpm for 15 min at room temperature to obtain the plasma for measuring different biochemical parameters. plasma glucose concentration was measured according to Trinder (1969). Protein content was determined by the Biuret method described by Wootton (1964). Total lipids were determined calorimetrically according to Knight *et al.* (1972). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, and creatinine were determined calorimetrically according to Reitman and Frankel (1975). Reproductive hormones as 17 ß estradiol (E2), testesterone (T) and follicle-stimulating hormone (FSH) measured using Radioimmunoassay procedure by Foster and Dunn (1974) also used for determination of thyroid hormones using ELISA Kit. Catalog No. CSB-E12045Fh (Cusabio Biotech Co., Ltd) procedure by Foster and Dunn (1974).

Statistical analysis:

The obtained data in the present study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to Snedecor and Cochran (1982). Differences among treatment means were compared using Duncan's multiple range tests (Duncan, 1995). Data were presented as mean ± SE and significant difference was declared at $P < 0.05$.

RESULTS

Reproductive parameters:

The fish ovulated in the group treated with 8 µg/kg GnRH was 80% and 100 % of the fish ovulated in the group treated with 12 µg/kg GnRH. Mean fecundity of *Clarias gariepinus* injected with GnRH at 4, 8 and 12 µg/kg was observed to range from 55200 eggs for fish with total weight (593 g), ovary weight (OW, 80 g) to 106700 eggs for fish with total weight (679 g), OW (153 g). There was a significantly increased in the fecundity of *Clarias gariepinus* injected with GnRH at 4, 8 and 12 µg/kg and control one Table 1. On the other hand, Mean spermiating of *Clarias gariepinus* injected with GnRH was observed increased than that control one. There was a significantly increased in the spermiating of *Clarias gariepinus* injected with GnRH and control one Table 2.

Table 1: Effect of 4, 8 and 12 µg/kg GnRH injection for a 30 days on the Gonadosomatic index (GSI) of Ovary and fecundity of *Clarias gariepinus.*

Fish	**Control**	**Female**		
	Saline	**4 µg/kg GnRH**	**8 µg/kg GnRH**	**12 µg/kg GnRH**
Number of fish	10	10	10	10
Ovulating	1 ± 0.2^{a}	5 ± 0.1^{b}	8 ± 0.2^{c}	10 ± 0.5^{d}
GSI%	4.82 ± 0.2^{a}	8.2 ± 0.2^{b}	10.9 ± 0.1^{c}	13.1 ± 0.9^{d}
Fecundity	10000 ± 1467^{a}	$47{,}000\pm2561^{b}$	$78{,}000\pm4352^{c}$	100.000 ± 6842^{d}

Data expressed as means ± SE, means with the same letter in the rows is not significant at $p<0.05$

Table 2: Effect of 4, 8 and 12 µg/kg GnRH injection for a 30 days on the Gonadosomatic index (GSI) of Testis and spermiating of *Clarias gariepinus*.

Fish	**Control**	**Male**		
	Saline	**4 µg/kg GnRH**	**8 µg/kg GnRH**	**12 µg/kg GnRH**
Number of fish	**10**	**10**	**10**	**10**
Spermating	$\mathbf{1\pm 0.1^{a}}$	$\mathbf{4\pm 0.7^{b}}$	$\mathbf{6\pm 0.5^{c}}$	$\mathbf{10\pm 0.7^{d}}$
GSI%	$\mathbf{0.82 \pm 0.1^{a}}$	$\mathbf{1.8 \pm 0.3^{b}}$	$\mathbf{2.1 \pm 0.7^{c}}$	$\mathbf{4.2 \pm 0.7^{d}}$
Sperm volume (µl)	$\mathbf{5.4 \pm 0.7^{a}}$	$\mathbf{13.29 \pm 1.7^{b}}$	$\mathbf{25.8\pm1.7^{c}}$	$\mathbf{47.22 \pm\pm 2.7^{d}}$
Spermatocrit Sct (%)	$\mathbf{15.1 \pm 4.7^{a}}$	$\mathbf{25.4 \pm 2.7^{b}}$	$\mathbf{27.2 \pm 1.5^{c}}$	$\mathbf{28.6 \pm 2.7^{d}}$

Data expressed as means ± SE, means with the same letter in the rows is not significant at $p<0.05$.

Follicular stimulating hormone (FSH), 17β-estradiol (E2) and testosterone (T) for the fish group treated with 4, 8 and 12 µg/kg GnRH were shown in Table 3. Similar pattern of changes was observed in all hormones. Steroid level for saline fish remained the same throughout the experiment. Initially the plasma of steroid level for fish treated with 4 µg/kg GnRH was low than that treated with 8 and 12 µg/kg GnRH were shown in Table 3. Plasma levels of 17β-estradiol (E2), follicular stimulating hormone (FSH) and testosterone (T) were determined. The increase of plasma E2 levels was stronger in females injected with GnRH than in males injected with GnRH. In both cases, plasma FSH increased than in control one. A similar effect on plasma FSH levels was observed in female fish injected with GnRH were increased than in male one, but plasma levels of testosterone (T) were much lower in control than those injected fish. The females injected with GnRH showed the lowest plasma T levels (Table 3).

Table 3: Effect of 4, 8 and 12 µg/kg GnRH injection for a 30 days on the FSH, E2 and T of *Clarias gariepinus.*

Fish	**Treatment**	**N**	**FSH mIU/ml**	**(E2) ng/ml**	**T ng/ml**
	Saline	10	9.2 ± 1.5^{a}	1.4 ± 0.6^{a}	0.22 ± 0.01^{a}
Female	4 µg /kgGnRH	10	22.2 ± 1.4^{b}	1.5 ± 0.3^{b}	0.28 ± 0.01^{b}
	8 µg /kgGnRH	10	24.6 ± 1.7^{c}	1.8 ± 0.4^{c}	$0.\ 30\pm0.01^{c}$
	12 µg /kgGnRH	10	25.2 ± 2.1^{d}	1.9 ± 0.3^{d}	0.33 ± 0.01^{d}
	Saline	10	6.5 ± 1.5^{a}	1.1 ± 0.8^{a}	0.48 ± 0.05^{a}
Male	4 µg /kgGnRH	10	10.9 ± 1.2^{b}	1.2 ± 0.1^{b}	0.58 ± 0.02^{b}
	8 µg /kgGnRH	10	12.8 ± 1.5^{c}	1.4 ± 0.8^{c}	0.63 ± 0.08^{c}
	12 µg /kgGnRH	10	15.7 ± 1.3^{d}	1.7 ± 0.5^{d}	0.73 ± 0.02^{d}

Data expressed as means ± SE, means with the same letter in the columns is not significant at $p<0.05$

Hematology Parameters

The red blood cells (RBCs) in the groups of the fish that injected GnRH at 4, 8 and 12 µg/kg for a period of 30 days were significantly increased compared to control group as showed in Table 4. There was a significant increase in the white blood cells (WBCs) in the groups of the fish that injected GnRH. Also, there was a significant increase in Hemoglobin (Hb) in the groups of the fish that injected GnRH at 4, 8 and 12 µg/kg for a period of 30 days compared to control group. Hematocrit (Hct) was a significantly increased in the groups of the fish that injected with GnRH compared to control group as shown in Table 4. There was a significant increase in mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean

corpuscular hemoglobin concentration (MCHC) in the groups of the fish that injected with GnRH at 4, 8 and 12 µg/kg for a period of 30 days compared to saline group.

Table 4: Effect of 4, 8 and 12 µg/kg GnRH injection for a 30 days on the hematology of *Clarias gariepinus.*

Fish	Control	Female			Control	Male		
	Saline	2 µg/kg GnRH	4 µg/kg GnRH	12 µg/kg GnRH	Saline	2 µg/kg GnRH	4 µg/kg GnRH	12 µg/kg GnRH
RBCs x 10^6	**1.8± 0.3^a**	**2.5± 0. 1^b**	**3.6± 0.7^c**	**4.5± 0.3^d**	**1.9± 0. 1^a**	**2.8± 0.3^b**	**3.4± 0.6^c**	**5.8± 0.3^d**
WBCs x 10^3	**3.6±0.6^a**	**3.8±0.2^b**	**4.3±0.4^c**	**5.6±0.6^d**	**3.8±0.3a**	**3.9±0.4^{ab}**	**4.6±1.6^b**	**5.2±0.6^c**
Hb gm/100ml	**4. 1±0.1^a**	**4.7±0. 9^b**	**5.2±0.1^c**	**5.5±0.6^d**	**4.5±0. 9^a**	**4.8±0. 4^{ab}**	**5.2±0. 6^b**	**6.5±0.7^c**
Hct %	**13.2±0.5^a**	**14±0.7^b**	**15.4±0.8^c**	**16.2±0.9^d**	**13.5±0.4^a**	**13.7±0.8^{ab}**	**14.2±0.7^b**	**16.2±1.3^c**
MCHC%	**33±0.5^a**	**36±1.5^b**	**39±2.1^c**	**42±1.5^d**	**35±1.7^a**	**38±1.4^b**	**43±1.5^c**	**51±2.4^d**
MCVµ3	**75±1.5^a**	**77±2.1^b**	**79±3.5^c**	**85±2.4^d**	**77±2.9^a**	**79±2.5^b**	**85±3.5^c**	**86±3.1^{cd}**
MCHpg x 10^3	**25±0.5^a**	**26±2.2^b**	**28±1.5^c**	**35±2.1^d**	**27±1.5^a**	**29±1.2^b**	**35±1.4^c**	**41±2.5^d**

Data expressed as means ± SE, means with the same letter in the rows is not significant at p<0.05

Biochemical Parameters

The activity of aspartate amino transferase (AST) in the plasma of fish that injected with 4, 8 and 12 µg/kg GnRH for a 30 days significantly increased than that control group. Also, there was increased in the plasma activity of alanine amino transferase (ALT) of the fish that injected with GnRH than that control group. The plasma uric acid level was significantly increased in the fish that injected with GnRH than that control one. There was significant increase in serum creatinine of the fish that injected with GnRH than that control one as shown in Table 5.

Table 5: Effect of 4, 8 and 12 µg/kg GnRH injection for a 30 days on the liver and kidney functions of *Clarias gariepinus.*

Fish	Treatment	AST U/ L	ALT U/ L	Uric acid mg %	Creatinine mg %
Female	Saline	27.4±0.5^a	20.1±1.6^a	5.8±0.6^a	1.5±0.1^a
	4 µg /kgGnRH	32.4±0.7^b	24.6±0.9^b	11.3±0.8^b	1.6±0.5^{ab}
	8 µg /kgGnRH	45.2±0.4^c	37.7±0.5^c	13.4±0.7^c	1.8±0.3^{bc}
	12 µg /kgGnRH	49. 6±0.6^d	41.1±0.8^d	17.6±0.8^d	1.9±0.4^c
Male	Saline	28.6±0.8^a	21.2±0.9^a	6.1±0.5^a	1.7±0.1^a
	4 µg /kgGnRH	34.3±0.9^b	28.6±0.6^b	12±0.4^b	2.1±0.6^b
	8 µg /kgGnRH	41.5±1.9^c	40.8±0.8^c	13.8±0.8^c	2.7±0.7^c
	12 µg /kgGnRH	45.1±1.8^d	45.6±0. 6^d	17.6±0.9^d	2.9±0.9^d

Data expressed as means ± SE, means with the same letter in the columns is not significant at p<0.05.

Glucose level was significantly increased in the fish which injected with 4, 8 and 12 µg/kg GnRH for a 30 days compared to control group. The plasma total protein of the fish that injected with 4, 8 and 12 µg/kg GnRH for a 30 days were significant decreased compared to control group as shown in Table 6. Also, the plasma total lipids of the fish that injected with 4, 8 and 12 µg/kg GnRH for a 30 days were significant decreased compared to saline group.

Table 6: Effect of 4, 8 and 12 µg/kg GnRH injection for a 30 days on the Glucose mg / dl, total protein g/dl and Lipids (g/dl) of *Clarias gariepinus.*

Fish	Treatment	Glucose mg / dl	Total protein g/dl	Lipids (g/dl)
Female	Saline	61.4±1.5^a	9.1±1.1^d	8.3 ± 0.1^d
	4 µg /kgGnRH	66.2±1.1^b	7.3±0.1^c	7.4 ± 0.2^c
	8 µg /kgGnRH	74±1.6^c	6. 6±0.8^b	6.7 ± 0.3^b
	12 µg /kgGnRH	78±1.5^d	5.3±0.6^a	5 ± 0.2^a
Male	Saline	71.4±2.5^a	10.2±1.6^d	9.1 ±0.1^d
	4 µg /kgGnRH	88±4.6^b	8.3±0.7^c	7.1 ±0.1^c
	8 µg /kgGnRH	94±3.7^c	6.4±0.4^b	6±0.3^b
	12 µg /kgGnRH	96±2.5^d	5.7±0.8^a	5 ± 0.1^a

Data expressed as means ± SE, means with the same letter in the columns is not significant at p<0.05

DISCUSSION

Fecundity defined as the number of eggs carried by a gravid female fish is a very important aspect of fish culture since it is concerned with the average reproductive characteristics of fish. Findings from this study have showed that fecundity in *Clarias gariepinus* can be influenced by hormone injection. Results of the present investigation clearly indicate that fecundity (number of eggs) of *Clarias gariepinus* injected with 4, 8 and 12 µg/kg GnRH. Equal size of fish in this study was found to have different ovary weight and fecundity. This agrees with Mylonas and Zohar (2001) who reported similar observation on the fecundity of *Mystus bleekeri* from the River Padma near Rajshahi City. The significant variation ($P < 0.05$) observed in the fecundity of injected with 4, 8 and 12 µg/kg GnRH may be due to the increased in steroid hormones. Also, injected with GnRH could be responsible for growth of ovaries and increase in number of eggs found in the ovaries of *Clarias gariepinus* in this study may be due to increase in the FSH and E2. Fecundity and ovary weight of *Clarias gariepinus* were affected by body size in fish injected with GnRH as evident in larger females who have the highest OW and fecundity. Extrinsic factors such as environment and food supply have been reported to affect fish fecundity Le Cren, 1951 and Armando, *et al.,* 2009. Comparing the results obtained in this study with the findings of Sedigheh, *et al.,* 2020 who used the same experimental variations in fecundity of other fish was noticed. In the present study, steroid hormones (FSH, E2 and T) of *Clarias gariepinus* injected with GnRH revealed a linear relationship with the biometric parameters (FBW and OW). This finding accedes with findings of Coccia, *et al.,* 2010 and Fobes 2013 who observed a direct relationship between fecundity and hormone that increase in the of female and male cat fish. Similar results were also reported in different fish Ramy, *et al*., 2014 and Shokr, 2015. In the present study, FSH, E2 and T hormones were found to be increased with the increase in OW which corroborates with findings of Sedigheh, *et al.,* 2020 and Maac, *et al.,* 2020. Based on the findings obtained from this study, it is concluded that fecundity of *Clarias gariepinus* reared in glass aquaria was significantly influenced by the injected with GnRH used in this study as evident in higher fecundity obtained from fish injected with GnRH compared to control one. Therefore, on the bases of affordability and availability to farmers, injected with GnRH is recommended as a cost-effective production of *Clarias gariepinus* female and male fish. The use of cGnRH-II is effective to induce oocyte maturation in African catfish as supported by Szabo *et al.* (2007). However, in this study, GnRH is only effective to induce ovulation and spermaiting in African catfish with a higher dosage which is 12 µg/kg GnRH. In the present study, saline-injected control group did not ovulate in captivity while fish treated with 12 µg/kg GnRH completed ovulation and spermiating within 30 days after hormone administration. The other treated groups (4 and 8 µg/kg GnRH) show 80% ovulation and spermiation. It was also observed in a study by Alok *et al.* (1999) that proved cGnRH-II manage to induce 100% ovulation in Indian catfish *Heteropneusteus fossilis* with 200 µg/kg. cGnRH-II has the most potent GtH releasing activity in fish exogenously (Zohar *et al.,* 1990) although it does not deliver directly by anatomy to the pituitary to induce GtH release (Gothilf *et al.,* 1996). The result clearly showed that the use of GnRH alone is sufficient to induce ovulation and spermiation due to the fact that dopaminergic inhibition plays a minor role in the regulation of ovulatory gonadotropin secretion in African catfish (De Leeuw *et al.,* 1985a &b) as well as other species; coho salmon, *Oncoryhncus kisutch* (Van der Kraak *et al.,* 1986),

Oncorhynchus keta (Park *et al.*, 2007), sea bass, *Dicentrarchus labrax L*. (Prat *et al.*, 2001) and loach, *Paramisgurnus dabryanus* (Lin *et al.*, 1985). In the present study, serum T, FSH and E2 levels in GnRH treated group were relatively higher than those of non treated group. Similar results were also reported in different fish Ramy, *et al*., 2014 and El-Sayed, 2015. In the present study, FSH, E2 and T hormones were found to be increased with the increase in OW which corroborates with findings of Sedigheh, *et al.*, 2020 and Maac, *et al.*, 2020. The comparison of GnRH and control resulted into ovulation in the group treated with GnRH. Therefore, only a smaller amount of this hormone (4 µg/ kg) is required to bring ovulation in African catfish compared to a large amount of dosage needed for GnRH which is 12 µg/kg. Native GnRH peptides were shown to rapidly degraded by peptidase whereby these enzymes cleave GnRH decapeptide at specifically position which consequently getting smaller and inactive fragments (Zohar *et al.*, 1990). The present study revealed that GnRH was able to enhance the potency of spawning induction due to the modification of the native GnRH with dextrorotatory amino acid which not only resistant to enzymatic degradation but also increasing receptor-binding affinity agreement with reported by Habibi and Peter, 1991and Brzuska, 2004. It was observed in the present study that this particular hormone has successfully induced spawning in African catfish. Therefore, the use of GnRH for the improvement of health induction therapies in cat fish is suggested. So, there was a significant increase in the red blood cells, hemoglobin, hematocrit, MCV, MCHC, MCH and white blood cells due to effect of GnRH on cat fish fecundity induction. This study indicated that there was an increase in the number of red blood cells and white blood cells. Also, an increased in the hematocrit value and hemoglobin content under the effect of 4, 8 and 12 µg/kg GnRH. An increase in the number of red blood cells and hematocrit and hemoglobin contents could also have contributed the results that reported by (Zohar *et al.*, 1990 ; ELSayed, 2015) who indicated that effect of hormones on tilapia causes increase in their hematology. The increase in the red blood cells, hematocrit and hemoglobin are agreement with (Sedigheh, *et al.*, 2020 ; Maac, *et al.*, 2020.) who indicated the blood parameters were increased in rainbow trout. Total protein and total lipids in *Calarias gerpineius* was decreased in this study and this may be due to effect of GnRH on the fish to increase synthesis and growing ovary and testis of cat fish. However, the ovulation and spermiation in *Clarias gariepinus* increased due to increase the doses of GnRH. This increase was agreement with the results of Gross and Wood (1988) who reported the total serum protein was increased in the fish that exposed to stress. This study observed that the decrease in the plasma proteins are agreement with (Abdelmegid *et al*. 2002) that reported the liver protein was increased in Tilapia zilii under the effect stress and agreement with Miligan & Wood, 1982 and ELSayed, 2015 who reported that increase of protein in tilapia with effect of hormone injection. This study revealed that the increase in the AST and ALT activities, creatinine, uric acid and plasma glucose level due to stress in the fish by affecting of GnRH. This increases due to the stress of hormone injection on synthesis of protein and lipids to increase the hormone secretion for growing of gonads in *Claries garepinus* which increase ovulation and fecundity. This studies are agreement with the result of (Goss and Wood 1988) who reported the validity of cortisol and glucose as indicators of the stress in the fish. The blood glucose levels have long been used as indicators of stress in fish as recorded by (Hattingh, 1976; Donaldson, 1981 and Wedemeyer & Mcleay 1981) who reported under condition of stress, hyperglycemia may provide additional energy during times of high metabolic need such as" fight or flight" response. This study observed that the increased in the creatinine and glucose level are agreement

with Abdelmegid *et al*. 2002 and Shokr, 2015) who reported that all mean difference total lipids, glucose and creatinine were significant increase and showed hyperglycemia and lipidimia and elevated levels of creatinine in plasma as compared to the control.

CONCLUSION

Hormonal treatment with GnRH through repeated injection was able to induce ovulation and spawning of *Clarias gariepinus* female and male, which usually fail to reproduce in captivity. It can be concluded from this study that disturbance in the RBCs and WBCs, hematocrit value, hemoglobin content, plasma total protein, glucose level, plasma activities of (AST, ALT) and (creatinine and uric acid) concentrations as a result of stress of GnRH injection on *Claries garepinus* female and male reflect the disturbance in all metabolic function. Therefore, multiple injection of hormonal therapies for this hormone is strongly suggested and as a matter of fact, reducing the amount of hormone dosage might be useful when using this method.

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ARABIC SUMMARY

تأثير هرمون الغدد التناسلية على التغيرات الفسيولوجية والهرمونات الإنجابية في اسماك القرموط الافريقي

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تم تقييم آثار هرمون إفراز الغدد التناسلية (GnRH) على معايير التكاثر والتكاثر في اسماك القرموط الافريقي وقد أجريت الدراسة الحالية لتحديد مدى فعالية GnRH في تحفيز النضوج والتبويض في اسماك القرموط الافريقي. تم استخدام الجرعات عند ٤ و٨ و ١٢ ميكروجرام / كيلوجرام من وزن الجسم المذاب في ٠.٦٪ من كلوريد الصوديوم تحت نظام درجات الحرارة الطبيعية. تم تغذية الأسماك بحمية ٣٠٪ من البروتين أثناء التجربة. تم حقن السمك من ٥٥٠ إلى ٦٠٠ جم من وزن الجسم بجرعات من الجونادوتروبين عند ٤ و ٨ و ١٢ ميكروجرام / كيلوجرام من وزن الجسم) ثلاث مرات في الأسبوع، وأظهرت النتائج ان التبويض حدث في غضون ٣٠ يومًا تقريبًا. مستويات١٧ -βاستراديول (E2) ، هرمون تستوستيرون (T) وهرمون محفز مسامي (FSH)كانت مرتفعة عند ٣٠ يوما بعد العلاج ، تسبق البويضات مع أعلى الخصوبة. توضح هذه النتائج أن تناول GnRH يحث على إنتاج عدد من البيض في الأسماك، حيث تزيد بنسبة ١٠٠٪ تقريبًا. وجد زيادة في خلايا الدم الحمراء (RBCs) وخلايا الدم البيضاء (WBCs) وقيمة الهيماتوكريت (Hc) ومحتوى الهيموجلوبين (Hb) على التوالي. أيضا، هناك زيادة في مستوى الجلوكوز في المصل، و نقص في البروتين الكلي و الدهن الكلي . وجد زيادة في الأسبارتات وأنشطة نقل الأمينية ASTو AL وزيادة تركيز الكرياتينين وحمض اليوريك بعد إعطاء .GnRH هذه البيانات تشير إلى أن الأسماك تستجيب للحقن بالهرمون GnRH عن طريق زيادةWBCs ، وأنزيمات وظائف الكبد وظائف الكلى. ووجد أنه فعال للحث على التبويض في اسماك القرموط الافريقي. كما لوحظ الإباضة بنسبة ١٠٠٪ للأسماك التي عولجت بـ .GnRH أيضا، ارتفع مستوى البلازما منE2 ، FSHو T بشكل كبير. تشير هذه الدراسة إلى أن تناول GnRH فعال في النضج الجنسي والتبويض في اسماك القرموط الافريقي .