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# The Effect of Low Level He-Ne Laser on Spawning Behavior in Common Carp Fish *Cyprinus carpio*

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## Abstract

Background: A lot of research were conducted to stimulate spawning of fish by using exogenous hormone and recently some researcher tended to use low level laser in that purpose. Method: A total of 30 sample of female common carp fish *Cyprinus carpio* were exposed to Low level He-Ne laser of 632 nm for (0.1, 0.3, 0.5, 1 and 1.5Joule) only or with half dose of hormone and 20 sample injected with full dose of carp pituitary gland (control positive) only. Result: the spawning percentage were (66.6%, 66.6%, 100%, 33.3% and 66.6%) respective in laser plus hormone treatment and 33.3% in 0.1J only compared with 80% in control positive group. Conclusion: low level laser was safety to use in stimulating spawning of fish and the best result obtained from 0.5J + hormone treatment.

## Keywords

LLL - Laser, Spawning, Carp Fish

## 1. Introduction

Changes in environmental factors like temperature and photoperiod simultaneously with internal signals stimulate the central nervous system to induce the maturation processes, the hypothalamus gland secretes GnRH, that stimulates the release of GtHs from the pituitary gland [7]. Many research aimed to spawn fish by using exogenous hormones. Dopamine antagonists (domperidone, sulpiride, and metoclopramide) induced spawning in mature female of Thai carp when injected alone or combined with Luteinizing hormone-releasing hormones (LHRH) analog Buserelin [19]. LHRH analogue with Haloperidol caused successful ovulation in minimal latency period in common carp fish *Cyprinus carpio* [2]. Hungarian strain 7 carp females stimulated with carp pituitary homogenate, Ovopel or Dagin, Ovopel give the highest weight of eggs and the lowest with carp pituitary homogenate [5]. human chorionic gonadotropin hormone (HCG) induce silver carp *Hypophthalmichthys molitrix* spawning with half dose in male and ovaprim with (HCG) show positive result in male only [18]. Ovaprim and Pregnyl accelerated the maturation of final Oocyte and ovulation percent grass carp *Ctenopharyngodon idellus* [11]. Domperidone is effective in stimulating the spawning in carp fish with different doses of the carp pituitary gland [1]. Some researchers have tended to use low level laser (LLL) in fish spawning, LLL is a light source generates single wave length light, its emits no heat, sound, or vibration, LLL may act via non thermal or photochemical reactions in the cells, Also act as photobiology or biostimulation [20]. Frequency of laser exposure was best for speed up the process of gonadal maturation, increasing the number of Leydig cells, motility and viability of sperm [10]. And low level Helium-Neon (He-Ne) of 5 mW, 632.8 nm can stimulate the release of gonadotropin hormones which affect the acceleration of gonad maturation and spawning [9]. Common carp is one of the most important freshwater fishes in the world, precious food in Asia (native), the Middle East and Europe so valuable in ponds, very adaptable, thrive in mud bottom of shallow lakes [3]. The present study conducted on common carp fish used Helium – Neon laser to stimulate spawning compared with stimulating by used carp pituitary gland hormone.

## 2. Materials and Methods

### 2.1. Subjects

A total of 50 sample of female common carp fish *Cyprinus*

*carpio* were used in this study between 14/3/2015 to 21/4/2015 in two farms (60 and 30 kilometer north Al-Hilla city middle of Iraq) the samples weight 1.25-7 kilogram. The samples were taken from the fish farm and put in aquarium supplied with oxygen aerator 24 hours for acclimation, every sample labeled with different color thread for distinguishes them and then perform the physical measurements, 15 sample treated with laser only and 15 sample treated with laser and half dose of hormone (carp pituitary gland 2mg/kg) and 20 sample treated with full dose of hormone (4 mg/kg).

### 2.2. Laser Treatment

Female fish of 30 samples were exposed for Low level He-Ne laser of 632 nm for (0.1, 0.3, 0.5, 1 and 1.5Joule) according to this equation:

$D = \frac{P*T}{A}$ were D: desired dose in joule (J), P: power in watt (W), T: time in second (S), A: target area in square centimeter ($cm^2$). Each sample were exposed for low level He-Ne laser first exposure and the second exposure after 8 hour.

### 2.3. Hormone Preparation

Four milligram of hormone was used for each kilogram of fish for control positive group and half this dose inject to fish with laser exposure. The dosage divided in two doses (10 and 90%) where the 10% prepared by dissolve 0.4 mg of stock hormone in 0.5 milliliter of normal saline per kilogram of fish, whereas 90% prepared by dissolves 3.6 mg in 0.5 milliliter of normal saline. The prepared hormone injected in muscles especially region below the dorsal fin in zero time and 8 hours for 10 and 90% of dosage respectively.

### 2.4. Spawning

After 12 hour of the second laser exposure and second dose of hormone the egg were collected from each sample by rubbing on the abdominal area (gonad area) toward the anus and collect the egg in container and measure the egg weight in kilogram and numerate the number of egg per gram for each sample.

***Table (1).*** *Spawning response and egg production in common carp fish by using different laser power exposure with hormone, 2mg /kg.*

| Treatment | Responding percentage | Weight average | Egg weight | Ratio of egg weight to mother |
|---|---|---|---|---|
| 0.1J + 2 mg/kg hormone | 66.6% | 3750 gm | 355 gm | 0.095 |
| o.1Jlaser | 33.3% | 2500 gm | 240 gm | 0.096 |
| 0.3J+ 2mg/kg hormone | 66.6% | 2000 gm | 203.5 gm | 0.101 |
| 0.5J + 2mg/kg hormone | 100% | 5833 gm | 468 gm | 0.080 |
| 1J+2mg/kg hormone | 33.3% | 6000 gm | 1150 gm | 0.191 |
| 1.5J +2mg/kg hormone | 66.6% | 3250 gm | 526 gm | 0.162 |
| Control + 4mg/kg hormone | 80% | 6333 gm | 1180 gm | 0.186 |

## 3. Results

The present study showed the percentage of spawning response in laser treatment group and laser with hormone (2mg/kg) group compared with control positive (4 mg/kg) group. Table (1) below show that the highest response 100% was in 0.5J+hormone group and the lowest response 33.3% was in group treated with 1J+hormone and group treated with 0.1J only, higher ratio of egg weigh to mother weight was 0.191 in 1J+hormone group while the lowest ratio was 0.080 in 0.5J+hormone.

The figure below show the spawning response percentage (%) in each group compared with control positive group of 80% response, figure (1) show that 0.1 J+hormone group response was 66.6% and 33.3% in 0.1J laser only, figure (2) and (5) show that 0.3J+hormone and 1.5 J+hormone have the same response 66.6% and in the group of 0.3J and 1.5J have no spawning response 0%, figure (3) show the 100% response in 0.5 J+hormone and no response 0% in 0.5 J only, figure (4) show 33.3% response in 1J +hormone group and 0% in 1J only. Figure (6) show the average of the number of eggs per gram 286 egg/gm in the total treatment responding group (0.1J+H, 0.1J, 0.3J+H, 0.5J+H, 1J+H, 1.5J+H) compared with 208 egg/gm in control positive group.

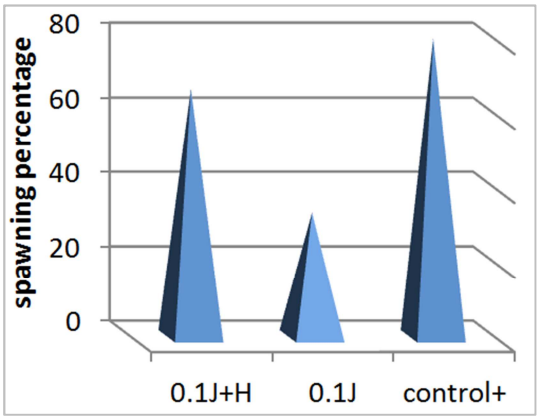


***Figure (1).*** *Percentage of Spawning behavior (%) in common carp fish treated with 0.1j with and without hormone as compared with control positive group.*

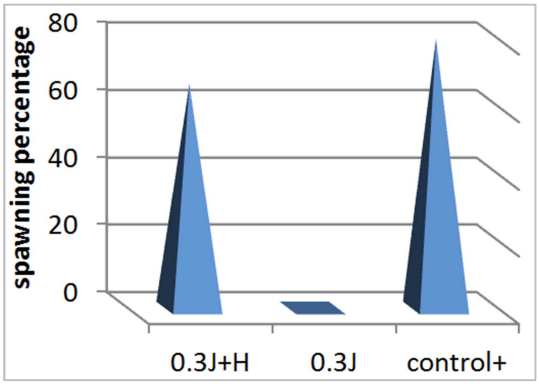


***Figure (2).*** *Percentage of Spawning behavior (%) in common carp fish treated with 0.3j with and without hormone as compared with control positive group.*

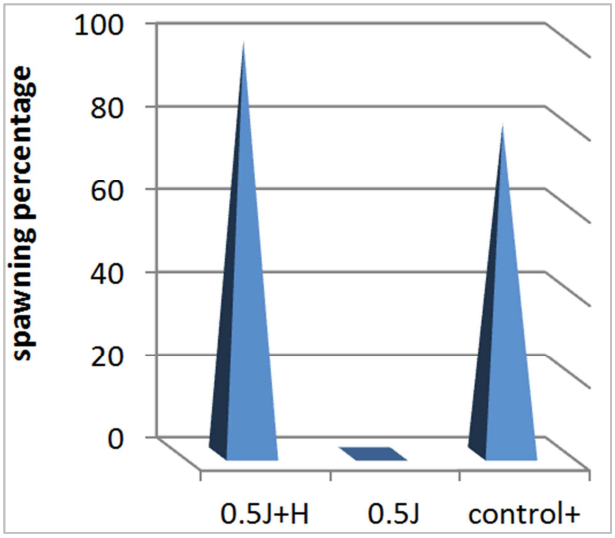


***Figure (3).*** *Percentage of Spawning behavior (%) in common carp fish treated with 0.5j with and without hormone as compared with control positive group.*

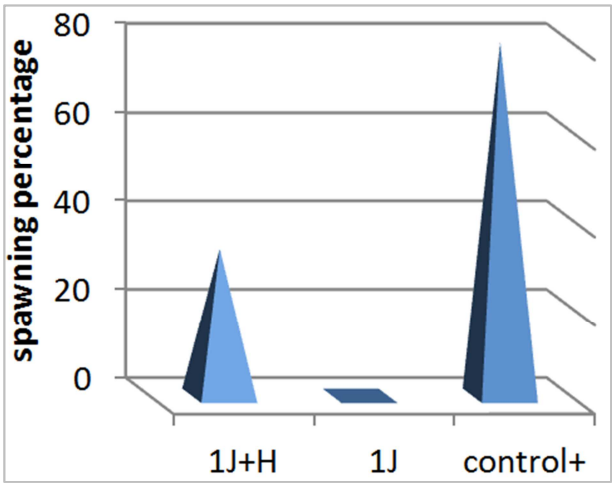


***Figure (4).*** *Percentage of Spawning behavior (%) in common carp fish treated with 1j with and without hormone as compared with control positive group.*

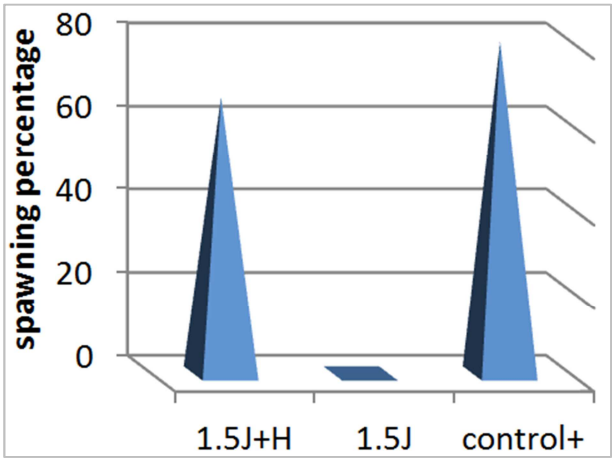


***Figure (5).*** *Percentage of Spawning behavior (%) in common carp fish treated with 1.5j with and without hormone as compared with control positive group.*

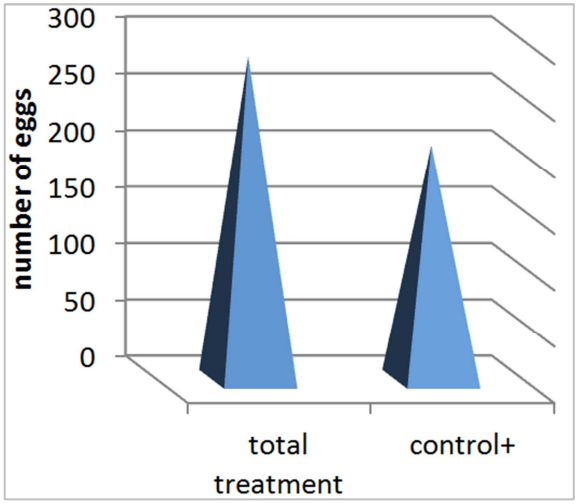


***Figure (6).*** *The number of eggs/gm (average) in total treatment compared with number of eggs /gm in control positive group.*

## 4. Discussion

The present study used low level laser a light source generates single wave length light, act as photobiology or biostimulation. The laser exposure was on the reproductive area (gonad area) can cause gonad maturation [8]; [20].The treatment group of laser induced spawning with the half dose of hormone at different percentage in different doses. The optimal dose was 0.5j+2mg/kg of hormone that responded 100% to treatment, this result is incompatible with [9] may be because of the presence of scale in common carp compared with absence of scale in catfish or the thickness of muscle layer that lead to disperse or more absorbance to the beam of laser retard its penetration to the gonads. Also radiated energy may be reflected off the skin surface, absorbed within, or penetrate through the skin and within the skin, part of the radiated energy is refracted and scattered in random directions [6]. Or difference in metabolic rate according to different temperature, metabolism is primarily modeled as functions of fish mass and water temperature, thermal adaptations would have provided for greater differences in metabolic rate in fish from more disparate latitudes [17]. The environmental conditions of ground water that sample acclimated to it 24 hour before experiments also may influence the action of laser. The gonad maturation and spawning of common carp fish after laser treatment may referred to the stimulation of gonadotropin releasing hormone in hypothalamus gland which stimulate the pituitary gland to release luteinizing hormone which stimulates early phases of gametogenesis and follicle-stimulating hormone that acts through gonadal membrane receptors to stimulate steroidogenesis and gametogenesis [22]. Also, the laser may be stimulate the mediators that are involved in regulation of GnRH synthesis and release, such as glutamate and γ-aminobutyric acid (GABA), dopamine (DA), serotonin neuropeptide Y (NPY), leptin and steroid hormones [7]. GABA has long been implicated as one of the major players in the regulation of GnRH neurons, although GABA is typically an inhibitory neurotransmitter in the mature adult central nervous system,

most mature GnRH neurons show the unusual characteristic of being excited by GABA. While many reports have provided much insight into the contribution of GABA to the activity of GnRH neurons [21]. Dopamine is one of the catecholamine neurotransmitters, and it is the only known factor having an inhibitory effect on LH secretion in the family Cyprinidae [14]. Several neurotransmitter serotonin (5HT) receptor subtypes have recently been identified to be involved in GnRH and LH release, for example subtypes 2C, 4 and 7 have been shown to mediate stimulatory effects of 5HT on GnRH release from the immortalized mouse neuronal cell line GT1–7, Only a 5HT2-like receptor is known to mediate stimulatory effects on LH release in goldfish [15]. Here we are hypothesized the pathway may be the laser exposure may be stimulates the spawning behavior, activation or inhibition, causes by this mediators. From other side, the laser exposure may be stimulates stress related peptides such as cytokines and hormones that are very sensitive to stress. There are some evidence that support an importance of IL-6 and IL-1beta in modulation of HPA axis especially the hypothalamus have IL-6and IL-1 receptor [16]. Also the feedback mechanism of L-6 and cortisol in the hypothalamus where the IL-6 can stimulate arginine vasopressin (AVP) in the hypothalamus affects in ACTH, which stimulates adrenal axis to secrete cortisol. In the ovariectomized ewe, cortisol suppresses pulsatile LH secretion by inhibiting pituitary responsiveness to GnRH rather than by suppressing hypothalamic GnRH release [4]. Other regulator Kisspeptin neurons express the estrogen receptor and the androgen receptor, and these cells are direct targets for the action of gonadal steroids in both male and female animals, kisspeptin signaling in the brain has been implicated in mediating the negative feedback action of sex steroids on gonadotropin secretion [13]. Inhibin have negative feedback effects on gonadotropin reduce the sensitivity to GnRH, negative feedback on FSH secretion reducing FSH output but not LH output selectively in response to GnRH-1, galanin released from the hypothalamus enhances release of LH, that contributing to the LH surge precedes ovulation [12].

## 5. Conclusion

Low level laser was safety to use in stimulating spawning of fish and the best result obtained from 0.5J and half dose of GnRH (2mg/kg) hormone treatment.

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