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ON THE EFFECTS OF THE PITUITARY GLANDS HOMOGENATE (PGH) OF THE NILE CATFISH (*SYNODONTIS SCHALL)* ON THE MATURATION OF THE WISTAR RAT (*RATUS NERVICUS*)

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ABSTRACT

Pituitary glands homogenate of the cat fish ,*S. schall* induced sexual maturation of immature males and females Wistar rat (*Ratus nervicus*. the homogenate administrated intra peritoneal for 6 weeks by 0.26g/ml and0.52g/ml for treated animals and saline solution(4%)for control group. The PGH of *S. schall* resulted in significant effects presented by changes in gonads weights as well as anatomical and histological progression . The concentration levels of sex hormones(testosterone& progesterone)in serum of rats using EISA, DAS EISA plate reader used confirmed the positive effects. However, the effects require specific duration to exposed. these results relieved strong resemblance related to phylogeny relationship of vertebrates.

Keywords: Pituitary glands homogenate, catfish (*S. schall)* , maturation gonads Wistar rat(*R. nervicus).*, levels of sex hormones.

1. INTRODUCTION:

Like higher vertebrates , fish pituitary gland also control directly or indirectly a wide variety of physiological processes by secreting a number of hormones. The most important ones being the gonad stimulating hormones FSH and LH which take part in stimulating the development and maturity of the sexual organs. The gonadotropic hormones produced by the pituitary gland are due to the cyclical changes in its concentration in sexually mature fish. The release of gonadotropins by the pituitary gland is ordered by the hypothalamus through the secretion of gonadotropin-releasing hormone GRH (Harris,1995). Numerous studies with several species had shown that implantation of anterior pituitary tissue from sexually mature females into sexually immature animals confer reproductive function.(Lunenfeld,2004). Interrelationships of gonads and the pituitary were shown very conclusively by classic works of first Smith and Engle(1927). Aschneim and Zondek(1928) are credited with the first experiments regarding reproductive behaviour in female mice and replacement therapy using pituitary implants .The first experiments in which the pituitaries of animals lower in the phylogeny order used as donors for higher animals in that series was done by Creaser and Gorbman 1936).

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The pituitary extracts and/or homogenates has been known for some time has containing growth hormones stimulate growth and increase lactation in animals (Gebelein,1990) .

Extracts with stated gonadotropins activity are available that could be used to standardize the technique. Small-scale farmers typically collect the pituitaries themselves from mature, often unrelated fish, and preserve these either in alcohol or dried, after acetone extraction of fats (Slnha,.1971).

Follicle stimulating hormone (FSH) is naturally produced by the pituitary gland and stimulates the recruitment and development of the ovarian follicles located on the ovaries, each of which contains an egg. The production of FSH and other reproductive hormones is controlled by the complex interaction of several hormones in a biologic feedback system known as the "hypothalamic-pituitary-ovarian" axis. The first FSH commercial gonadotropin, Pergonal, is derived from the urine of post-menopausal women and purified for injection.. Pergonal also contains luteinizing hormone (LH) which produces many effects including higher estrogen levels (Penn,2002). The Practice Committee of the American Society for Reproductive Medicine(CASRM ,2008) reported that modern highly purified urinary and recombinant gonadotropin products have clearly superior quality, specific activity, and performance in comparison to crude extracts."

2. MATERIALS AND METHODS

2.1. Preparation of pituitary glands homogenate (PGH)of fish

Glands were collected from freshly killed mature *S. schall*, and immediately crushed by mortar .,and homogenizer with 4.0% normal saline . Then the suspension was centrifuged in Electro mag. centrifuge Model M19 (serial No:4030808A) at 300 rpm . The doses of pituitary glands homogenates were calculated by consider the number of the pituitaries gland to the volume of normal saline used as medium. Accordingly, two doses were considered: 0.26g/ml and 0.52g/ml beside 4.0% normal saline as control .

2.2. Experimental design

36 Wistar rats (*R. nervicus*) 4 weeks old ; length range 14-17 cm and body weight range 17-23g were randomly selected from lab-bred Wistar rats . Rats acclimatised to lab condition at temperature 25−30℃ for one week, then divided randomly in 3 groups. Two groups were injected for 6 weeks intra peritoneal with PGH of *S. schall* of concentrations of 0.26g/ml, 0.52g/ml, respectively and with normal saline(4%) for control group. The injections repeated at weekly intervals. Random samples were scarified after 3 and 6 weeks. Rats were dissected for examining the gonads maturation indicated by Gonosomatic indices i e. gonads relative weights as well as the accompanied morphological and histological changes. Sex hormones (testosterone and progesterone) concentrations in the blood sera were assessed By EISA method using DAS EISA plate reader . Blood sera were separated from blood collected by cardiac puncture and centrifuged at 1200r/min for five minutes and preserved at 4.0°C. For histological studies ,gonads removed fixed in formal saline(10%) paraffin wax imbedded and H.E. stained.

3. RESULTS:

The results shown that the PGH of *S. schall* was successfully led to maturation of Wistar rats(*R. nervicus*) indicated by significant change in Gonosomatic indices ($P<0.05$) for the two sex. (figures 1a,1b). These changes varies with dose concentrations and durations of time.

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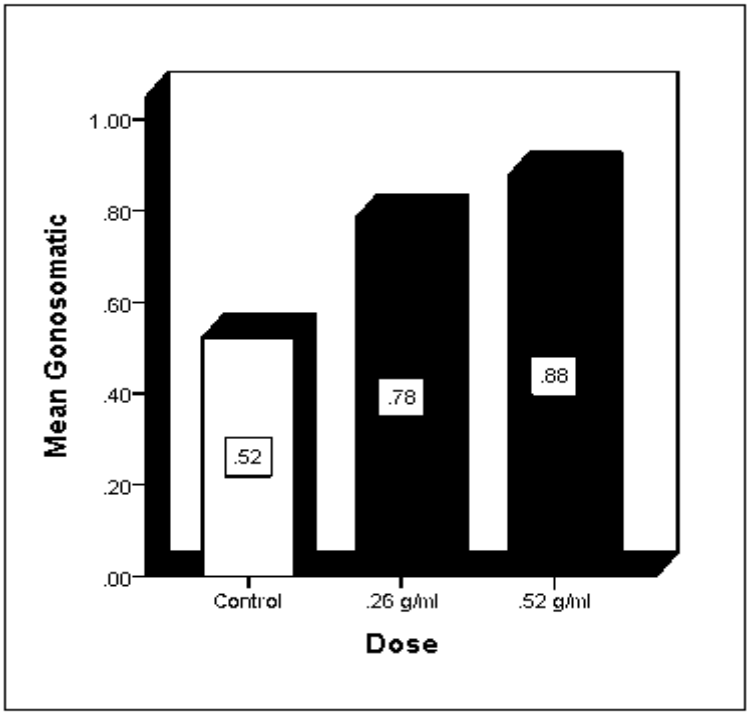
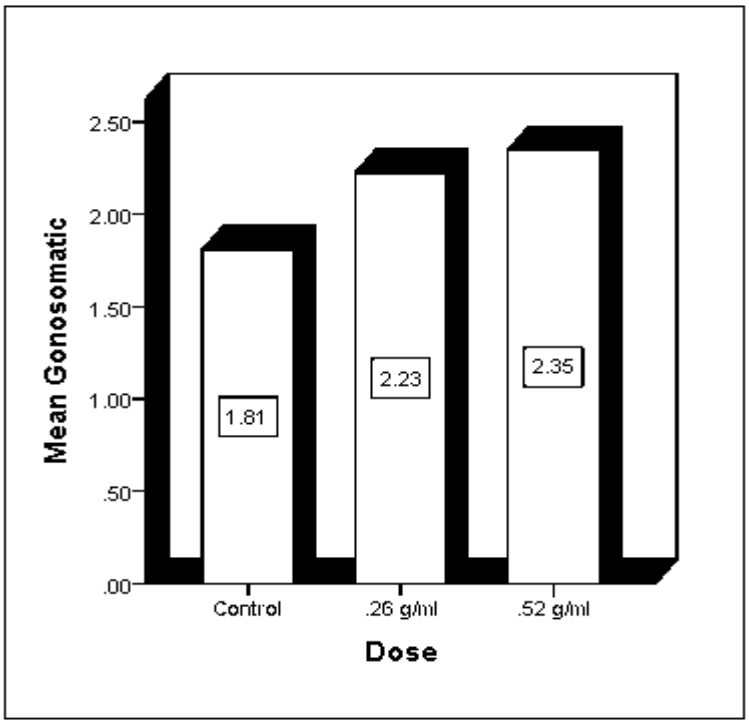


"Fig1a"Gonosomatic indices of *R. nervicus* males injected weekly with various doses of PGH of *S. schall* for 3 weeks.



"Fig1b" Gonosomatic indices of *R. nervicus* females injected weekly with various doses of PGH of *S. schall* for 3 weeks

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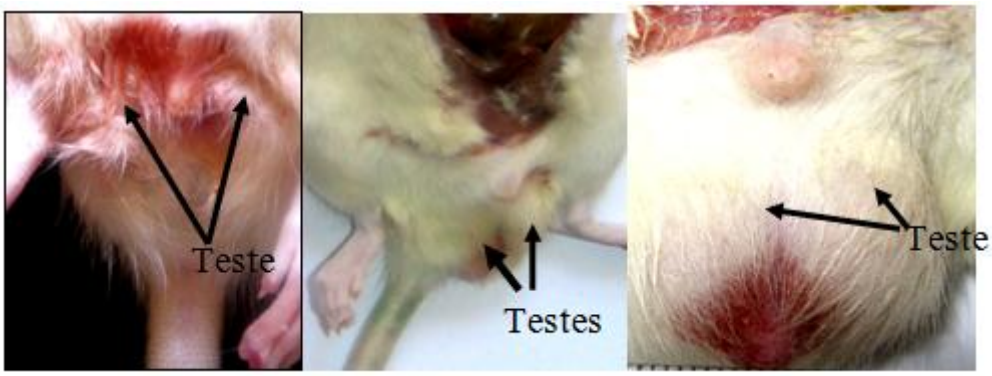
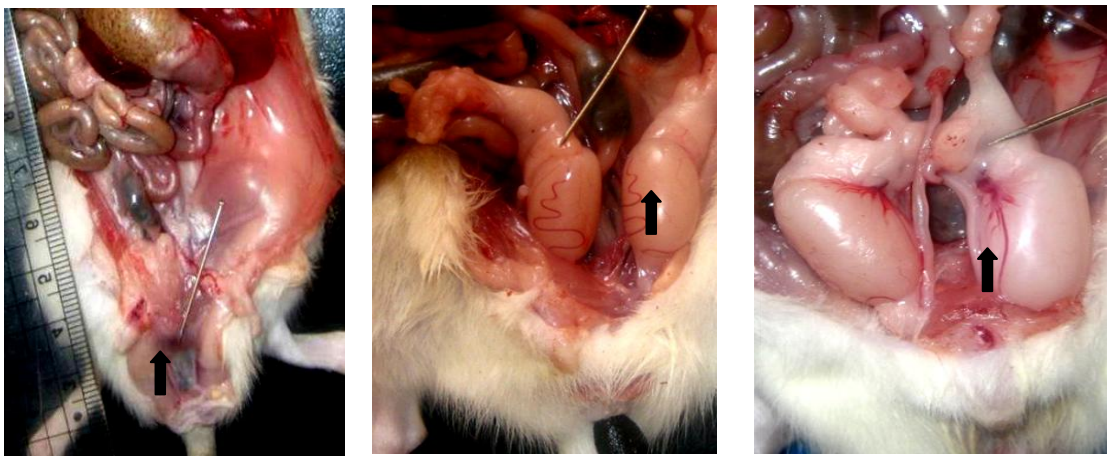
Morphological changes presented by the changes in the secondary sexual characteristics accompanied maturation were shown in plates (1a,b,c) where the descending of testes to scrotal sacs were reviewed.



A B C

"Plate1a"Show the morphological changes due to *R. nervicus* testes injected with PGH of *S. schall.* plate .a. control group for saline solution(4%)dose pate .b. for single dose, plate .c. for double doses for 6 weeks treatment.

Anatomical investigation of maturation of male and female *R. nervicus* exhibited by plates (2a,b,c) and plates (3a,b,c,) ,respectively where there were successive progress of testes growth for males and thickening of uteri walls (endoutrium) and uterus glands differentiation.



A B C

"**Pate2a" Show the anatomical changes due to *R. nervicus* testes treated with PGH of *S. schall.* Plate .a. control group for saline solution(4%)dose, plate .b. for single dose,Plate.c. for double doses for 3 weeks**

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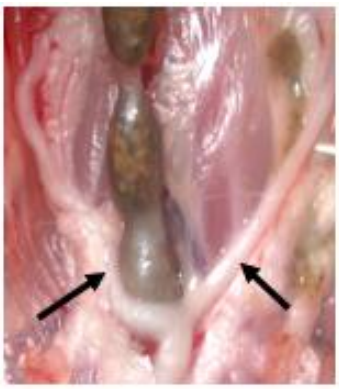
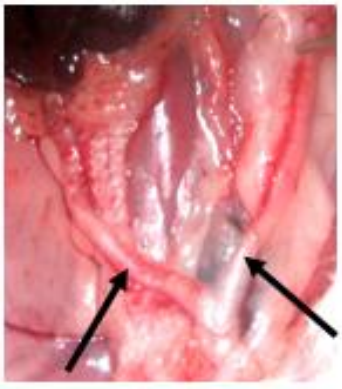
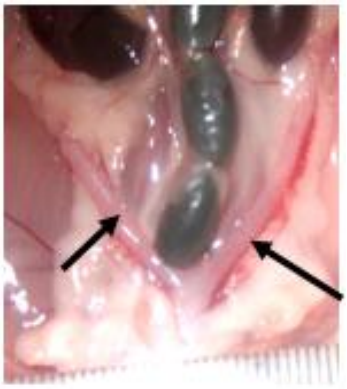
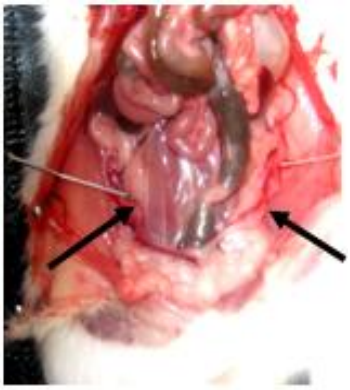
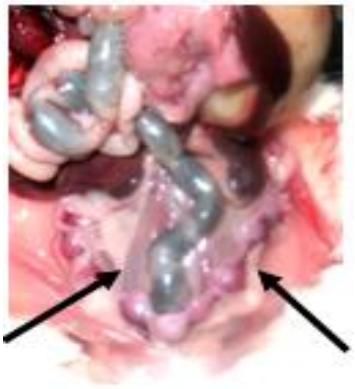
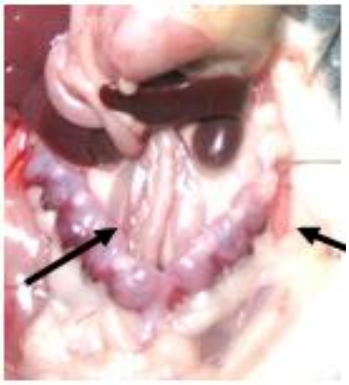




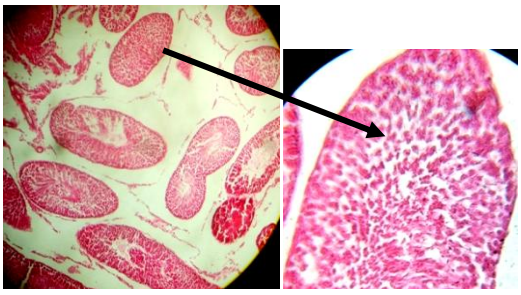
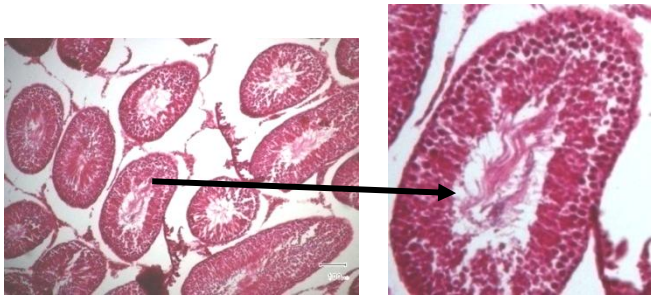
1A 2A 3A

1B 2B 3B

"Plate3a,b" Show the anatomical changes due to *R. nervicus* uteri treated with PGH of *S. schall.* (1. control :normal saline 4% dose, 2: low dose 0.26g/ml,3: high dose 0.52g/ml treatments after 3 weeks and after 6 weeks ..

Histological investigations reveal advanced stages of spermatogenesis of testes of treated male rats with PGH of *S. schall* expressed by the primordial sex cells that developed to spermatid and spermatozoa stages "plate 4"

"Pate4" T.S. of seminferous tubules of *R. nervicus* testes (H&Ex10&x40) showing early(1) in control and late stages (2) of spermatogenesis induced by injection with PGH of *S. schall* for 6 weeks .

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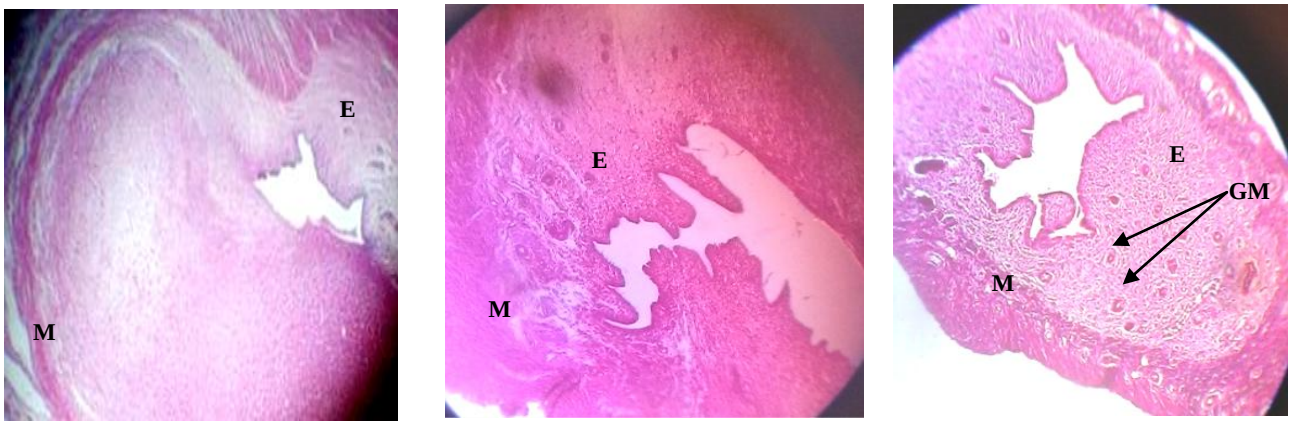
Transverse section for the uteri of control and PGH treated females of *R. nervicus* for 6 weeks exposed endometrium (E)and myometrium(M)) thickness as well as distinct differnation of uteri glands(UG) in treated female rats resulted due to attained pregnancy "Plate 5".



"Plate4" T.S. for the uteri in female *R. nervicus* control: 1 treated with 1 , dose:2, and 2 doses:3 of PGH of *S. schall* after 6 weeks treatment.

The levels of male sex hormones (testosterone) in the sera of rats treated with PGH of *S. schall* in comparison to untreated ones were significantly higher*(p*=0.022*).* Fig.(2a). Both doses Resulted in increase for the first 3 weeks treatment followed by significant drop((P= 0.023) for the 6 weeks treatment. Concentration of testosterone in the sera of rats control for the period 6 weeks was 1.030±0.020, and for low dose was 1.050±0.507,and for high dose was 4.357±4.688 Fig.(2b).

For the females, almost the same results were recorded concerning the concentration of progesterone of rats treated with PGH of *S. schall*. The mean concentration of progesterone after 3 weeks treatment for control was 17.183±1.458 for low dose (0.26g/ml) was 48.33±0.577, and for high dose (0.52g/ml) mean was 54.25±7.583. Fig.(3a). These changes in concentration of sera progesterone of PGH treated significant (P =0.0001) Fig.(3a).After 6 weeks treatment there was a drop where the mean for control was 15.003±1.995, for low dose 29.333±8.387and 42.897±3.383 for the high dose Fig.(3b).

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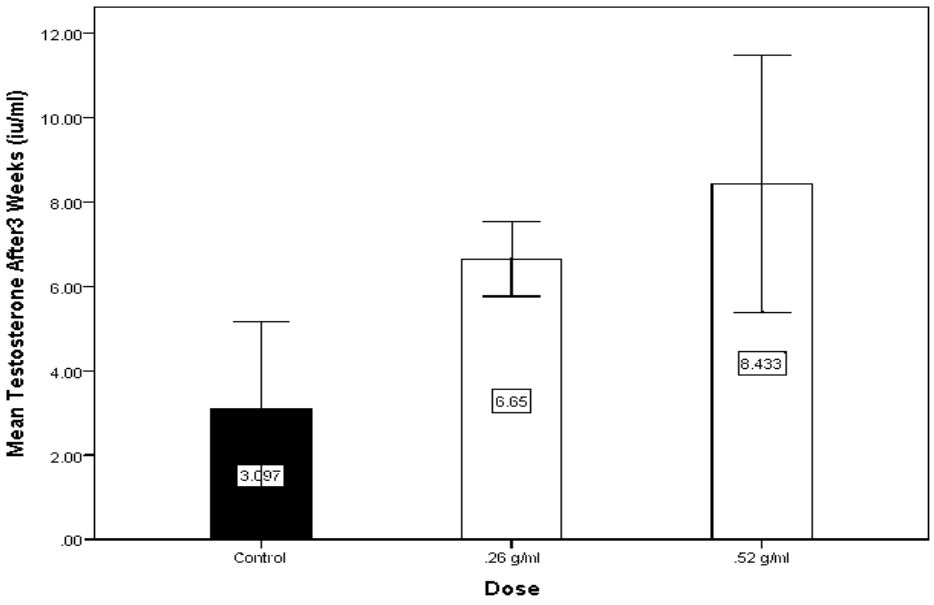
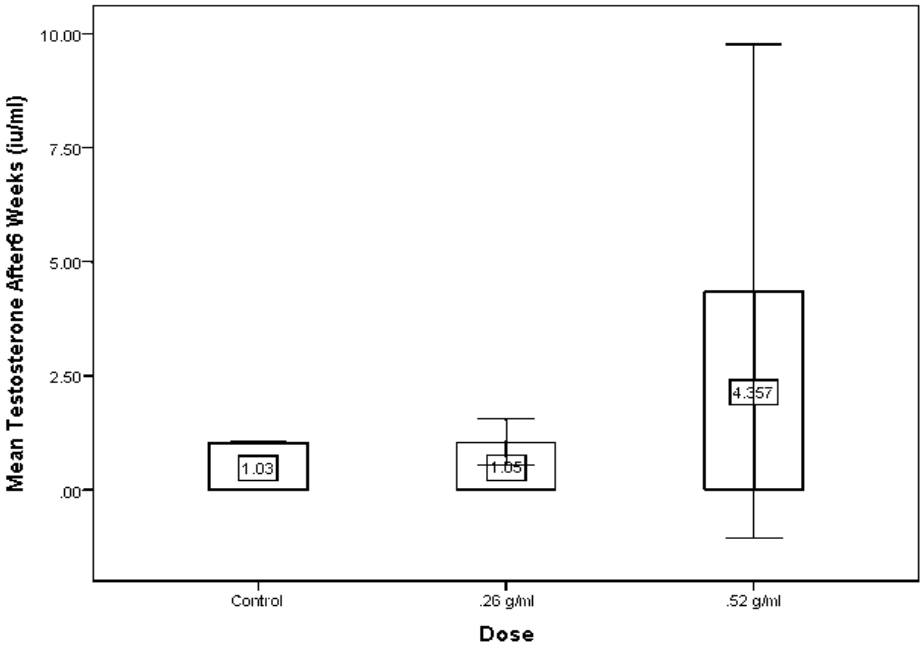


a



b

"Fig2".Show the effect of PGH of *S. schall* on the concentrations of testosterone in the serum of *R. nervicus* due to varies treatment after 3 weeks in Fig.2a. and 6 weeks in fig.2b.

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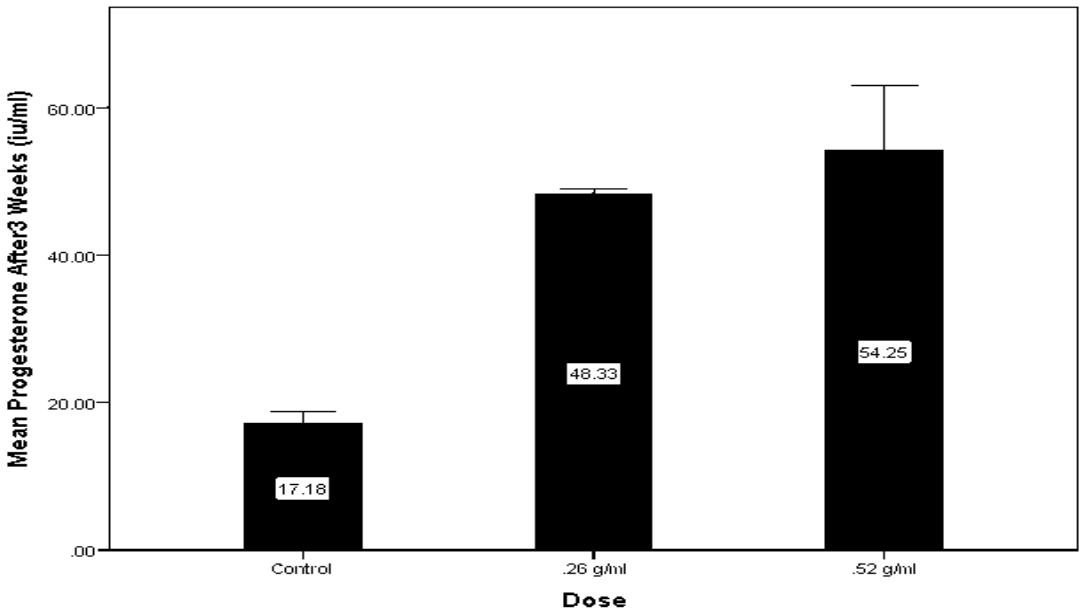

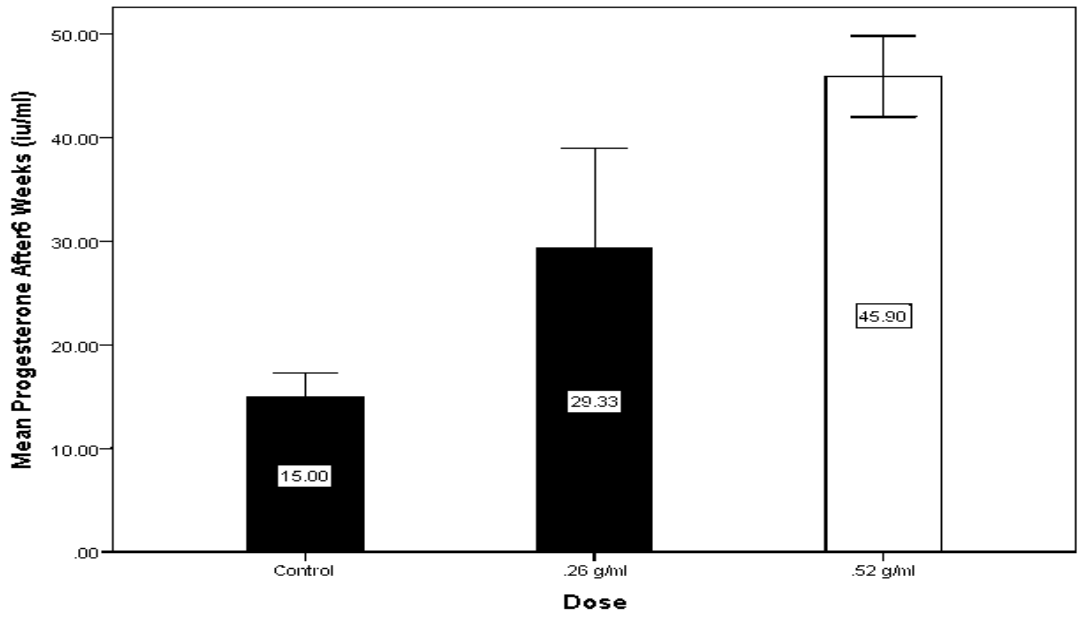

"Fig.3"Show the effect of PGH of *S. schall* on the concentrations of progesterone in the serum of *R. nervicus* due to varies treatment after 3 weeks in fig.3a. and 6 weeks in fig .3b.

4. DISCUSSIONS:

The present investigation reported positive effects of PGH of catfish *S. schall* led to sexual maturity of immature Wistar rats treated for 6 weeks. This Results confirmed by number of studies . Smith and Engle, (1927)noted that daily

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transplants of anterior pituitary tissue from a number of mammals rapidly induced sexual maturity in immature mice. They also reported that female white mice showed uteri and vagina precocious developed after injections beside increase in the size of the ovaries of immature female mice after four or five daily implantation of homoplastic pituitary body. They stated that the usual number of follicles of transitional size between those with small atrium follicle and that of the more advanced follicles has been a accelerated to a mature condition . Bishop *et. al.* (1949) showed an increased of ovri-uteri weight in immature mice after injection with extract fish pituitary. The histological change in tissues of ovary and uteri of females mice Confirmed that result.

Geschwind (1967) has reported that the pituitary of lung fish *Protopterus aethiopicus* showed good maturity promoting activity in the rat. Helen ,*et. el.* (2008)shown that treated mouse for 50 days with recombinant human FSH was significantly increased seminferous tubule volume and testis weight. Hammam, (2010) recorded that the injection of purified FSH of Buffalo increased the number of follicles in ovaries female of Albino mice compared to the control. Sasaki *et. el*.(2000) noted that the FSH was promoting sertoli cells division when injected into immature rats. Nuti *et. al.* (1973) reported that subcutaneously injection of purified ovine FSH and LH effect progesterone concentration of immature rats from 2.5ng/ml prior to ovulation to 6.6 and 8.3ng/ml in FSH-and LSH ,respectively.

concentration of testosterone was varies with the duration of treatment as observed by Martijn (1979) when administered immature rabbit with LH stimulated hormones increased to 6% after 25 days then to 3% after 180 days measured by radioimmunoassay .Similar findings were observed in the present study where there was rice of testosterone concentration after 3 weeks treatment followed by a drop after rats reached maturation.

Purvis *et. al.* (1974) demonstrated that intravenous injection of human chorionic gonadotropin resulted in an increase peripheral concentration of plasma testosterone content of the testes 5-1 min after injection.

5. ACKNOWLEDGEMENTS:

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