Asian Pacific Journal of Reproduction

Effects of *Lepidium sativum* supplementation on growth and gonadotropins secretion in ovariectomized, estrogen−implanted rabbits

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ARTICLE INFO

ABSTRACT

Objective: To test the effects of dietary supplementation of *Lepidium sativum* (LS) seed powder on growth performance and gonadotropins secretion in ovariectomized, estradiol-implanted rabbits. **Methods:** Ovariectomized, estradiol-implanted Chinchilla rabbits were assigned into four experimental groups: LS seed powder was included into normal rabbit chow at 0% (control), 5% (low), 7% (mid) and 10% (high) w/w. Experimental feed and water were given *ad−libitum* for 3 weeks. Weekly body weights and daily feed intake of rabbits were recorded. Twenty-one days post-feeding, blood samples were collected at 15-minute interval for 3 h (Period Ⅰ) after which 2.5 μg gonadotropin-releasing hormone (GnRH) was injected intravenously and the sampling continued for another hour (Period Ⅱ). Plasma was harvested and analyzed for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by radioimmunoassay. **Results:** Feed intake was significantly ($P<0.05$) increased in LS-supplemented rabbits. However, the increase in feed intake did not result in significant body weight gain. LS seed supplementation significantly ($P<0.001$) increased mean plasma LH dose-dependently from the low- to the mid-LS level and then decreased LH at the high-LS level. LS supplementation increased ($P<0.001$) plasma FSH secretion. Injection of GnRH had no effect on plasma LH, however significantly ($P<0.05$) decreased overall plasma FSH secretion. **Conclusions:** LS seed supplementation stimulates feed intake and gonadotropins secretion in rabbits. Gonadotropins effect may be mediated through LS seeds phytosterols through the activation of estrogen receptors thereby producing agonistic effects resulting in LH and FSH secretion. The differential responses of gonadotropins to GnRH in LS-supplemented rabbits suggest differential regulation of the synthesis and secretion of these gonadotropins.

1. Introduction

Traditional uses of medicinal plants and their associated properties have been documented by several authors. One of the medicinal plants that has been reported to have multi-system effects and also possess important biological activities on reproductive characteristics is *Lepidium sativum* (LS)[1–3]. LS, also known as garden cress, has been recognized to possess properties such as abortifacient[4], aphrodisiac[5], teratogenic[6] antifertility[7], antiovulatory[8] and

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lactogenic effects[9,10]. This plant reportedly increased sexual stamina and sexual retentivity in both human males and females[4].

Recently, there has been an increased interest in unravelling the mechanism of action of LS on reproduction. Available evidence indicates a divergent effect of LS on reproduction. Although proceptive and receptive activities of the seed were reported in female rat[11], mammogenic and lactogenic activities in female rat[10] and in ewe[12], contraceptive effects have also been reported in female mice[3,12,13]. Further, administration of tocopherol extract increased epididymal sperm concentration, sperm count, sperm motility, grade activity, sperm viability and decreased abnormal sperm morphology percent in rabbit[14,15]. Therefore, the effect of LS on reproduction may depend on the organism, the physiological state and the mode of administration of the seed or the extract.

Reproduction in all mammals is controlled by the hypothalamic-pituitary gonadal axis through which gonadotropin-releasing hormone (GnRH) from the hypothalamus regulates the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. These gonadotropins in turn regulate gonadal gametogenesis and the secretion of gonadal hormones. To date, there is a dearth of information on the effects of LS on the synthesis and secretion of gonadotropins and specifically on its pulsatility. Therefore, this study was carried out to determine the effects of LS seed supplementation on growth performance and gonadotropins secretion in ovariectomized, estradiol-implanted (OVX+E$_2$) rabbits.

2. Materials and methods

2.1. Plant material and preparation

LS (garden cress) seeds were purchased from a local market in Gombe, Nigeria and confirmed in the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria. The seeds were ground into fine powder using electric mill and kept in air-tight container until required for use.

2.2. Experimental animals

The research was carried out at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Ethical approval (COLANIM/ANP; April 22, 2015) for this study was obtained from the Research Ethics Committee, Federal University of Agriculture, Abeokuta, Ogun State. Twenty primiparous Chinchilla rabbits [mean weight: (2.10 ± 0.15) kg] were used in this study. All rabbits were bilaterally ovariectomized under ketamine-xylazine and diazepam anesthesia and then received subcutaneous estradiol implants at the neck region. The animals were housed individually in a wooden cage where feed and fresh water were given *ad–libitum*. The house was naturally ventilated and maintained under clean environmental condition.

2.3. Experimental design and blood sampling

Twenty-one days post ovariectomy, the animals were randomly assigned to receive either normal rabbit chow (control; *n* = 5) or rabbit chow supplemented weight for weight with LS seed powder at either 5% (low; *n* = 5), 7% (mid; *n* = 5) or 10% (high; *n* = 5). All animals were fed the control or experimental diet. Daily feed intake and weekly live body weight were recorded. Blood samples were collected after 21 days of feeding through an indwelling marginal ear vein catheter[16] every 15 min for 3 h (Period Ⅰ) after which GnRH (2.5 μg/animal) was injected intravenously and then sampling continued for 60 min at the same frequency (Period Ⅱ). After each sampling, the blood was replaced by an equal volume of heparinized saline solution (10 U heparin/mL). Blood samples collected were centrifuged at 3 000 rpm in refrigerated centrifuge (4 ℃) to obtain plasma. Plasma was stored at -20 ℃ until hormonal assay was done.

2.4. Radioimmunoassay of LH

LH concentration was determined using techniques described by Bernard *et al*[17]. The LH used for the standards and iodination reaction was obtained from Sigma Aldrich Chemicals, St. Louis, Missouri, USA. The antiserum AFP C5288113 (obtained from Dr. A. F. Parlow) was used at 1: 35 000 dilution as the first antibody and Goat Anti-Rabbit Immunoglobulin G (GARGG, EQUITECH-BIO INC, Kerrville, Texas, USA) was used as the second antibody.

Briefly, on day 1, standards, antibody (100 μL) and tracer ^{125}I-LH (18 000-20 000 cpm/100 μL) were pipetted into 12 mm × 75 mm glass tubes (VWR International, Pennsylvania, USA) containing 150 μL of blood plasma and incubated at 4 ℃ for 48 h. Thereafter, 100 μL of GARGG diluted with phosphate buffer saline-ethylene diamine tetraacetic acid and 500 μL 6% polyethylene glycol were added. Tubes were centrifuged at 3 000 rpm at 4 ℃ for 15 min. The supernatant was discarded. The tubes containing pellets were loaded and counted in a Packard Cobra Ⅱ Gamma counter (Perkin Elmer, Waltham, MA, USA). The minimum LH detected by assay was 0.4 ng/mL, and average intra- and interassay coefficients of variation were 5.1% and 7.4%, respectively.

2.5. Radioimmunoassay of FSH

Plasma FSH concentration was determined using a modified heterologous radioimmunoassay[18] in duplicates of 50 μL volume in a 5-day assay. The FSH used for the standards and iodination reaction was obtained from Sigma Aldrich Chemicals, St. Louis, Missouri, USA. The iodinated FSH (NIDDK-oFSH-19-SIAFP, AFP 4117A) and the first antibody (NIDDK-anti-oFSH-1, AFP-C5288113) were obtained from Dr. A.F. Parlow, and Goat anti-rabbit Gamma Globulin (GARGG, EQUITECH-BIO INC, Kerrville,

Texas, USA), the second antibody, were used in the assay.

Briefly, on day 1, 50 μL plasma and antibody (100 μL of 1:15 000 AFP-C5288113) were pipetted into 12 mm 75 mm glass tubes (VWR International, Pennsylvania, USA) and incubated for 24 h at 4 ℃. Then, 125I-oFSH (18 000-2 0000 cpm/100 μL) was added on day 2 and incubated at 4 ℃ for another 24 h. Thereafter, 200 μL of the second antibody, GARGG was added and tubes were incubated for 48 h at the same temperature. Then, 3 mL of 0.01M phosphate-buffered saline was added and tubes were centrifuged at 3 600 rpm at 4 ℃ for 1 h. The assay detection limit was 0.125 ng/mL and intraassay coefficient of variation was 4.2%.

2.6. Statistical analysis

Data were statistically analyzed using the general liner model program of SAS[19]. Multiple comparisons were done with Tukey's test. The main effects of treatments on plasma LH, FSH concentrations, feed intake and body weight gain were determined. Differences were considered significant at $P<0.05$. All the data were reported in Mean ± standard error (Mean±SE).

3. Results

3.1. Effects of LS supplementation on growth performance in OVX+E$_2$ rabbits

Average daily feed intake was significantly higher ($P<0.001$) in LS-supplemented ovariectomized rabbits compared to normal rabbit chow-fed controls. However, changes in body weight between LS-supplemented and control rabbits were similar, although there was a trend toward an increase in the earlier group (Figure 1).
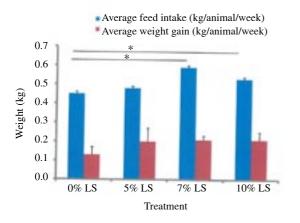

Figure 1. Effects of LS seed supplementation on growth performance indices in OVX+E$_2$ rabbits.
Animals were fed either normal rabbit diet or LS seed supplemented diet for 21 days. Data are Mean ± SE; Bars with asterisks are significantly different; LS: *Lepidium sativum*.

3.2. Effects of LS supplementation on LH and FSH secretion in OVX+E$_2$ rabbits

Figure 2 showed the plasma LH levels in control and LS-supplemented ovariectomized rabbits obtained during a 4-hour frequent blood sampling period after 21 days of normal rabbit chow or LS seed powder supplementation.
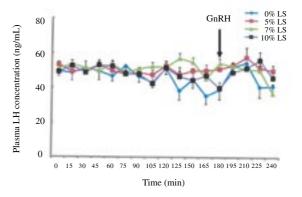

Figure 2. Plasma LH concentrations in OVX+E$_2$ rabbits fed normal rabbit diet or LS seed-supplemented diet for 21 days.
Blood samples were obtained at 15-interval for 3 h before 2.5 μg GnRH injection (*i.v.*) and sampling for 1 h at the same frequency; LH: luteinizing hormone; LS: *Lepidium sativum*.

Figure 3 showed the mean plasma LH levels during the entire 4-hour window sampling period. The window sampling period consisted of a 3-hour baseline component followed by a 1-hour post-GnRH injection sampling component.
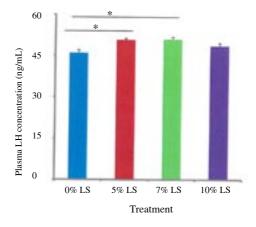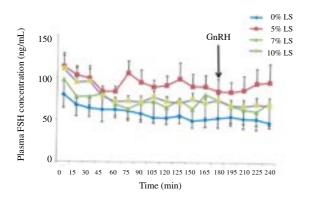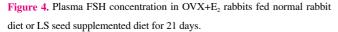

Figure 3. Main effects of LS seed supplementation on plasma LH concentrations in OVX+E$_2$ rabbits.
Data showed Mean ± SE; Bars with asterisks are significantly different; LH: luteinizing hormone; LS: *Lepidium sativum*.

Corresponding values for plasma FSH were shown in Figures 4 and 5 respectively. Both basal plasma LH and FSH levels were very high in the rabbits (Figures 2 and 4). Pulsatile LH secretion tended to be increased by LS seed supplementation (Figure 2 and Table 1) and overall, LS seed supplementation significantly ($P<0.001$) increased mean plasma LH concentration dose-dependently, increasing from low- to mid-LS supplementation and then decreasing at the highest level of LS supplementation. Basal pulsatile plasma FSH secretion was significantly increased ($P<0.001$) by LS supplementation and this increase was dose-dependent (Table 1).

The study results showed that there was a tendency for LS seed supplementation to affect the basal temporal secretion of LH in Period Ⅰ (before GnRH injection) and to significantly affect mean levels of LH during our 4-hour sampling period. With respect to FSH, baseline FSH concentrations were significantly affected by LS seed supplementation during Period Ⅰ and this effect was dose-dependent, with the lowest LS supplementation having the most effect.

Figure 5. Main effects of LS supplementation on plasma FSH concentrations in OVX+E$_2$ rabbits.

Data showed mean ± SE; Bars with asterisks are significantly different; FSH: follicle-stimulating hormone; LS: *Lepidium sativum*.

Figure 4. Plasma FSH concentration in OVX+E$_2$ rabbits fed normal rabbit diet or LS seed supplemented diet for 21 days.

Blood samples were obtained at 15-interval for 3 h before 2.5 μg GnRH injection (*i.v.*) and sampling for 1 h at the same frequency; FSH: follicle-stimulating hormone; LS: *Lepidium sativum*.
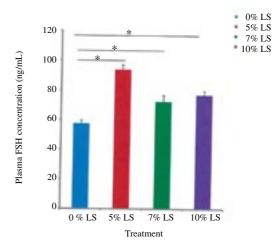
3.3. Effects of GnRH injection in OVX +E$_2$ rabbits fed LS supplemented diet

Table 1 presented the effects of GnRH injection on LH and FSH secretion in LS-supplemented OVX+E$_2$ rabbits. GnRH administration had no effect on plasma LH secretion but significantly decreased ($P<0.05$) overall plasma FSH levels in comparison to pre-GnRH injection levels. Further, there was a tendency ($P=0.053$) for the plasma LH concentration to be dependent on the interaction between LS supplementation and GnRH stimulation.

4. Discussion

Generally, administration and implantation of estrogen have been reported to reduce gonadotropins in ovariectomized rats[20,21], rabbits[22] and monkey[23] by exerting a negative feedback effects on the LH and FSH secretion[24]. In the current study, LS supplementation increased plasma LH and FSH secretions in OVX+E$_2$ rabbits in a

Table 1

Effect of graded doses of LS seed supplementation and GnRH injection on LH and FSH secretion in OVX+E$_2$ rabbits.

Groups	LH (ng/mL)		FSH (ng/mL)	
	Period Ⅰ	Period Ⅱ	Period Ⅰ	Period Ⅱ
0% LS	45.7 ± 1.21	47.1 ± 2.55	59.1 ± 2.86[a]	51.7 ± 3.03
5% LS	50.0 ± 0.48	53.1 ± 1.55	94.6 ± 3.40[c]	90.8 ± 8.06
7% LS	51.9 ± 0.84	47.7 ± 2.25	74.3 ± 4.55[b]	65.9 ± 10.86
10% LS	47.9 ± 1.05	51.0 ± 2.47	79.6 ± 2.98[b]	68.3 ± 2.37

Data represents Means ± SE. Period Ⅰ is 3 h before GnRH injection. Period Ⅱ is 1 h after GnRH injection.

[a,b,c] Means within a column with different superscripts are significantly different ($P < 0.000\ 1$).

dose dependent manner. Stimulation of LH and FSH may be ascribed to phytosterol constituent of LS, which has estrogenic activity. The effect could be through temporary or permanent alteration of the feedback loop in the hypothalamus, pituitary and the gonad by mimicking the effects of endogenous estrogen which then trigger their specific receptors, thereby resulting in stimulation of FSH and LH secretion. Phytosterol has been reported to increase basal gonadotropins secretion in immature male and female rabbits[25]. In addition, plasma estrogen levels in the OVX+E_2 rabbits were very high which was undoubtedly due to estradiol implantation. This report is in agreement with the results from a previous study[10] where the increases in LH, prolactin, estrogen and progesterone were observed in young adult virgin rats fed with LS-supplemented diet for only 14 days. However, reduction in LH and FSH levels at high level of LS supplementation may be as a result of adenohypophysial negative feedback of the estrogenic action of phytosterol, which induces a decrease or loss of progressive sensitivity or the affinity for estrogen receptors binding and then causes subsequent LH and FSH inhibition.

In the OVX+E_2 rabbits, pre-GnRH injection levels of LH and FSH were high and suppression of only FSH was observed following GnRH challenge. Although previous studies indicate importance of species differences in the sensitivity of the gonadotropins to GnRH, the dose of GnRH used in this study, much to our chagrin, did not stimulate further increase in the secretion of gonadotropins. This result indicates that LS-supplemented estrogen-primed pituitary did not respond to GnRH stimulation. The lack of pituitary response may be due to the high level of baseline LH level and therefore a depletion of releasable gonadotropins. Besides, the high level of estrogen observed with estrogen implantation may reduce the responsiveness of the gonadotropins to GnRH stimulation. In our hands, plasma estradiol-17 β level in implanted rabbits was similar to the level observed in pregnant rabbits. Thus, the sub-maximal gonadotropins response to GnRH in this study may be due to the high negative estrogen feedback or a diminished anterior pituitary responsiveness to GnRH. The level of estrogen measured in OVX+E_2 New Zealand white rabbits is of many magnitudes lower than the value measured in this study with OVX+E_2 Chinchilla breed. Thus, there may be a breed effect observed in this study. Most rabbit experiments have used the New Zealand breed of rabbit[21,26]. Further, the dose of GnRH injected may be subthreshold; the dose used was based on a previous study[27] in which the Fauve de Bourgogue strain was used. In contrast, the Chinchilla breed was used in the present study.

The differential response of LH and FSH to GnRH stimulation in this study confirms that the synthesis and release of the gonadotropins are differentially regulated. This difference must relate to the differences in the GnRH-induced responses in LH- β and FSH- β genes. It has been suggested that this may reflect differences in the frequency of GnRH stimulation. Many studies have indicated that LH- β gene promoter activity is stimulated to a greater extent at higher GnRH pulse frequency[28–30]. These higher frequencies of GnRH increase GnRH receptor number which reportedly preceded the differential regulation of LH- β and FSH- β genes.

Significant increase in feed intake in OVX+E_2 rabbits may indicate the positive influence of LS on the appetite of the rabbits and nutrient digestion. The lack of difference in weight gain between control- and LS-supplemented rabbits suggests that LS seed may contain anti-nutritional factors which hampered the bioavailability and utilization of the nutrients. It has been previously reported[31] that whole LS seed flour contains tannins, phytic acids, oxalic acid and cyanogens which might obstruct the bioavailability of nutrients. This result is in agreement with previous findings[32,33]. Whereas weight decreasing effect of LS supplementation in rats was reported[34], other studies in poultry[35,36] have found positive effect of LS on weight gain. It will appear that the effect of LS on weight gain may be dependent on the animal model, the dosage, fractions and method of LS administrations.

In conclusion, these findings suggest that biological activities of LS and mechanism underlying its effects on reproduction may be mediated through multiple pathways which depend greatly on the amount of LS ingested. Increases in feed intake without observable increase in body gain deserve further investigation.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

References

[1] Raval N. A comprehensive review of *Lepidium sativum* Linn, a traditional medicinal plant. *World J Pharm Pharm Sci* 2016; **5**: 1593-1601.

[2] Doke S, Guha M. Garden cress (*Lepidium sativum* L.) seed–An important medicinal source: A review. *J Nat Prod Plant Resource* 2014; **4**: 69-80.

[3] Sharma M, Agarwal N. Nourishing and healing prowess of garden cress (*Lepidium sativum* Linn): A review. *Indian J Nat Prod Resources* 2011; **2**: 292-297.

[4] Jabeen A, Rani S, Ibrahim M, Mohammad A. A review on *Lepidium sativum*. *Indo Am J P Sci* 2017; **4**(8): 2223-2227.

[5] Kirtikar KR, Basu BD. *Indian medicinal plants.* Allahabad, Uttar Pradesh,

India: Popular Prakashan Pvt. Ltd; 2006, p. 174-175.

[6] Lather A, Valecha R, Sharma K, Garg M. World wide potential of plants causing teratogenicity- An overview. *Spatula DD* 2011; **1**(2): 101-106.

[7] Falana H, Nofal W, Nakhle H. *Lepidium sativum* (garden cress). A review article. 2014. [Online] Available from: https://www.researchgate.net/publication/262914046.

[8] Al-khazraji SM. Biopharmacological studies of the aqueous extract of *Lepidium sativum* seeds in alloxan-induced diabetes in rats. Proceeding of the Eleventh Veterinary Scientific Conference 2012; p. 158-163.

[9] Kumar S, Baghel R, Khare A. Effect of chandrasoor (*Lepidium sativum*) supplementation on dry matter intake, bodyweight and milk yield in lactating Murrah buffaloes. *Buffalo Bulletin* 2011; **30**: 262-266.

[10]Al-Yawer MA, Al-Khateeb HM, Al-Khafaji FA. Garden cress seed could be a factual galactagogue. *Iraqi Postgrad Med J* 2006; **5**(1): 62-67.

[11]Kagathara VG, Shah KK and Anand IS. Effect of methanolic extract of seeds of *Lepidium sativum* Linn. on proceptive and receptive behaviors of female rats. *Int J Pharm Pharm Res* 2015; **4**(1): 101-112.

[12]Alshawabkeh K, Herzallah SM, Al-Fataftah AA. Supplemental effect of plant extracts of *Lepidium sativum* and Brassica juncae seeds on milk production and composition of Awassi ewes. *Jordan J Agric Sci* 2013; **9**(3): 363-368.

[13]Sharief M, Gani ZH. Garden cress (*Lepidium sativum*) seeds as oral contraceptive plant in mice. *Saudi Med J* 2004; **25**: 965-966.

[14]Naji NS, Abood FN. Effect of tocopherol extraction of *Lepidium sativum* seeds in sperm parameters of white male rabbits. *J Biol Agric Healthc* 2013; **3**(8): 43-48.

[15]Naji NS, Shumran FM. The Effects of tocopherol extraction from *Lepidium sativum* seeds on the histology of testis, epididymis, and seminal vesicles of adult male rabbits. *J Biol Agric Healthc* 2013; **3**(6): 97-100.

[16]Marongiu ML, Gulinati A. Ultrasound evaluation of ovarian follicular dynamics during early pseudo pregnancy as a tool to inquire into the high progesterone (P4) syndrome of rabbit does. Proceedings of the 9th World Rabbit Congress Verona, Italy; 2008, p. 393-397.

[17]Bernard C, Valet JP, Beland R, Lambett RD. Prediction of bovine ovulation by a rapid radioimmunoassay for plasma LH. *J Reprod Fertil* 1983; **68**: 425-430.

[18]Bolt DJ, Rollins R. Development and application of a radioimmunoassay for bovine follicle-stimulating hormone. *J Anim Sci* 1983; **56**: 146-154.

[19]SAS Institute. *SAS/STAT(R) 14.1 User's guide*. Cary, North Carolina, USA: SAS Institute Inc.; 2015.

[20]Hwang C, Pu HF, Hwang CY, Liu JY, Yao HC, Tung Y-F, et al. Age-related differences in the release of luteinizing hormone and gonadotropin-releasing hormone in ovariectomized rats. *Neuroendocrinology* 1990; **52**: 127-132.

[21]Dufy-Barbe L, Dufy B, Vincent JD. Serum gonadotropin levels in the ovariectomized rabbit: Effect of acute and chronic administration of estradiol. *Biol Reprod* 1978; **18**: 118-124.

[22]Parillo F, Zerani M, Maranesi M, Dall'Aglio C, Galeati G, Brecchia G,

et al. Ovarian hormones and fasting differentially regulated pituitary receptors for estrogen and gonadotropin-releasing hormones in rabbit female. *Microsc Res Tech* 2013; **77**(3): 201-210.

[23]Chongthammakun S, Terasawa E. Negative feedback effects of estrogen on luteinizing hormone-releasing hormone release occur in pubertal, but not prepubertal, ovariectomized female rhesus monkeys. *Endocrinology* 1993; **132**: 735-743.

[24]Shaw ND, Histed SN, Srouji SS, Yang J, Lee H, Hall JE. Estrogen negative feedback on gonadotropin secretion: evidence for a direct pituitary effect in women. *J Clin Endocrinol Meta* 2010; **95**(4): 1955-1961.

[25]Register B, Bethel MA, Thompson N, Walmer D, Blohm P, Ayyash L, et al. The effect of neonatal exposure to diethylstilbestrol, coumesterol and β -sitosterol on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. Proceedings of the Society for Experimental Biology and Medicine; 1995, p. 72-77.

[26]Yang F, Hu K, Wang X, Liu Z, Hu Q, Li J, et al. Combination of raloxifene, aspirin and estrogen as novel paradigm of hormone replacement therapy in rabbit model of menopause. *Acta Pharmacol Sin* 2011; **32**(8): 1031-1037.

[27]Dufy-Barbe P, Franchimont B, Faure JMA. Effects of ovariectomy and estradiol benzoate treatment on LH-RH induced LH and FSH release in the female rabbit. *Horm Res* 1975; **6**: 372-379.

[28]Kaiser UB, Sabbagh E, Katzenellenbogen RA, Conn PM, Chin WW. A mechanism for the differential regulation of gonadotropin sub-unit gene expression by gonadotropin-releasing hormone. Proceedings of the National Academy of Sciences; USA; 1995, p. 12280-12284.

[29]Bedecarrats GY, Kaiser UB. Differential regulation of gonadotropins sub-unit gene promoter activity by pulsatile gonadotropins releasing hormone (GnRH) in perifused L β T2 cells: Role of GnRH receptor concentration. *Endocrinology* 2003; **144**: 1802-1811.

[30]Haisenledger DJ, Dalkin AC, Ortolano GA, Marshall JC, Shupnik MA. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin sub-unit genes: Evidence for differential regulation of transcription by pulse frequency *in–vivo*. *Endocrinology* 1991; **128**: 509-517.

[31]Agarwal N and Sharma S. Appraisal of garden cress (*Lepidium sativum* L.) and product development as an all pervasive and nutrition worthy food stuff. *Annal Food Sci Technol* 2013; **14**(1): 77-84.

[32]Juma AHA. The effects of *Lepidium sativum* seeds on fracture-induced healing in rabbits. *Med Gen Med* 2007; **9**(2): 23.

[33]Shawle K, Urge M, Animut G. Effect of different levels of *Lepidium sativum* L. on growth performance, carcass characteristics, hematology and serum biochemical parameters of broilers. *Springerplus* 2016; **5**(1): 1441.

[34]Bafeel SO, Ali SS. The potential liver toxicity of *Lepidium sativum* seeds in Albino rats. *J Biol Sci* 2009; **4**(12): 1250-1258.

[35]Nada KHA. Studies on effect of *Lepidium sativum* on the performance of laying hens [MSc. Thesis]. University of Khartoum, Sudan; 1999.

[36]Adamu M, Chaiwat B. Effect of *Lepidium sativum* L. (garden cress) seed and its extract on experimental Eimeria tenella infection in broiler chickens. *Kasetsart J Nat Sci* 2014; **48**(1): 28-37.