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Effects of *Lepidium sativum* Seed on Reproductive Characteristics in Rabbit Bucks

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ABSTRACT

Lepidium sativum (LS) has been documented to possess numerous properties but little is known as regards its mechanism on male reproductive features which the study aimed to clarify by evaluating its effects on luteinizing hormone, testosterone, sperm characteristics, and histology of reproductive organs in rabbit bucks. Twenty mature, intact bucks were randomly assigned into four treatment groups and fed either normal rabbit feed or diets containing LS seeds powder at varying levels for 63 days. Blood samples were collected from each rabbit weekly to determine LH and testosterone levels. Semen was collected three times for evaluation of sperm characteristics; weight and histological examination of reproductive organs were evaluated at the end of the experiment. Inclusion of LS seed in the feed of rabbit bucks significantly increased plasma LH concentrations in a bimodal dose-dependent manner with no significant difference in the levels of testosterone. Percentage motility and live sperm percent were significantly reduced in all rabbits fed LS based diet while the control rabbits showed an increasing pattern in percentage motility and live sperm. Sperm abnormality percent was significantly increased in all rabbits in LS dose-dependent manner. There were no significant effects of LS on the relative weights of all bucks accessory glands but significantly affected the relative weights of paired testis and caudal epididymis. Marked alterations in the testis were also noted. The findings suggest that mechanism underlying the effects of LS seed on reproduction is mediated through multiples pathways which depend greatly on the amount of LS seed ingested.

Keywords: Rabbit, *Lepidium sativum*, LH, Testosterone, Sperm, Histology.

INTRODUCTION

Historically, medicinal plants have been used widely for preventive and therapeutic purposes [1]. The natural chemicals embedded in the plants have provided the basis for their medicinal and phytotherapeutic potencies. There is no or little scientific confirmation supporting the assertions attributed to some medicinal plants and many times, the adverse effects associated with remedies are unknown due to lack of safety proof and efficacy.

One of the plants been used over a period of time with a significant reputation in different traditions, especially in the Middle Eastern parts of the world as a therapeutic medicinal plant to cure various ailments is *Lepidium sativum* (LS), also known as garden cress. It has also been recognized and frequently consumed in Europe and some parts of Africa. Traditionally, the decoction of LS seed with a tablespoonful of honey is given as an effective medicine to increase breast milk in female [2], sexual stamina and sexual retentivity in both male and female subjects [3, 4].

The gear towards discovering the mechanism of action of the plant on the reproductive system particularly towards the safety evaluation, curative and protective effects has increased but the reports are controversial. A significant increase in all testicular sperm characteristics were observed in New Zealand white male rabbits after administering tocopherol extract of LS for 50 days [5]. Similarly, Naji *et al.*, [6] reported no histological changes in reproductive organs of rabbit administered tocopherol extract of LS seed. More so, no macroscopic or microscopic alterations were observed in the gonads and other vital organs (liver, lungs, kidney, spleen, brain, adrenals and heart) of both male and female rats when LS seed powder was administered through diet for 90 days [7]. However, Fagbani *et al.*, [8] reported antispermatogenesis activity of LS seed in rat testis. Sharief and Gani, [9] also reported 100% oral contraceptive effects of *L. sativum* seed in mice.

Moreover, there is a dearth on information on the effects of LS seed on the synthesis and secretion of luteinizing hormones and testosterone since these are major factors regulating gonadal gametogenesis. Therefore, this study investigated the effects of dietary inclusion of *L. sativum* seed on LH, testosterone, sperm characteristics, weight and histological examinations of reproductive organs using rabbit bucks as animal model.

MATERIALS AND METHODS

Plant material and preparation

The seeds of *Lepidium sativum* (Garden cress) were purchased from a local market in Gombe, Nigeria, further identified and authenticated in the Herbarium of the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria. The seeds were ground into fine powder using an electric mill and kept in an air-tight container until required for animal treatments.

Experimental site, animal selection and maintenance

The research was carried out at the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria. Ethical approval for this study was obtained from the Research Ethics Committee, Federal University of Agriculture, Abeokuta, Ogun State. Twenty, Chinchilla rabbit bucks (mean weight, 2.0 ± 0.1 Kg; 6 months of age) were used in this study. The rabbit bucks were equally distributed into four treatment groups containing five (5) rabbits in each. The animals were fed either standard rabbit feed (0% LS; Control, group I) or rabbit feed containing 5% LS seed powder, 7% LS seed powder or 10% LS seed powder for 63 days. The animals were housed individually in a wooden cage where feed and fresh water was given *ad-libitum*. The rabbits were housed in a natural open-sided pen and reared under clean environmental condition.

Data collection

Blood samples were collected from each animal once a week into lithium heparin tubes. Blood samples collected were centrifuged at 3000 rpm in a centrifuge (4°C) to obtain plasma. Plasma was stored at -20°C until LH and testosterone assays were done using radioimmunoassay and ELISA techniques, respectively. Semen samples were collected three times (day 0, 28 and 56) from each rabbit buck with the aid of an artificial vagina. In each ejaculate, the semen volume, percentage sperm motility, live sperm, and abnormal spermatozoa were evaluated using procedures reported by El-Tarabany *et al.*,^[10] and Daramola *et al.*,^[11]. At the end of the study (day 63), 3 animals from each group were euthanized. Reproductive organs (paired testis, epididymis, seminal vesicles, prostate gland and bulbourethral) were excised, blotted weighed and the relative weight was calculated and recorded. A portion of the testis and caudal epididymis were fixed in Bouin's fixative prior to the histological assessment. The standard histological assessment was done following the procedures of Abadjeva *et al.*,^[12].

LH assay

LH concentration was determined using techniques described by Bernard *et al.*,^[13]. The LH used for the standards and iodination reaction was obtained from Sigma Aldrich Chemicals, St. Louis, MO. The antiserum AFP C5288113 (obtained from Dr. A. F. Parlow) was used at 1:35,000 dilutions as the first antibody and the GARGG, Goat Anti-Rabbit Immunoglobulin G (EQUITECH-BIO INC, Kerrville, Texas, USA) were used as the second antibody. The samples were run in duplicates in 3-day assay.

Briefly, on day 1, standards, antibody (100 μ L of diluted 1:35K AFP C5288113) and tracer 125I-LH; 18,000-20,000cpm/ 100 μ L) were pipetted into 12x75mm glass tubes (VWR International, Pennsylvania, USA) containing 150 μ L of blood plasma and incubated at 40C for 48 hours. After the 48hrs, the second antibody, GARGG (100 μ L of 1:1

dilution of GARGG with PBS-EDTA) and 500 μ L 6% polyethylene glycol was added. Tubes were centrifuged at 3000 rpm at 40C for 15 minutes. The tubes were decanted and loaded into Auto Gamma Counter where the values of LH concentrations were recorded. 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.

Testosterone assay

Plasma testosterone was measured using Testosterone Enzyme Immunoassay (EIA) Test Kits (purchased from TECO DIAGNOSTICS, 1268N, Lakeview Ave., Anaheim, California, 92807, USA) according to the manufacturer's instructions. Briefly, standards and plasma samples were run in duplicate of 10 μ L. 100 μ L of Testosterone-horseradish peroxidase (HRP) conjugate reagent and 50 μ L of rabbit anti-Testosterone reagent were pipetted into each designated well and the plates were incubated for 90mins at 370C. The content inside each well plate was removed, 300 μ L of deionized water was added into each well, decanted and drained. Later, 100 μ L 3, 3', 5, 5'-Tetramethylbenzidine (TMB) reagent solution was added into each well and then the reaction was stopped with 100 μ L stop solution after 20 minutes. Each well plate was loaded into plate reader and read at 450nm within 15 minutes of adding top solution and absorbance for each well was recorded. The standards curve was done and the equation for the line was obtained which helps to determine the testosterone concentrations of each sample. The testosterone ELISA kit had a sensitivity of 0.06ng/ml.

Statistical analysis

Data were analyzed using the General Linear Model (GLM) Program of SAS^[14]. Multiple comparisons were done with Tukey's Test. The main effects of treatments on plasma LH concentrations, testosterone concentrations, and sperm characteristics were determined and a p-value less than 0.05 were considered statistically significant in all analyses. Data were reported in Mean \pm Standard Error.

RESULTS

Effects of dietary inclusion of LS seed on plasma LH and testosterone in rabbit bucks

The modulation of plasma LH was significantly ($p < 0.05$) higher in all LS groups (fig. 1) during the sampling period. Similarly, average plasma LH concentration in rabbit bucks fed LS based diet was higher compared with the control rabbits (fig. 2). The highest value of LH concentration was obtained in 7% LS group followed by 10% LS groups. The statistical values of control and 5% LS groups were similar. However, there was no significant ($p > 0.05$) difference in the average plasma testosterone concentrations in all groups (table 1).

Effects of dietary inclusion of LS seed on sperm characteristics in rabbit bucks

The semen volume of the experimental animals were not significantly ($p > 0.05$) different across the dietary treatment groups.

An increased pattern of sperm motility percent was observed in the control and 5% LS groups. There was a significant ($p < 0.05$) decrease in percentage sperm motility in 7% LS group at day 56 only. However, the percentage of sperm motility significantly ($p < 0.05$) decreased at day 28 and further decreased at day 56 in 10% LS group (table 2).

The live sperm percent in control bucks increased throughout the duration of the study.

However, the live sperm percent in 5% and 7% LS groups significantly ($p < 0.05$) decreased at day 28 and showed no significant ($p > 0.05$) difference at day 56. However, live sperm cells percent of the rabbits in 10% LS group significantly ($p < 0.05$) reduced on day 28 and later improved on day 56.

There was a significant ($p < 0.05$) increase in the percentage total abnormality of spermatozoa in all rabbit groups and the increase is LS dose-dependent.

Effects of dietary inclusion of LS seed on relative weight and histological examination of reproductive organs of rabbit bucks

LS seed did not significantly ($p > 0.05$) affect the relative weight of the prostate gland, seminal vesicles, bulbourethral and total epididymis in rabbits (Table 3). However, there was a significant ($p < 0.05$) reduction in the relative weight of paired testis of bucks fed different levels of LS seed. The relative weight of caudal epididymis was significantly ($p < 0.05$) decreased in 5% and 10% LS groups. An increase in the relative weight of caudal epididymis was observed in 7% LS group but comparable with the control group.

Plates 1a and 1b show the histological slides of caudal epididymis. Caudal epididymal histological examination revealed no microscopic alteration across the dietary treatment groups. In contrast, LS seed inclusion into the feed of rabbit bucks caused pathological alteration in the testis (Plate 2a and 2b). All testes from rabbits fed LS seed-based diet showed diffuse degeneration of the germinal epithelial cells characterized by irregular and disordered cell arrangement.

DISCUSSION

Reproduction in mammals is regulated by the hypothalamic-pituitary-gonadal axis. Specifically, both LH and FSH regulate gonadal steroidogenesis and gametogenesis. In this present study, LS seed dietary inclusion increased plasma LH secretion in rabbit bucks. Stimulation of LH may be ascribed to phytosterol constituent of LS seed through temporary or permanent alteration of the feedback loop in the hypothalamus, pituitary and the gonad by mimicking the effects of endogenous estrogen and trigger their specific receptors, thereby resulting in increased LH secretion. Phytosterol reportedly increased the secretion of the basal gonadotropins in immature male and female rabbits [15]. This report is in agreement with the findings of [16] where an increase in luteinizing hormone was observed in young adult rats fed LS seed-based diet for only 14 days. Despite the documented potential of LS as an aphrodisiac [17] the results of this study indicate that aphrodisiac effects of LS seed may be through some other pathways, but most certainly not through increased testosterone levels. LS seed increases the peripheral LH level without altering the levels of testosterone.

Ajayi *et al.*, [18] established the effect of nutrition on sperm characteristics of rabbits. In this present study, the percentage motility and live sperm percent in LS groups are within normal recommended values of rabbit sperm for good reproductive potential and fertility in either normal mating or in artificial insemination. However, the

percentage motility and live sperm percent drastically dropped below the minimum level of the recommended values [19] in 10% LS inclusion level at day 56 and 28, respectively. Furthermore, the percentage abnormalities in all LS groups at day 28 and 56 of the experiment were higher than the upper limit of 20% recommended as the minimum for good reproductive potential and fertility in either normal mating and or in artificial insemination [20]. The negative effects of LS seed on sperm qualities especially high sperm abnormality in rabbit may be attributed to metabolites in LS seed such as caffeine, which have been reported to interfere or disrupt in the stages of spermatogenesis [21, 22], particularly the process of spermatids formation, which lead to damage in the process of the sperm head configuring, forming abnormal sperm and perhaps obtained as a result of genetic overlap and cause abnormalities in sperm. Phytosterol has been reported to inhibit sperm motility in goat [23]. No known study on the effects of LS seed on semen quality at different collection periods, especially as related to the rabbit spermatogenic cycle. The only study seen is on testicular and epididymal content. At the end of their study, there was no proving that LS seed affects sperm characteristics [5]. Conflict in the reports may be due to phenolic extract of LS seed used, the process of extraction (which may have reduced or eliminated some natural chemicals that can tamper with spermatogenesis process), breed of rabbit and also the dosage may be small to inclusion levels of the LS seed used in this present study. However, these present results support the findings of [8], who reported spermatogenesis impairment and decrease in germinal epithelial height in diabetic induced rat testis administered extract of LS for 21 days.

The knowledge of basic morphometric characteristics of the reproductive tract has been found to provide valuable information in the evaluation of breeding and fertility potential of the animals [24]. Decreased weights of testis of rabbit fed LS seed-based diet indicate widespread and or diffuse loss of seminiferous epithelial cells as described by Morton [25]. These were evident in the histological examination of the testis; it was found that the higher the LS seed inclusion levels in the feed of rabbits then more severe the constraints on the testis. Phytosterol has been documented to decrease testicular weight [26], increase epididymal weights in rats [27], alter reproductive organ in gilt [28]. This present study seems to confirm similar attributes of phytosterol, which is abundant in LS seed. These results support the outcome of [29], but contrary to the report of [7], where no macroscopic and microscopic changes were observed in the reproductive organs of the rat. Also, against the report of [6], where no pathohistological changes in the testis of rabbits administered tocopherol extract of LS seed were observed. Dosage and fraction of *Lepidium sativum* seed may be ascribed to the differences in the outcomes. Although *L. sativum* is traditionally used to treat a sexual disorder, the adverse effects observed in sperm characteristics and histology of the testis may suggest a possible antispermatogenic property.

Table 1: Statistical summary of effect of dietary inclusion of LS seed on plasma testosterone in intact rabbit bucks

Experimental Diet	Testosterone (ng/mL)				
	Week 0	Week 2	Week 4	Week 6	Week 8
0%LS	21.01 ± 0.01	21.06 ± 0.02	17.00 ± 4.03	21.03 ± 0.04	21.04 ± 0.02
5%LS	21.01 ± 0.03	21.05 ± 0.03	21.01 ± 0.04	21.04 ± 0.04	20.99 ± 0.04
7%LS	21.04 ± 0.07	21.01 ± 0.03	20.95 ± 0.05	20.96 ± 0.02	20.98 ± 0.01
10% LS	20.94 ± 0.03	19.79 ± 0.09	20.71 ± 0.17	20.87 ± 0.13	20.89 ± 0.01

No significant difference between control and LS seed fed rabbits.

Table 2: Effects of dietary inclusion of varying levels LS seed on sperm characteristics in intact rabbit bucks

Parameters	Treatment	Time of Collection		
		Day 0	Day 28	Day 56
Semen Volume (mL)	0% LS	0.86 ± 0.16	0.64 ± 0.09	0.62 ± 0.11
	5% LS	0.78 ± 0.07	0.58 ± 0.11	1.68 ± 1.08
	7% LS	0.62 ± 0.10	0.84 ± 0.19	1.04 ± 0.37
	10% LS	0.59 ± 0.00	0.64 ± 0.11	0.46 ± 0.02
Progressive Motility (%)	0% LS	76.14 ± 2.43 ^{b,B}	78.74 ± 2.30 ^{bc,A}	81.60 ± 2.47 ^{b,A}
	5% LS	80.36 ± 1.71 ^{b,B}	84.62 ± 1.82 ^{b,A}	84.20 ± 1.46 ^{ab,A}
	7% LS	88.68 ± 1.78 ^{ab,B}	93.24 ± 1.10 ^{a,A}	89.20 ± 1.30 ^{a,B}
	10% LS	93.24 ± 1.10 ^{a,A}	73.46 ± 3.72 ^{c,B}	69.68 ± 2.40 ^{c,C}
Live Sperm (%)	0% LS	84.50 ± 1.35 ^{c,B}	91.50 ± 1.31 ^{a,A}	91.00 ± 1.76 ^{a,A}
	5% LS	91.50 ± 1.67 ^{b,A}	80.50 ± 2.23 ^{b,B}	82.50 ± 1.90 ^{b,B}
	7% LS	93.00 ± 1.47 ^{ab,A}	78.00 ± 1.72 ^{b,B}	81.00 ± 1.91 ^{b,B}
	10% LS	96.00 ± 1.12 ^{a,A}	58.45 ± 3.01 ^{c,C}	75.50 ± 4.32 ^{b,B}
Sperm Abnormality (%)	0% LS	11.0 ± 0.14 ^{ab,B}	17.2 ± 0.13 ^{c,A}	18.6 ± 0.12 ^{d,A}
	5% LS	12.6 ± 0.12 ^{a,C}	28.4 ± 0.14 ^{b,A}	25.4 ± 0.16 ^{c,A}
	7% LS	9.0 ± 0.07 ^{b,B}	32.4 ± 0.16 ^{b,A}	32.6 ± 0.11 ^{b,A}
	10% LS	8.4 ± 0.07 ^{b,C}	40.8 ± 0.16 ^{a,B}	48.4 ± 0.12 ^{a,A}

^{a, b, c, d} means in a column with no common superscript (s) differs significantly (Treatment effect). ^{A, B, C, D} means in a row with no common superscript (s) differs significantly (Interaction between days of semen collection and treatment). (*p*<0.05).

Table 3: Effects of Dietary Inclusion of LS Seed on the Relative Weight of Reproductive Organ in Intact Rabbit Bucks

Organ Weight	Diet			
	0% LS	5% LS	7% LS	10% LS
Paired Testis (g/KgBW)	2.28 ± 0.07 ^a	1.60 ± 0.00 ^b	2.02 ± 0.21 ^{ab}	1.86 ± 0.15 ^{ab}
All Epididymis (g/KgBW)	0.71 ± 0.17	0.56 ± 0.14	0.93 ± 0.08	0.79 ± 0.04
Paired Caudal Epididymis (g/KgBW)	0.45 ± 0.08 ^{ab}	0.28 ± 0.06 ^b	0.55 ± 0.05 ^a	0.30 ± 0.09 ^b
Prostate Gland (g/KgBW)	0.29 ± 0.05	0.25 ± 0.04	0.35 ± 0.05	0.21 ± 0.07
Seminal Vesicles (g/KgBW)	0.07 ± 0.03	0.11 ± 0.03	0.17 ± 0.09	0.08 ± 0.02
Bulbourethral (g/KgBW)	0.61 ± 0.28	0.70 ± 0.21	0.55 ± 0.11	0.51 ± 0.14

^{a, b} means in a row with no common superscript(s) differ significantly (*p*<0.05).

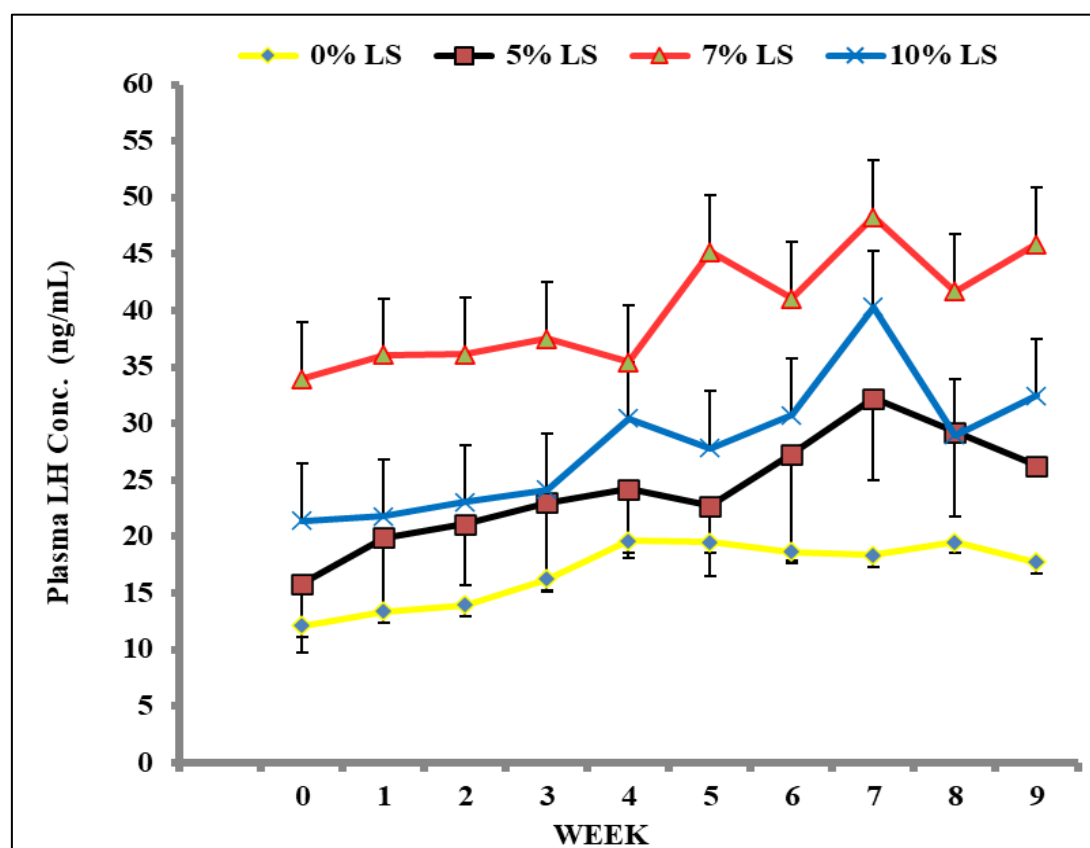


Figure 1: Effects of dietary inclusion of LS seed on LH secretion in rabbit bucks

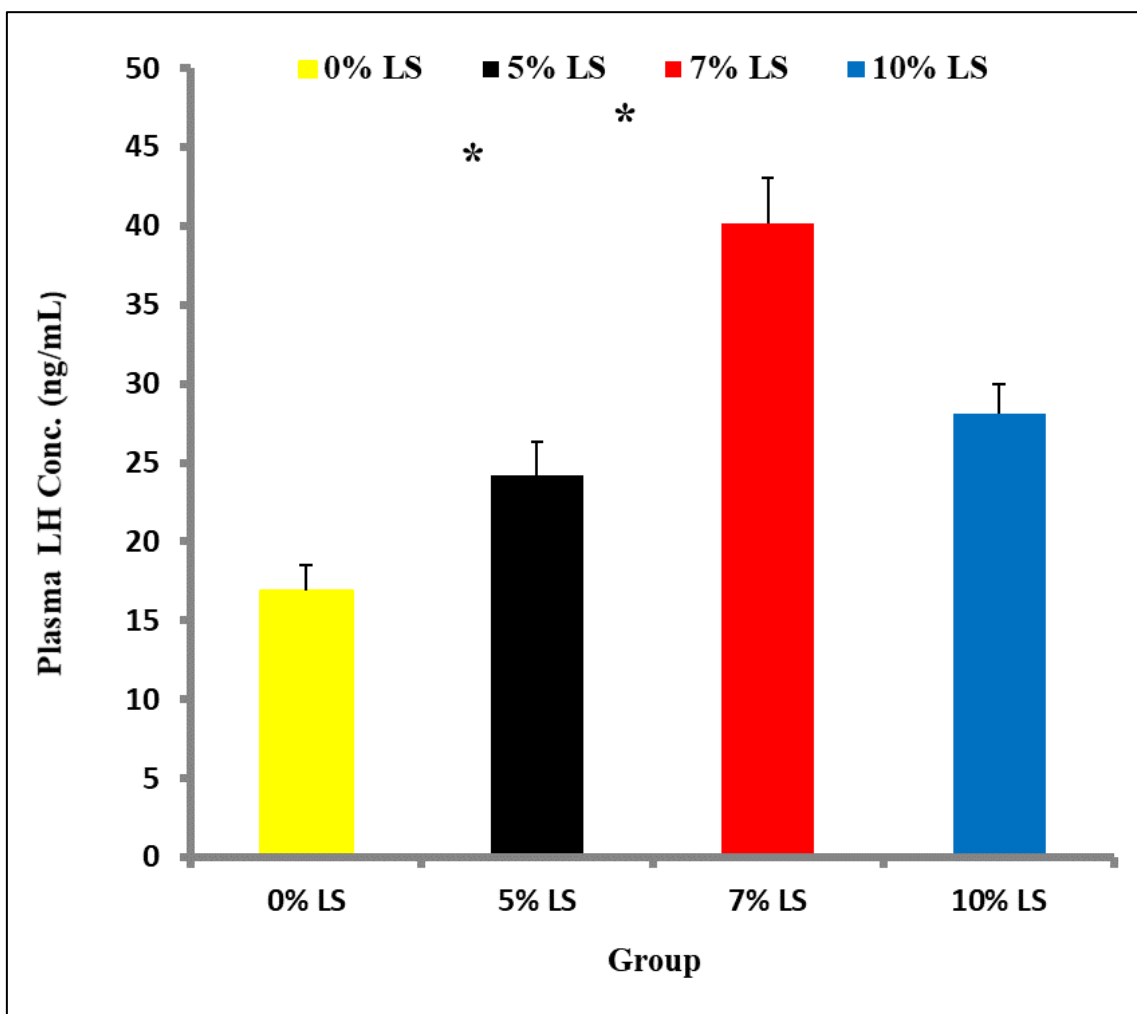


Figure 2: Dietary *Lepidium sativum* seed Inclusion and Mean Plasma LH Concentrations in Rabbit Bucks. Bars with asterisks are significantly different.

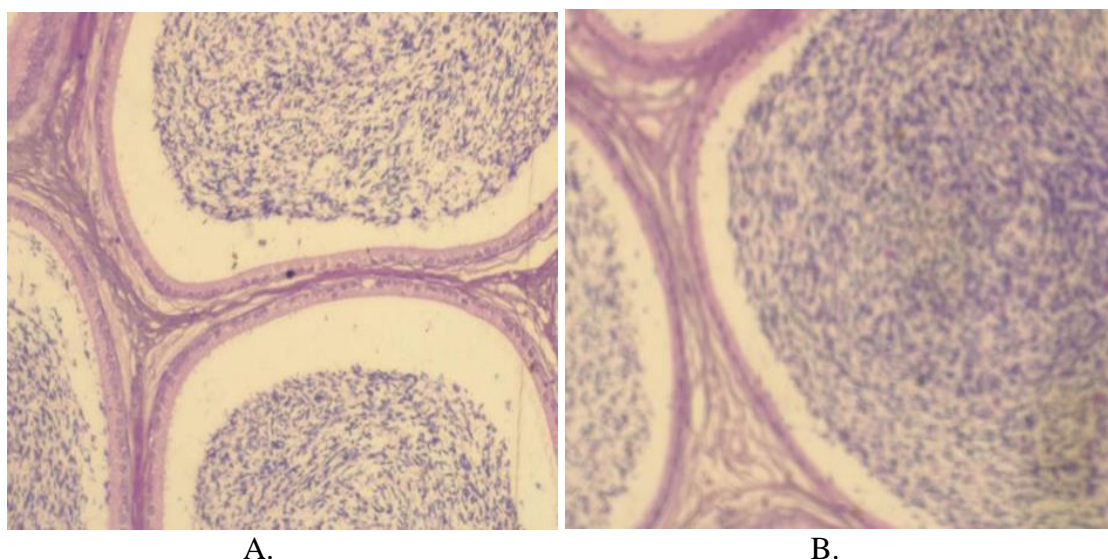


Plate 1a: Epididymis from a rabbit on a normal diet (A) and on 5% LS inclusion (B) showing no microscopic changes. Epididymal tubule of rabbit lined by ciliated pseudostratified columnar epithelium with numerous sperms in the lumen

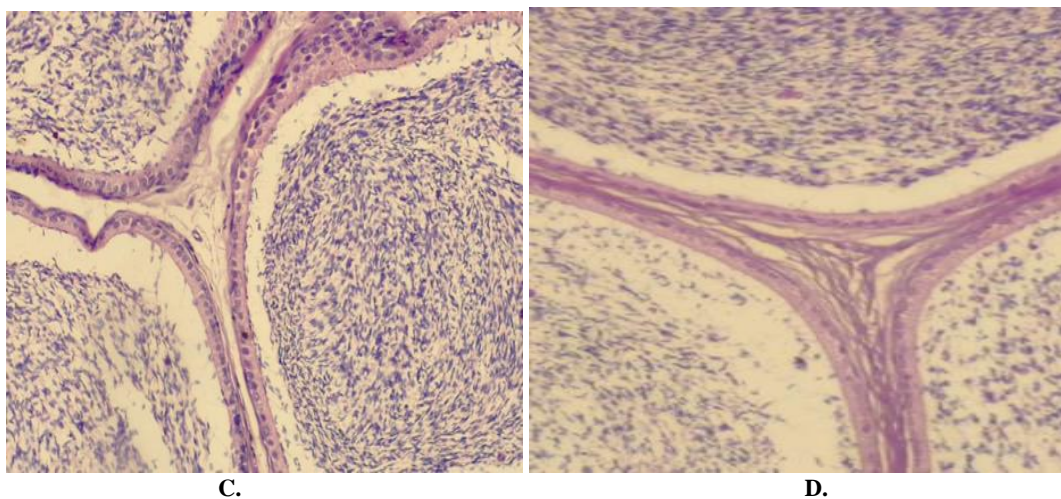


Plate 1b: Epididymis from a rabbit on 7% LS inclusion (C) and on 10% LS inclusion diet (D) showing no microscopic changes. Epididymal tubule of rabbit lined by ciliated pseudostratified columnar epithelium with numerous sperms in the lumen.

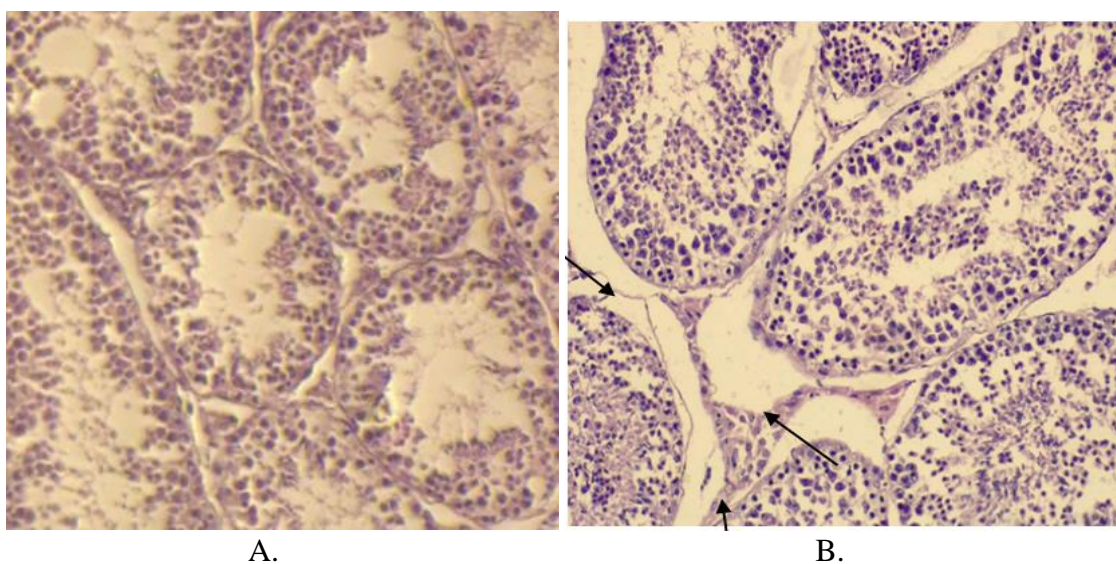


Plate 2a: Testis from a rabbit on a normal diet (A), showing no pathohistological changes and on 5% LS seed inclusion diet (B), showing mild less impact cells, irregular and disordered cell arrangement

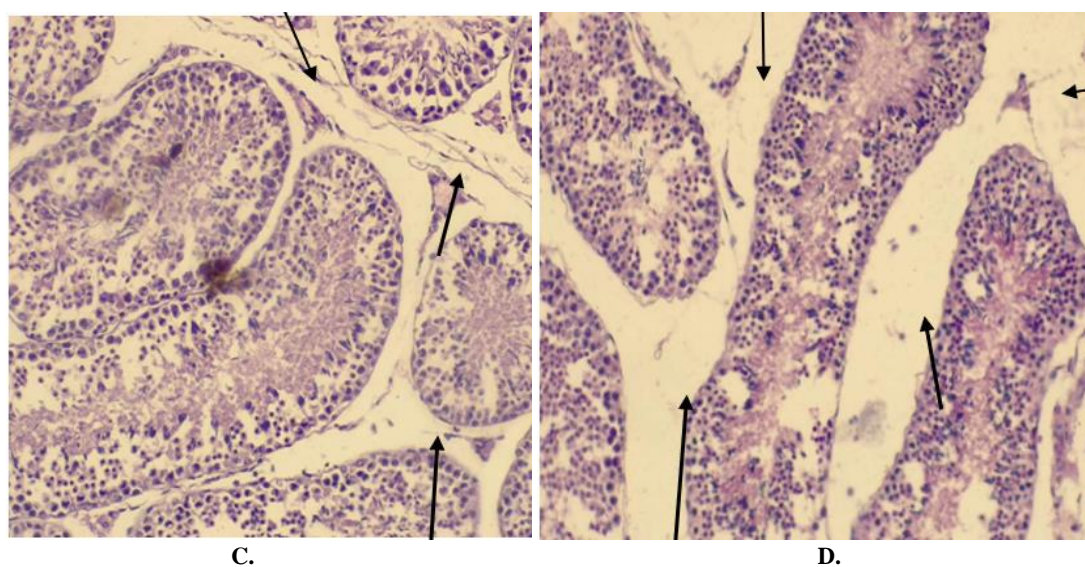


Plate 2b: Testis from a rabbit on 7% LS seed inclusion diet (C) and on 10% LS seed inclusion diet (D) showing pathohistological changes (less impact cells, irregular and disordered cell arrangement). Arrows showing diffuse degeneration of the germinal epithelial cells

CONCLUSION

In conclusion, these findings show the toxicity effects of LS seed on sperm quality and testis in rabbit bucks, especially when the high amount is ingested. The toxicity effects are indicated to occur due to some phytoconstituents of the plant which tend to have deleterious effects despite its positive role on gonadotropins. Further studies are required to carefully investigate the potential effects of LS seed as a therapeutic agent against the reported adverse effects to gear towards exploiting the pharmacological and medicinal potentials of *L. sativum*.

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