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Research Article

Effects of dietary inclusion of *Lepidium sativum* (garden cress) seed on plasma luteinizing hormone and reproductive performance in female rabbits

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ABSTRACT

Background: *Lepidium sativum* (LS) has been reported to have multi-purpose medicinal uses. For instance, rubefacient, antihypertensive, hepatoprotective, galactagogue and aphrodisiac properties are well documented; however, there are few controversial reports particularly as related to reproduction. Also, no known study has identified the mechanisms underlying the action of this plant on reproduction. This study was aimed at evaluating the effects of dietary supplementation with LS on luteinizing hormone and reproductive performance in female rabbits. **Methods**: A total of twenty, nulliparous Chinchilla female rabbits (6.5-month old) were randomly assigned to four treatment groups with five (n=5) rabbits in each group. Diet containing either LS seed powder at 0% (Control), 5%, 7% or 10% were given *ad-libitum* to female rabbits two weeks pre-pregnancy and throughout the entire period of pregnancy. Blood samples were collected through the marginal ear vein from each female rabbit weekly during pre-pregnancy and pregnancy periods to evaluate Luteinizing hormone (LH). Conception rate, gestation length, litter size, litter weight, number of stillbirths and runts were recorded. Data obtained were analyzed using ANOVA. **Results**: LS seed inclusion significantly increased (P<0.05) conception rate and plasma LH concentration in dose dependent manner, from 5%- to the 7%- and then decreased at 10%-LS seed level. There was a significant decrease (P<0.05) in gestation length in does fed 10% LS-based diet. Furthermore, the litter weights and number of live kid born were significantly (P<0.05) decreased in all LS groups. In contrast to zero incidence in control rabbits, high incidence of stillbirth and runt were recorded in all LS-based groups. **Conclusion**: The results show that inclusion of LS seed in rabbit chow increases conception rate and plasma LH levels which were attributed to the phytoestrogens constituent in the seed. Despite the positive role of LS seeds on conception rate and LH, LS seed may possess fetal developmental toxicity effects due to high incidence of stillbirth and extremely low birth weight of newborn recorded.

INTRODUCTION

For many years, various ethnic communities understand and have secret ethnomedicinal knowledge about many plants available in their environments and these plants have been serving them with their advantages (Taid *et al*., 2014). The biological activities of the medicinal plants have been accredited to chemical constituents that induce or suppress particular physiological action in the body system (Faraz *et al*., 2003). Though, lot of the acclaimed properties and physiological actions of plants used in traditional system of medicine are not scientifically defined especially those ones related to reproduction.

Lepidium sativum (LS), also known as garden cress in English, Jan Algaro or Lansir in Hausa (Northern Nigeria), Habburshad (Arabian), Chandrasur (Hindi), Lepido (Spanish) and Cresson de fontaine in French is been reported to possess various multi-system effects in traditional medicine, such as anti-rheumatism (Ahsan *et al*., 1989); anti-diarrhoea (Rahimi *et al.,* 2010); anti-febrifuge (Ageel *et al*., 1987); rubefacient and galactagogue (Kirtikar and Basu, 1995) etc. Potentials

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of LS as related to reproduction were also documented. For instance, LS seeds roasted and mixed with sugar are given as an effective medicine to increase breast milk (Pullaiah, 2006). It's also reported to increase sexual stamina and sexual retentivity (Duke, 1992; Mali *et al*., 2007; Jabeen *et al*., 2017). The plant is considered as abortifacient in human (Baquar, 1989; Duke, 1992). It is also recorded to possess teratogenic (Nath *et al*., 1992), antifertility (Falana *et al*., 2014) and antiovulatory effects (Satyavati, 1984). As a result of the aforementioned reproductive properties of LS, some scientific studies were carried out on the various parts of the plant. Supplementation of LS seed powder into the feed of lactating buffaloes increases their total milk yield (Abo El-Nor *et al.,* 2007; Kumar *et al*., 2011). A mammogenic and lactogenic activity of LS was reported in rats administered 1.6mg/gm BW of LS seed powder for 14 days (Al-Yawer *et al*., 2006). Moreover, proceptivity and receptivity property was reported in ovariectomized rat when 200 and 400 mg/Kg BW of methanolic extract of LS were given orally for 21 days (Kagathara *et al.,* 2015). Similarly, it was reported to increase prolactin, progesterone and luteinizing hormone in ovariectomized rats (Kagathara *et al.***,** 2015). However, Shareif and Gani, (2004) reported 100% oral contraceptive effects of *L. sativum* seed in female mice.

There is a divergence of opinion on the effects of *L. sativum* seed on reproduction. Furthermore, information on efficacy, safety and mechanism of action is lacking. Moreover, little is known about it effects on LH secretion and reproductive performance in mammals. Hence, this study was undertaken to investigate the effects of dietary inclusion of *Lepidium sativum* seed on plasma LH and reproductive performance in female rabbits.

MATERIALS AND METHODS

Plant material and preparation

Lepidium sativum (Garden cress) seeds were purchased from a farmer at local market, Gombe, Gombe State, Nigeria. The LS seeds were identified and authenticated in the Herbarium of the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria by Dr. J.A. Soaga. The seeds were ground into fine powder using electric mill and kept in an airtight container for later use.

Experimental animals

Twenty (20) nulliparous Chinchilla rabbits (mean weight, 2.05 ± 0.1 Kg; 6.5 months of age) were obtained from a reputable breeder farm at Abeokuta, Ogun State, Nigeria. The rabbits were randomly assigned into four different groups, animals in group I, II, III and IV (n=5 per group) were fed with diet containing *Lepidium sativum* seed powder at 0% (Standard rabbit feed, Control), 5%, 7% and 10%, respectively (w/w). The animals were housed individually in a wooden cage where feed and water were given *ad libitum*. The research was carried out at the Department of Animal Physiology, Federal university of Agriculture, Abeokuta, Ogun State, Nigeria. The animals were housed in a natural open-sided pen and reared under clean environmental condition at Favour farms, Abeokuta, Ogun State. Ethical approval for this study was obtained from the Research Ethics Committee, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Data collection

The animals were given experimental diet for two weeks before mating and throughout the entire period of pregnancy. The rabbits were synchronized by injecting 40 IU (standard approved by The European Agency for the Evaluation of Medicinal Products) Pregnant Mare Serum Gonadotropin (PMSG, obtained from Sigma Aldrich Chemicals, St. Louis, MO, USA) subcutaneously 72 hours before mating. 72 hours post-PMSG injection, mating was done naturally by taking rabbit buck to each doe cage. Mating was visually observed and all rabbits were receptive. Pregnancy was tested through palpation after 14 days of mating. Blood samples were collected from each animal through the marginal ear vein into lithium heparin tubes once a week throughout the period of experiment. Blood samples were centrifuged at 3000 rpm in refrigerated centrifuge (4 ^{o}C) for 15 mins to obtain plasma. Plasma was stored at -20 ^{o}C until LH assay was done using radioimmunoassay technique. Conception rate, gestation period, litter size (total, alive and dead), number of runt, litter weight of the kids at birth and number of stillbirth were recorded.

Conception rate

Conception was tested at 14-day post mating through palpation. Conception rate was calculated as a ratio of the number of does conceived to the total number of does mated multiplied by 100.

Gestation length and litter size

This was recorded as the interval between mating and kindling (Oguike and Okocha, 2008). Litter size was measured by direct counting of newborn immediately after kindling. It included number of stillbirth (Paci *et al*., 2012).

Litter weight

The newborns were weighed at birth using a sensitive weighing balance (Oguike and Okocha, 2008). Number of stillbirths denote the number of dead newborns at the time of parturition while Number of runt denote number of newborns with extremely low birth weight; usually less than average weight of Chinchilla rabbit at birth (< 40g).

LH assay

Plasma LH concentration was determined using radioimmunoassay technique described by Bernard *et al*. (1983). The LH used for the standards and iodination reaction was obtained from Sigma Aldrich Chemicals, St. Louis, MO. The antiserum AFP C5288113 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. The samples were run in duplicates in 3-day assay. 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.

Statistical analysis

Data are presented as Means ± SEM and were analyzed using statistical program of SAS (2002). Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The main effects of treatments on plasma LH concentration and reproductive performances were determined and a p – value less than 0.05 was considered statistically significant in all analysis.

RESULTS

Effects of Dietary Inclusion of LS Seed on LH Concentrations in Female rabbits

Averaged all over sampling periods, dietary inclusion of LS seed at 5% and 7% significantly (P<0.05) increased plasma LH levels compared with the control or 10% LS inclusion (fig. 1).

Effects of dietary inclusion of lepidium sativum seed on reproductive performances in female rabbits

Effects of dietary inclusion of *Lepidium sativum* seed on reproductive performances in female rabbits are presented in table 1. The results show a statistically significant (P<0.05) higher conception rate in 5% LS and 7% LS groups (100%) while that of control and 10% LS groups were lower (60%).

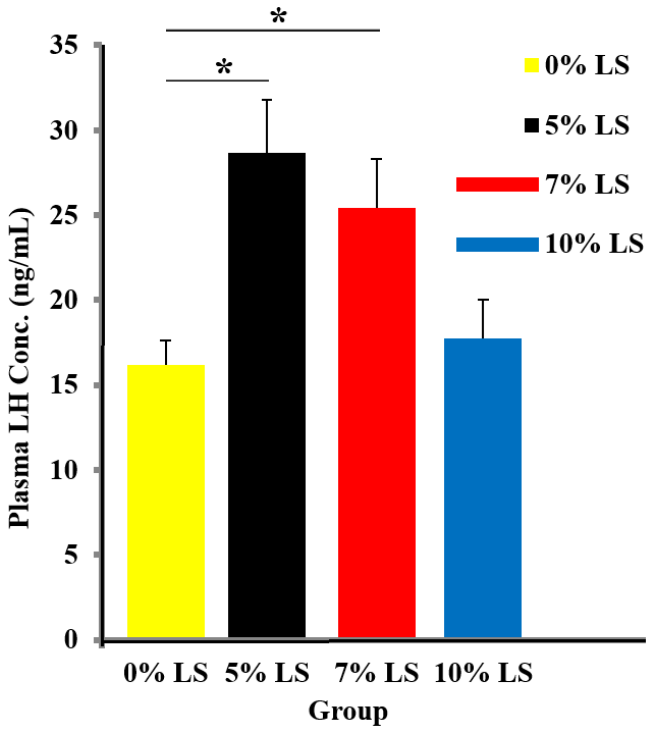

Figure 1: Effects of Dietary Inclusion of *Lepidium sativum* Seed on Plasma LH Concentrations in Female rabbits. Data shown mean ± SEM; Bars with asterisks are significantly different.

The gestation length of control, 5% and 7% LS groups were not significant (P>0.05) while gestation length in 10% LS group was statistically (P<0.05) lower compared with the control and other LS groups. No significant (P>0.05) difference in the litter size was observed among the four treatment groups.

Litter weight and number of live newborn of the control group were significantly (P<0.05) higher than the LS groups. Control rabbits recorded neither runt nor stillbirth. 5% LS group had the highest percentage of runt (9.1%) with 15.2 % of stillbirth, 7% LS group recorded 3.6% of runt with 7.1% of stillbirth while 10% LS group had the highest number of stillbirth with no runt.

DISCUSSION

In this present study, 5%- and 7%-LS seed inclusion in the diet of female rabbits increased plasma LH secretion while a decrease in plasma LH concentration was observed in 10% LS rabbits. Stimulation of LH may be ascribed to phytosterol constituent of LS 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. This report is in agreement

Table 1: Effect of Dietary Inclusion of *Lepidium sativum* Seed on Reproductive Performance in Female rabbits

Parameters	0% LS	5% LS	7% LS	10% LS
Conception (%)	60^{b}	100^{a}	100^{a}	60^{b}
Gestation Length (Day)	31.6 ± 0.50^{a}	31.2 ± 0.20^{a}	32.0 ± 0.32^{a}	28.0 ± 1.38^{b}
Litter Size	7.0 ± 0.84	6.6 ± 0.75	5.6 ± 1.17	6.2 ± 2.11
Litter Weight (g)	322.0 ± 36.11^{a}	210 ± 30.98^{ab}	224.2 ± 53.28^{ab}	131.4 ± 39.69^{b}
No of Live	7 ± 0.84^{a}	5.6 ± 1.17^{ab}	5.2 ± 1.11^{ab}	3.4 ± 1.17^{b}
Runt (%)	0.0^{c}	9.1^{a}	3.6^{b}	0.0^{c}
Stillbirth (%)	0.0^{d}	15.2^{b}	7.1^{c}	60.9^{a}

[a, b, c, d] Values in the same row with no common superscript(s) differ significantly ($P<0.05$)

with the findings of Al-Yawer *et al*. (2006) which was the only known available research work that evaluated effects of LS on reproduction at the hormonal level. The authors reported increase in luteinizing hormone in young adult female virgin rats fed LS supplemented diet for only 14 days. However, reduction in LH levels at 10% level of LS inclusion in this present study may be as a result of adenohypophysial negative feedback of the estrogenic action of phytosterol, inducing a decrease or loss of progressive sensitivity or the affinity for estrogen receptors binding which then causes subsequent LH inhibition.

The outcome of this study establishes that LS seed inclusion into the feed of rabbits did not inhibit ovulation, fertilization or implantation because all the rabbits were receptive at the time of mating, conception took place except for lower conception rate recorded in 10% LS group but still within the normal percentage rate of conception in nulliparous rabbits and the does carried the pregnancy to term. This is in contrast to the antiovulatory, contraceptive and abortifacient reports by Pande *et al*. (2002); Shareif and Gani, (2004) and Duke *et al*. (2002), respectively. The use of different animal model, administration method, fraction and dosage of LS seed in these studies could be the reason for the differences. Previous studies (Duke *et al*., 2002; Kagathara *et al*., 2015) reported receptivity action of LS seed and this activity may contribute to the high rate of conception observed in 5 and 7 percent LS groups in this present study. The receptivity effect may be ascribed to phytosterols in LS, which mediate their activity through estrogen receptors by producing agonistic effects thereby resulting in LH secretion which is required for the rupture of follicles. The percentage conception rate of the control group is within the normal percentage rate of conception in nulliparous rabbits. The reduction in conception rate in rabbits fed at the 10 percent LS seed inclusion level suggests that addition of LS seed at that high level could alter ovulation and fertility, similar to documented report by Falana *et al*. (2014). It is therefore speculated that this may be more pronounced at high level of LS seed more than 10%.

LS seed inclusion into the diet of female rabbits decreased average litter weight and number of live kid born. The decrease in average litter weight and number of live kid born in LS groups may be due to maternal nutrient during pregnancy. Maternal nutrient intake before and even during pregnancy is critical for foetal development. Nutrition of the foetus begins at conception and foetal development may be arrested at any stage due to many factors including nutrition (Mertz, 1986). LS seed contain antinutritional factors which might have hampered the bioavailability of nutrients. Agarwal and Sharma (2013) reported that whole LS seed flour contain tannins, phytic acids, oxalic acid and cyanogens which might obstruct the bioavailability of nutrients. Maternal exposure to inadequate or excessive amount of some nutrients may cause malformations or irreversible effects, stillbirth, early death or live birth of weak kids.

LS seed inclusion into the diet of female rabbits increased number of runts and incidence of stillbirth. Furthermore, the occurrence of stillbirth was bimodal with highest incidence seen in the 5%- and 10%-LS seed inclusion. In addition, all runt kids died within 48 hours of delivery. The death of the runt kids is attributed to the extremely low birth weight. Low birth weight kids are physiologically immature when compared to littermates of average birth weights. They are also at greater risk from hypothermia and cannot compete well for milk against their larger litter mates. The number of stillbirth and runt observed in rabbits fed LS in this present study may indicate fetal developmental toxicity effects of LS seed. This may be attributed to the abundance of phytosterols in LS seed and the most potent, phytoestrogens. Phytoestrogens possess goitrogenic activity, a reported property of LS (Ensminger *et al*., 1994; Okpala, 2015). A goitrogen is a substance that disrupts the production of thyroid hormones by interfering with the uptake of iodine in the thyroid gland, thereby causing hypothyroidism. Uncontrolled hypothyroidism or thyroid failure during pregnancy can lead to low birth weight and stillbirth (Abalovich *et al*., 2002; Brent, 2012). This may due to reduced foetal thyroxine which may cause disruption in the pituitary-thyroid gland axis of the new born. The foetuses are highly sensitive to environmental chemical exposures thereby affecting organogenesis, rapid growth and extensive tissue differentiation that occur during these developmental periods and therefore small perturbations can have important consequences. In addition, metabolic processing and elimination mechanisms are immature in the foetus, so

detoxification is inefficient (Beath, 2003; Chen *et al*., 2006). Therefore, a culmination of these factors may precipitate the stillbirth observed in the LS-supplemented rabbits in this study. Further studies should be done on the reversible effects and the impacts of LS seed on organ systems of offspring born/survived by mother fed LS based diet during pregnancy in rabbit.

CONCLUSION

The results of the current study showed that *Lepidium sativum* seed inclusion in the diet is capable of stimulating LH and increasing conception rate in female rabbit through the secondary metabolites but however may pose harmful effects on developing rabbit foetus due to low birth weight, reduction in live born kits, high rate of stillbirths observed.

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