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Research Article

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Alteration in Serum Sex Hormonal Level after Long-Term Administration of *Lagenaria siceraria* Fruit Powder in Female Rats

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ABSTRACT

Background. This research work was to emphasis the consequence of Lagenaria siceraria fruit powder (LSFP) on serum estrogen, FSH, LH and progesterone hormonal level in Wistar rats. Method. In present research female rats received 300, 500 and 1000 mgkg^{-1} body weight of the LSFP. Female rats were separated into four groups; control group (vehicle only), low dose group (300 mgkg^{-1}), medium dose group (500 mgkg^{-1}) higher dose group (1000 mgkg^{1}). Suspension of LSFP in 2% gum acacia was administered orally for ten weeks. 24 h. from the last doses of LSFP, blood samples were withdrawn by puncturing retro-orbital plexus and serum estrogen, FSH, LH and progesterone hormonal levels were assessed by using ELISA immunoassay technique. Results. A significant rise in serum estrogen level at the doses 1000 mgkg^{-1}, while reduction in serum FSH and LH levels at 500 mgkg^{-1} and 1000 mgkg^{-1}, furthermore, fall in serum progesterone levels at a dose of 1000 mgkg^{-1} of LSFP treated rats were observed. Organ morphology depicted rise in ovarian uterine weights at the doses 500 mgkg^{-1} and 1000 mgkg^{-1}. Histological observation not revealed any changes in architect of ovary. All results from treatment group were compared with that of control group rats. Conclusion. An effect of LSFP on serum sex hormonal profile in female Wistar rats might be due to the phytosterols or 5-alpha-reductase enzyme inhibition.

Keywords: Estrogen; FSH; LH; Progesterone; Hypothalamo-pituitary gonadal axis; Ovarian weight

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INTRODUCTION

Reproductive hormones are responsible for regulating the reproductive activities and functional characteristics in both the sexes [1]. Much more studies have depicted an altered estrogen level associated with the advancement of several pathological states. Various phytochemicals that already proven to possess advantageous influence with reduced side effect on endocrine events in both sanimals and humans [2]. Some plant phytochemicals elicited their effect on hypothalamo-pituitary gonadal axis or undeviating hormonal consequences on reproductive organs by altering steroidogenesis [3-5].

Various plant parts or decoctions had been used medicinally in ancient system of medicine over the centuries to compensate various reproductive disorders such as aphrodisia, fertility, menstrual disorders, impotence, complaints of the pituitary gland etc. Plant phytochemicals having estrogenic property can influence pituitary functions by outlying modulation of FSH and LH, their secretions and obstructing ovulation [6,7]. Any fluctuation in female reproductive hormones or effects on ovarian-uterine axis is indication of alteration in maturation and ovulation of ovarian follicles along with changes in the pattern of reproductive cycles. Serum estrogen, FSH and LH indices help in estimation of a variety of menstrual or reproductive dysfunctions in females [7]. Measurement of serum sex hormonal contour is therefore very useful while determining the reproductive integrity in both animals and humans [1]. Phytochemical evaluation of *Lagenaria siceraria* fruit revels that it contains phytosterols such as β-sitosterol, campesterol, fucosterol, kaempferol and 5α-reductase enzyme inhibitors such as oleic acid, linoleic acid, stearic acid, palmitic acid [8]. The phytochemicals having 5α-reductase enzyme inhibitor activity and phytosterols can be evaluated for their effects on sex hormonal profile through their antiandrogenic and estrogenic activity in rat animal models [9-16]. Thus stated research

was to evaluate the effect of *Lagenaria siceraria* fruit powder (LSFP) on estrogen, FSH, LH and progesterone hormonal profile in female Wistar rats.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The fresh fruits of *Lagenaria siceraria* were procured from market of Pusad, Maharashtra, India. The plant material was authenticated by Dept. of Botany, Nagpur University, Nagpur, India (Voucher no. 9257).

Fruit Powder Preparation

Whole fruits were cut in small slices, shed dried and processed in pulveriser to make coarse powder.

LD50 Determination

Acute toxic study was carried out for determination of LD50 as per the guideline of OECD 423. Suspension of LSFP in 2% gum acacia was administered in a single dose of 5000 $mgkg^{-1}$ (LD50 Cut-off dose) by oral route, after dosing animals were observed initially for first 30 minutes and intermittently during the first 24 h. In all cases no mortality was observed within first 24 h of dosing. Other observations were done like changes in fur and skin colour, eyes and mucous membranes, circulatory, respiratory, central and autonomic nervous systems, behavioral pattern along with motor activity. Attention was also given to observation of tremors and convulsions, righting reflex, pinna reflex, corneal reflex, body weight or any other clinical abnormalities. Thus 1/10th of LD50 dose was consider as therapeutic dose [17].

Experimental Design

The animals were accustomed to the laboratory setting for two weeks before experiment. Animals had free admittance to food and water, accommodated in a natural light-dark cycle. The experimental procedures for present study was sanctioned by the Institutional Animal Ethics Committee. Female Wistar rats were divided in different groups as Control group (vehicle only), Low dose group (300 $mgkg^{-1}$LSFP), Medium dose group (500 $mgkg^{-1}$ LSFP), High dose group (1000 $mgkg^{-1}$ LSFP), each group containing 05 animals, weighing 170-200 g. Suspension of LSFP in 2% gum acacia was administered daily orally for 10 weeks. 24 h. after the administration of last dose, animals were weighed and blood was collected by retro orbital method in heparinized tubes. Heparinized tubes were kept aside for 15 min. and centrifuged for 20 min. at 3000 rpm to collect serum samples. Serum levels of estrogen, FSH, LH and progesterone were assessed by ELISA immunoassay [7,18-20] at Metropolis diagnostics lab Mumbai, India. Uterus and ovaries were dissected out at autopsy, weighed up to the nearest 0.001 g and estimated for gonadal morphology [21,22] as;

Ovarian Weight [21]: Average weight of two ovaries weighed up to the nearest 0.001 g of each Wistar rat was taken.

Uterine weight [21]: Weighed up to the nearest 0.001 g.

Relative weight of uterus [21]: calculated by the formula

$$(\text{Weight of uterus/body weight}) \times 100.$$

Histopathological Investigation

At autopsy the ovaries of each animal were isolated, preserved and fixed in 10% formalin for two days. Then after washed in running water for about 12 h formalin is removed. Followed by treatment with isopropyl alcohol of increasing concentration for dehydration for 12 h. Final dehydration was carried out using absolute alcohol with about three changes for 12 h each to remove all traces of water. Further alcohol was removed by using chloroform and chloroform removed by paraffin infiltration. The cleaning was ended by using chloroform with two changes for 15 to 20 min. each. Embedding in paraffin was done by melting hard paraffin and pouring it into L-shaped blocks. The ovaries were then quickly plunged into molten paraffin and allow cooling. Then after the slicing and staining with haematoxylin and eosin was performed at department of veterinary science, Vasantrao Naik University, Parbhani, India conferring as per Roste et al. [21] and Cajuday and Pocsidio [20] method. The stained sections were examined for histological changes.

Statistics

All the results were expressed as mean ± SD. (n=5). The statistical significance between means was analyzed using one-way analysis of variance [ANOVA] followed by Dunnett's multiple comparison post-test by using Graph pad software. $p<0.05$ were considered significant.

Ethical Matter

Animal experiment was performed according to ethical guidelines of Institutional animal ethics committee (729/02/a/CPCSEA) and the care of the animals was done in accordance with the CPCSEA guidelines by Indian Govt.

RESULTS

Serum estrogen levels was estimated after 10 weeks of LSFP treatment and no significant effects were witnessed at the doses of 300 and 500 mgkg^{-1} of LSFP in treated group rats (Figures 1-6). Further it was observed that LSFP at the dose of 1000 mgkg^{-1} revealed noteworthy increase in the levels of serum estrogen when compared with control group (Table 1). Serum FSH (Table 2) and LH (Table 3) levels were noticeably reduced after LSFP treatment in animals dosed with 500 and 1000 mgkg^{-1}, whereas significant reduction in serum progesterone levels was observed in rats treated with 1000 mgkg^{-1} as compared to control group (Table 4).

While a significant increase in ovarian uterine weights (Tables 5 and 6) was found at 1000 mgkg^{-1} of LSFP treated group as compared to control group. In histopathological findings [21] haematoxylin and eosin stained, light microscopic evaluation of ovaries had no significant differences regarding number of corpora lutea or number of secondary follicles in 300, 500 and 1000 mgkg^{-1} of LSFP treated rats compared to control group rats. This reveled normal oestrous cycle in the treated group Wistar rats.

Table 1: Effect of LSFP on serum estrogen level in Wistar rats

Group		Dose	Estrogen (pg./ml)
I	Control	--	55 ± 2.56
II	Low dose	300 mgkg^{-1}	56 ± 1.03
III	Medium dose	500 mgkg^{-1}	58 ± 3.89
IV	Higher dose	1000 mgkg^{-1}	94 ± 3.05*
The values were given as MEAN ± SD (n=5). *indicates significant ($p<0.05$) when compared with control group. One-way ANOVA followed by Dunnett's multiple comparison tests			

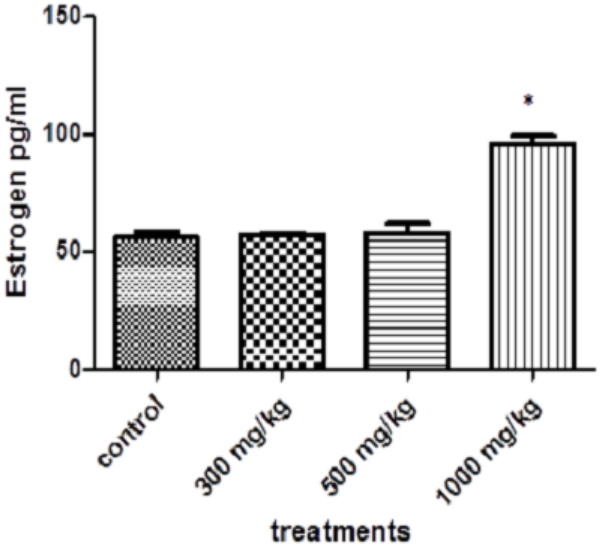

Figure 1: Effect of LSFP on serum estrogen level in Wistar rats

Table 2: Effect of LSFP on serum FSH level in Wistar rats

Group		Dose	FSH (mIU/ml)
I	Control	--	0.73 ± 0.09
II	Low dose	300 mgkg^{-1}	0.73 ± 0.10
III	Medium dose	500 mgkg^{-1}	0.30 ± 0.09*
IV	Higher dose	1000 mgkg^{-1}	0.38 ± 0.07*
The values were given as MEAN ± SD (n=5). *indicates significant ($p<0.05$) when compared with control group. One-way ANOVA followed by Dunnett's multiple comparison tests			

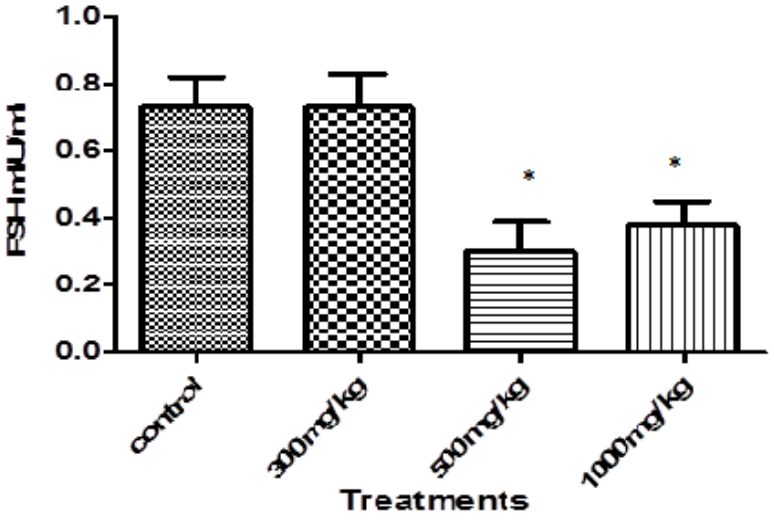

Figure 2: Effect of LSFP on serum FSH level in Wistar rats

Table 3: Effect of LSFP on serum LH level in Wistar rats

Group		Dose	LH (mIU/ml)
I	Control	--	0.22 ±0.09
II	Low dose	300 $mgkg^{-1}$	0.22 ± 0.07
III	Medium dose	500 $mgkg^{-1}$	<0.07*
IV	Higher dose	1000 $mgkg^{-1}$	<0.07*
The values were given as MEAN ± SD (n=5). *indicates significant (p<0.05) when compared with control group. One-way ANOVA followed by Dunnett's multiple comparison tests			

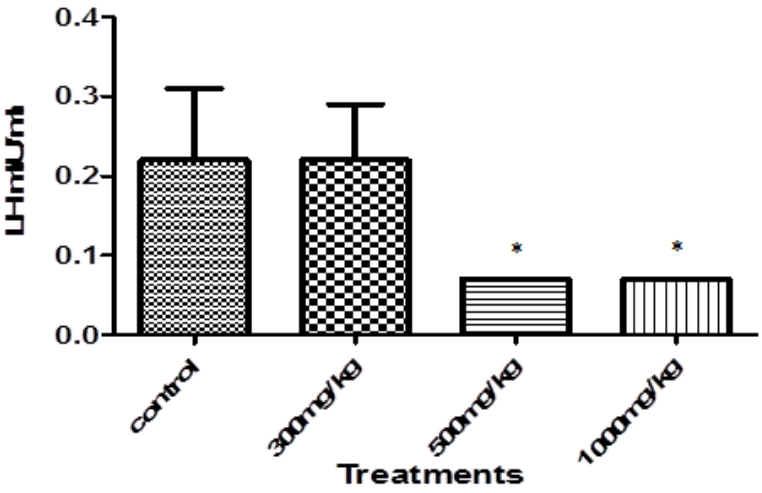

Figure 3: Effect of LSFP on serum LH level in Wistar rats

Table 4: Effect of LSFP on serum progesterone level in Wistar rats

Group		Dose	Progesterone (ng/ml)
I	Control	--	20.79 ± 4.54
II	Low dose group	300 $mgkg^{-1}$	22.50 ± 3.22
III	Medium dose group	500 $mgkg^{-1}$	15.44 ± 3.57
IV	Higher dose group	1000 $mgkg^{-1}$	12.83 ± 2.01*
The values were given as MEAN ± SD (n=5). *indicates significant (p<0.05) when compared with control group. One-way ANOVA followed by Dunnett's multiple comparison tests			

Table 5: Effect of LSFP on ovarian weight in Wistar rats

Group		Dose	Ovarian Weight (mg) (Per 100 g body weight)
I	Control	--	18 ± 3
II	Low dose	300 $mgkg^{-1}$	19 ± 2
III	Medium dose	500 $mgkg^{-1}$	23 ± 2*
IV	Higher dose	1000 $mgkg^{-1}$	25 ± 2*
The values were given as MEAN ± SD (n=5). *indicates significant ($p<0.05$) when compared with control group. One-way ANOVA followed by Dunnett's multiple comparison tests			

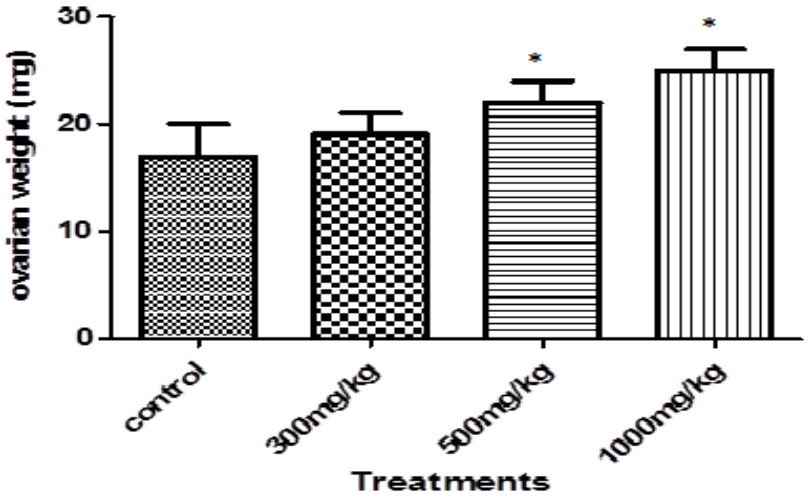

Figure 4: Effect of LSFP on ovarian weight in Wistar rats

Table 6: Effect of LSFP on relative weights of uterus in Wistar rats

Group		Dose	Relative Weight of Uterus (mg)
I	Control	--	66.28 ± 8.43
II	Low dose	300 $mgkg^{-1}$	65.77 ± 6.57
III	Medium dose	500 $mgkg^{-1}$	89.54 ± 5.18*
IV	Higher dose	1000 $mgkg^{-1}$	110.41 ± 6.39*
The values were given as MEAN ± SD (n=5). *indicates significant ($p<0.05$) when compared with control group. One-way ANOVA followed by Dunnett's multiple comparison tests			

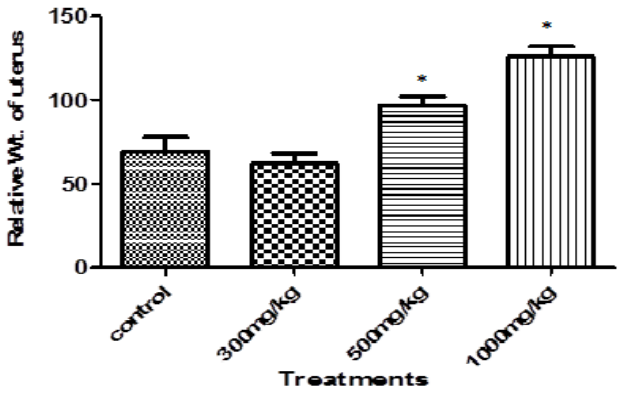

Figure 5: Effect of LSFP on relative weight of uterus on Wistar rats

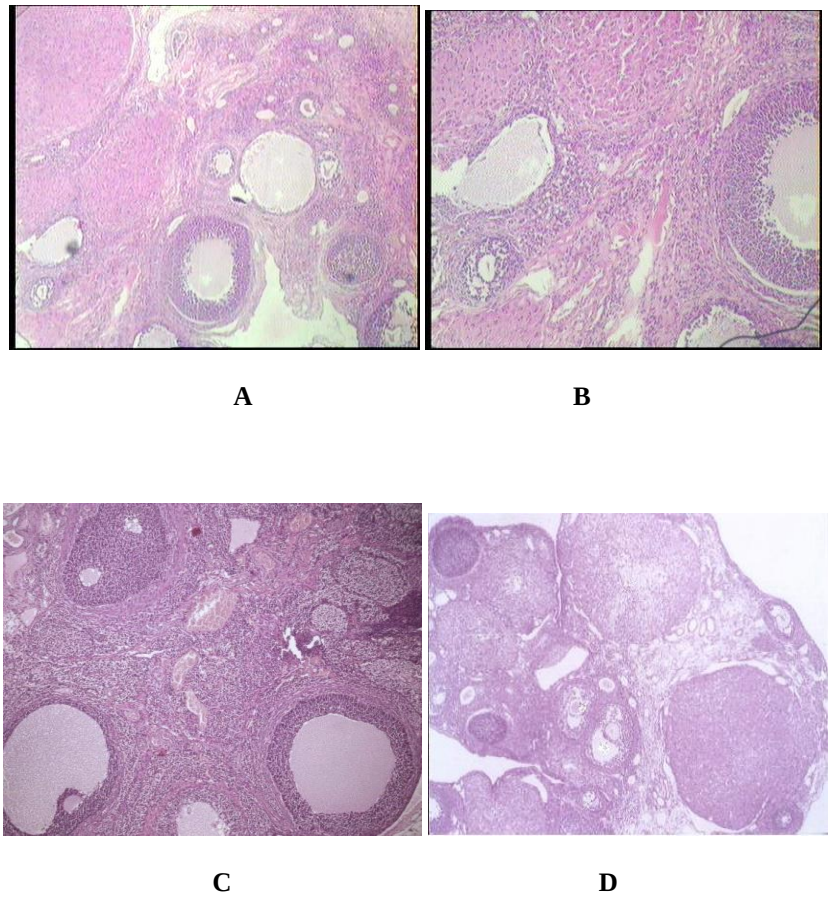

Figure 6: Photomicrographs of haematoxylin and eosin stained ovaries of Wistar rat; (A) Control (B) 300 mgkg^{-1} (C) 500 mgkg^{-1} (D) 1000 mgkg^{-1}. Animals treated with LSFP not revealed any significant change in the ovarian architecture.

DISCUSSION

The phytochemical analysis [8] of *Lagenaria siceraria* fruit revels the major chemical constituents as phytosterols (β-sitosterol, campesterol, fucosterol), flavonoids, alkaloids and 5-alpha-reductase enzyme inhibitors (oleic acid, linoleic acid, palmitic acid and steric acid). The Phytosterols, glycosides, saponins, alkaloids have a marked effect on estrogenic activities in female. Shows synergetic actions with estrogens, acts as estrogen agonists. Thus it may be the reason for alteration in circulating sex hormones levels [7]. During the preovulatory phase of the menstrual cycle estradiol helps to build the uterine lining, causing it to thicken. It is well recognized that, for the growth and development of reproductive organs estradiol is responsible. Phytochemical with estrogenic property may directly stimulate pituitary action by peripheral alteration of FSH and LH, decreasing their secretion and further blocking ovulation [6]. β-sitosterol and coumesterol have synergetic actions with estrogens [10,12], it was evidenced in increased ovarian and relative uterine weights [22] (Figures 4 and 5). Decrease in serum FSH levels (Figure 2) possibly due to negative feedback mechanism on HPG axes [7]. It might be due to exerted effect of LSFP on the anterior pituitary or the hypothalamus, since FSH secretion is controlled by the gonadotropic releasing hormone. Reduction in serum LH levels (Figure 3) indicated centrally mediated effect of phytochemicals or might be due to effect of triterpenoid or glycoside [7]. Substances capable of preventing this release could aggravate interruption of ovulation by reducing number of mature follicles or induce an oestrous cycle disruption at rest. Therefore, the decrease in the levels of serum LH may be clarified by an inhibitory effect of LSFP on the release of LH which may trigger ovulation disruption. This may end with impairment in oestrous cycle. Whereas, fall in serum progesterone levels might be possibly due to phytosterols [12,14,22,23] or alkaloids have equally responsible for inhibition of cellular progesterone synthesis. Thus it may have connected with the alkaloidal part of the LSFP. So, LSFP showed some significant changes in the amounts of serum sex hormones in female rats.

CONCLUSION

The long term administration of LSFP reveled an effect on serum sex hormonal profile in female Wistar rats, which might be contributed by the phytosterols, 5-alpha-reductase enzyme inhibitors. Further study is required to ascertain the molecular mechanism to find out exact mode of action of such phytoconstituents for such effect.

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CONFLICT OF INTEREST

No Conflict of Interest.

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