10.5005/jp-journals-10056-0010

ORIGINAL ARTICLE

An Experimental Study to identify the Source Plant of Lakshmana with Reference to Its Action on Female Infertility

Vinod Patange

ABSTRACT

Female infertility is an important medical problem which attached a sort of social stigma to mankind who are afflicted by it. Ayurveda has mentioned different medicinal plants sources to treat female infertility and one of the repeated drug mentioned in from ancient Ayurveda texts is "Lakshmana" in female infertility. But, there is a bit controversy in identifying the original Lakshmana because different medicinal plant sources have been taken into consideration as Lakshmana. This controversy can be resolved by doing animal experiments. Animal experiments on infertile female obese rats done with significant results on female infertility. Out of the three plants selected, with the control group, Euphorbia fusiformis is showing statistically significant results.

Keywords: Animal experiments on infertile obese rats, Female infertility, Lakshmana.

How to cite this article: Patange V. An Experimental Study to identify the Source Plant of Lakshmana with Reference to Its Action on Female Infertility. Int J Educ Res Health Sci 2016;2(4):57-60.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Bareness or inability to bear children is a major social stigma for women in our society. This condition can be psychological or physiological and such infertility can be corrected by appropriate treatment, modern medicine, or traditional medicine. Ayurved have tackled this problem by using different medicinal plants. Lakshmana is a drug commonly mentioned in several Ayurvedic texts that appear to have a role in enhancing fertility in females.[1-3] Different plant sources were suggested from time to rime as Lakshmana[4] and is felt that problem could be solved by doing animal experiments. Human clinical trials would have been decided, but due to ethical reasons cannot be attempted. For the study, a mutant rat strain called WNIN/ob developed at Animal facility of National Institute of Nutrition was selected. These are the first obese rats of its kind to be developed from the Wistar strain of rats, an internationally well-known strain of rats used in biomedical research all over the world.

These mutant obese rats were reported to be infertile, the colony being raised by carrier (+/–) rats from mutant stock. While the male infertility was reversible, it was found to be difficult with respect to female. The female hormonal insufficiency leading to the problem of infertility. The females have delayed puberty as judged by the time taken for the opening of vagina; 80 to 85 days in obese rats compared with 35 to 40 days seen in the lean (+/+) phenotype as well as original Wistar rats. The obese females were found to be have irregular estrus cycle, with predominantly only two-stage Pro-estrus and Di-estrus. The ovary and uterine horns are found to be smaller in size.

Thus this particular rat model has all the symptoms of infertility, which makes them suitable model for testing efficacy of the plants selected to be real Lakshmana as described in the literature.

The plants selected belong to three different groups, Euphorbia fusiformis from Euphorbiaceae family, Solanum xanthocarpum (white flowered) from the Solanaceae family and Ipomoea sepiaria from the family Convolvulaceae, and are showing morphological features similar to original plants described in the literature. It was decided that these plants processed in cow milk will be prepared in powered form as described in standard Ayurveda texts and fed at 1% through the diet to the WNIN/ob female rats at weaning age 21 to 90 days (the day by which the laboratory rats normally attains sexual maturity). The following reproductive parameters were selected for the investigation in these animals to know the phytoestogenic effects of these plant extracts, days taken for opening of vagina, estrus cycle duration, weights of reproductive organs like ovaries, uterus, and length of the fallopian tubes. Further histopathology of reproductive organs with the major organs was included to see whether these extracts have any toxic effects on these animals.

Assistant Professor

Department of Dravyaguna, SC Mutha Aryangla Vaidyak Mahavidyalaya, Satara, Maharashtra, India

Corresponding Author: Vinod Patange, Assistant Professor Department of Dravyaguna, SC Mutha Aryangla Vaidyak Mahavidyalaya, Satara, Maharashtra, India, Phone: +919028067171, e-mail: vinodpatange@rediffmail.com

MATERIALS AND METHODS

Animals

As per the original experimental design, 21 days old WNIN/ob female rats (–/–) type were supposed to be used. But the animals turned out to be heterozygous carrier rats of (+/–) phenotype. Due to lack of time, the study was continued with these animals, which was not evident earlier, till they attained 3 days of age. However, in each of the experimental groups, 1 homozygous obese female rat was included. These rats were housed in standard polypropylene cages in stainless steel top grill having facilities for keeping powdered diet cup and water bottles. An ambient temperature of 22 ± 1°C along with humidity of 50 to 60% and light dark cycles of 12 hours duration with 200 to 400 lux light intensity were maintained throughout the experiment. A standard rats chow as provided *ad libitum* to the animals.

Preparation of the Drugs

Three plant sources of Lakshmana were selected for the study. These are:

1. Euphorbia fusiformis useful part tuber
2. Solanum xanthocarpum (white flowered) useful part whole plant
3. Ipomoea sepiaria useful part whole plant.

These three plant sources were processed in cow milk as described by the ancient texts and dried under shade and a made a powder by pounding.

Experimental Protocol

Rats are divided into four groups containing seven female rats (six carrier rats and one homozygous obese rat).

Group I received standard diet containing adequate protein, fiber, carbohydrate, vitamins, and minerals in powdered form as given to the other rats in NCLAS facility.

Groups II, III, and IV received milk processed plant Euphorbia fusiformis, Solanum xanthocarpum (white flowered), and Ipomoea sepiaria at 1% of the above diet respectively.

Parameters

Weight and Food Intake

A total of 28 animals of 21 days old consisting of 24 carriers (+/–) and 4 obese (–/–) were taken from the colony. The animals were weighed weekly on an electronic balance (Essae Digi 0.1 gm sensitivity) from 21 to 90 days of age.

For food intake the observations were made from 21 to 90 days of age. The food was given to the rats in the cups daily. The remaining food was weighed next day using an electronic balance. Food efficiency ratio was calculated as per the method of floover and Nelson.

Physical Examination

Here pubertal attainment is judged by noting the days taking for opening the vagina. All the rats were observed daily for this purpose from 28 days onwards.

Preparation of the Vaginal Smear

Vaginal smear preparation: The days of the estrus cycle was observed by preparing vaginal smears as described by Nicholas. For this, first thin, small coconut broomstick, the heads of which is wrapped in cotton wool plugs (moistened with saline), was prepared. The rat was held loosely on left hand, while the vaginal margins are separated and the prepared broomstick pledge was introduced. The pledge was then rotated inside the rectum clockwise once. The material obtained from the pledge was transferred to the microscope slide. The slide was stained with methylene blue for 7 minutes. The slide was then washed to remove excess stain and the stained slide was left in a slanting position at room temperature for 10 minutes. The slide was later examined for the various stages of estrus cycle under the microscope.

Organ Weighing

At the age of 90 days animals were sacrificed by CO_2 inhalation. The major organs like liver, kidneys, and spleen along with reproductive organs were collected from the viscera of the animals and rapidly removed. They were trimmed of fat and trimmed on the blotted paper and were weighed to the nearest milligram on Essae Digi analytical balance.

HISTOPATHOLOGICAL EXAMINATION

The removed organs were fixed in the appropriate fixatives and processed for histological studies. In the present study, 10% buffered formalin was used.

Preparation of 10% formalin: This was made by mixing concentrated formaldehyde (37–48% in water) to 90 cm^3 of distilled water along with a phosphate buffer to give a pH around 7 to 7.2. This fixative was used for major organs like liver, kidney, spleen, and reproductive organs. All the organs were subjected to routine processing and 4 micron paraffin embedded sections were stained with hematoxylin and eosin for microscopic analysis.

Experimental Design

The animals were weighed weekly and food intake was monitored daily. The animals were observed from 4th week onwards daily for opening of vagina, and estrus

cycle was monitored in animals, as and when vagina opened. At the end of 90 days of feeding animals were sacrificed after weighing. All the major organs including reproductive organs were dissected and weighed. The uterine horn lengths were measured both in control and experimental group. After weighing the organs all the organs were fixed in proper fixation as described above and processed for histological analysis.

RESULTS

Weekly Weight and Food Intake

The weight and the food intake of the animals were not affected; it clearly shows the safety of the plant fed to the animals. All the data are presented in Table 1.

Pubertal Attainment

It was observed that there was no difference in the days taken for vaginal opening in experimental groups (II, III, and IV) compared with the control group. However, in homozygous obese rats of groups II and III, the vaginal opening was decreased by several days (group II – 45 days, group III – 59 days, group IV – 77 days and group I – 78 days).

Estrus Cycle

In the experimental group, 83% (/56) of group II female shows regular estrus cycle (4–5 days cycle with all 4 stages) compared with 66% (4/6) in group III, 33% (2/6) in group IV, and 16% (1/6) in control group I.

Reproductive Organ Weights

The reproductive organ weights are given in Table 2. There was a significant difference in the weight of the uterus in experimental group II (0.54 ± 0.03) as compared with control group I (0.46 ± 0.02), group III (0.47 ± 0.03), and group IV (0.44 ± 0.02).

The length of the uterine horn is slightly increased in group II (3.2 ± 0.20) as compared with groups I, II, and IV which were 3.18 ± 0.09, 3.3 ± 0.23, and 3.4 ± 0.24 respectively. The weight of the ovary found to be increased significantly ($p < 0.01$) as compared with group I (0.09 ± 0.005), III (0.08 ± 0.01), and IV (0.09 ± 0.004).

In the homozygous obese rats, it was seen that the weight of the uterus was improved in group II, which was 0.40 gm as compared with control group (0.31 gm), III (0.30 gm), and group IV (0.30 gm). The length of the uterine horns was also increased in group II (4.2 cm) as compared with control group I (3.0 cm), III (3.7 cm), and IV (3.9 cm). The weight of the ovaries also was increased in group II (0.10) as compared with control (0.07), III (0.08), and IV (0.07).

Histopathology

It was observed that there was no gross histological change in any of the organs studied in all groups. However, on microscopic examination of various tissues, it was observed that all 4 carriers (4/5) in groups I and II carriers in group II (2/5) showed presence of focal calcification at tubular levels in the kidneys. The calcification was also confirmed by special stain (Von Kosaa method)

Table 1: Weights and average food intake

Groups	*21st day body weight*	*90 days body weight*	*Weight gain*	*Average food intake per week*	*FER gain in body weight 100/food intake*
I	52.5 ± 10.50	255 ± 7.62	202	115	175.65
II	42.16 ± 14.72	249 ± 7.13	203	118	176.52
III	47.16 ± 9.37	241 ± 8.10	195	116	165.25
IV	47.33 ± 12.34	239 ± 6.19	188	115	162.06

FER: Food efficiency ratio

Table 2: Organ weights

Groups		*Uterus weight with fallopian tubes*	*Ovary weight*	*Uterine horn length in cm*	*Liver weight*	*Spleen weight*	*Kidney weight*
I	∑	2.76	0.54	20.4	63.94	4.13	11.76
	σ	0.46 ± 0.04	0.09 ± 0.01	3.4 ± 0.59	10.65 ± 1.25	0.68 ± 0.03	1.96 ± 0.1
II	∑	3.26	0.68	21.1	66.53	4.06	12.11
	σ	0.54 ± 0.06	0.11 ± 0.02	3.52 ± 0.48	11.09 ± 0.70	0.68 ± 0.02	2.02 ± 0.14
III	∑	2.80	0.50	19.1	63.54	3.84	11.51
	σ	0.47 ± 0.04	0.08 ± 0.02	3.18 ± 0.22	10.59 ± 0.87	0.64 ± 0.03	1.92 ± 0.13
IV	∑	2.65	0.52	19.8	64.63	3.75	11.05
	σ	0.44 ± 0.13	0.09 ± 0.01	3.3 ± 0.57	10.77 ± 0.33	0.63 ± 0.03	1.84 ± 0.08

Values are mean ± standard deviation; $p < 0.003$ significant by Duncan multiple ANOVA. One group is significant

where calcific areas appear black. Rest of the kidneys were normal. All the remaining organs were essentially unremarkable and showed no toxicological changes, which could be attributed to the use of herbal products fed in their respective diets.

DISCUSSION

The objective of the experiments was to find out whether the three plant sources selected based on the literary review has phytoestrogenic activity. And among the three, which can be the real source of Lakshmana based on the observation made on the experimental animals. The obese mutant rats were selected as ideal animal models for the study as homozygous females are having morphological and physiological change in their reproductive organs. It would have been ideal to have homozygous obese rats (–/–) only for the study but unfortunately the animals selected based on the body mass index (at 21 days) turned out to be heterozygous carrier type and only single homozygous obese female assigned in each of the group. In spite of this major drawback, this preliminary study still allowed to draw the correct interference, which was made earlier by the literary review.

Earlier the literature survey had indicated that the local plant Euphorbia fusiformis could be real source of Lakshmana mentioned in Ayurvedic literature. The experimental study described earlier also supports this view even in carrier rats (+/–) and this plant substantially increased the weight of the uterus, ovary, and regularized the estrus cycle compared with the control and other experimental groups. This was also reflected in homozygous female (–/–) that was fed the same plant. The carrier (+/+) and homozygous obese rats in other experimental groups (group II to IV) did not show much physiological and morphological changes compared with control (group I). The other two plants may be used as possible substitute for the actual Lakshmana represented by Euphorbia fusiformis. Histological analysis of major organs along with reproductive organs did not show any toxicological effects from the plant except for the calcification of the kidney, both in the control and one of the experimental groups, which anyway cannot be attributed to any toxicological effect on the part of the plant. The weights and the food intake of the animals were also not affected; it clearly shows the safety of the plant fed to animals.

CONCLUSION

However, there are certain limitations to the present study, like unavailability of homozygous obese animals in large numbers for the study, which would have proved the point more conclusively. Yet, the indication given in this study is worth pursuing in more detail and see that the plant Euphorbia fusiformis can really be used for reversing infertility first in animal models like obese rats and later in humans as well. The histopathological analysis of various organs in experimental groups did not show any toxicological effect and this drug can be used well for its therapeutic use. Though the study was preliminary in nature, it gave enough pharmacological indications by way of action to identify Euphorbia fusiformis as the possible source of Lakshmana.

ACKNOWLEDGMENTS

- Dr M Vidyasagar, Retd. Professor, Guide; Dr BRKR Govt Ayurved College, Hyderabad.
- Dr NV Giridharan, Co Guide, NCLAS, Asst Director, NIN (ICMR), Hyderabad.
- NCLAS, National Institute of Nutrition (ICMR) for providing Animal Experimental facility.

REFERENCES

1. Krushnamurthy MS. Basavarajiyam. 1st ed. Chapter 15, quotation 13. Choukhamba Orientalia; 2014. p. 388.
2. Gadnigraha, Vandhyadhikar, quotation 5. Ayurvediya Granthamala no 12, Vaidya Jadavji Trikamji Acharya, ed. 1915 AD; p. 613.
3. Rajamartand, Streerogadhikar, quotation 318. Dvitiya Sanskaran, Vaidya Jadavji Trikamji Acharya, ed. 1924. p. 48.
4. Nighantu B, Varga G, quotation 39. Commentary by Chunekar KC, Choukhamba Bharati Academy, Reprint 1999. p. 290.