AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Anti-Ovulatory Activity of Siddha Polyherbal Formulation Maavilingathy Mathirai in Female Wistar Albino Rats

B. Sathya[1*], V. Velpandian[1], M. Pitchiah Kumar[1], M. Ramani[1], V. Banumathi[1]

1. Post graduate department of Gunapadam (Pharmacology), Government Siddha Medical College, Chennai, Pin- 600 106, Tamilnadu, India

ABSTRACT

The present study was aimed to evaluate the antiovulatory activity of the Siddha polyherbal formulation Maavilingathy Mathirai in female Wistar albino rats. Female adult mature non pregnant Wistar albino rats weighing 120 to 200 g with at least three regular estrous cycles were administered two doses of 100 and 200 mg/kg orally for 15 days and the control group received 2 ml/kg of CMC solution. On the 16th day all the animals were sacrificed. The effect of the drug on estrous cycle was studied. The proestrous phase of the rats at dose levels of 100, 200mg/kg were found to be highly significant ($P < 0.01$) when compared with the control. The decrease in median duration of the estrous phase of the rats at dose levels of 100, 200mg/kg were found to be highly significant ($P < 0.01$) when compared with the control. Metestrous phase was also significantly reduced at dose levels of 100, 200mg/kg as compared to the control and the p value was found to be extremely significant.The results also showed that the p value of ovarian weight at dose levels of 200 mg/kg was found to be highly significant. The cholesterol value of the ovarian tissue at the doses of 100, 200 mg/kg were found to be significant ($p<0.05$) when compared with the control.Hence the drug proved to have a better antifertility effect on the administration of 100, 200 mg/kg dose levels.

Keywords: antiovulatory, Maavilingathy Mathirai, estrous phase, Wistar albino rats

*Corresponding Author Email: sathyagsmc@gmail.com

Received 03 September 2014, Accepted 7 September 2014

Please cite this article in press as: Sathya B *et al*., Anti-Ovulatory Activity of Siddha Polyherbal Formulation Maavilingathy Mathirai in Female Wistar Albino Rats. American Journal of PharmTech Research 2014.

INTRODUCTION

The biggest ever threat faced by the people nowadays, is boom in population. India is the 2nd populous country in the world comprising about 1.21 billion according to 2011 census[1]. The world's rising population has severely depleted the land required for forests, water, air, minerals and other natural resources resulting in scarcity of food and bags the reason for accumulation of organic waste, pesticides, industrial waste, radioactive nuclear waste and other man-made pollution. These reasons forced mankind to implement contraception to cut off population size and it has become not only an important environmental issue, but also an essential topic of human existence. So in this regard, a significant progress has been made in the field of family planning methods in which many chemical formulations including hormonal and synthetic steroids have been used. Most of the medicines bear many side effects with them. In 2005, the World Health Organization officially classified oral contraceptives as Group I carcinogens [2] causing high blood pressure, blood clots, stroke, heart attack, depression, weight gain, migraines, irregular menstrual cycles. The hormonal contraceptives can also cause prolonged cycles and result in unexplained fertility when they are discontinued. Hence the community is in great need of an adverse free, safe medicine. To handle with this situation herbal preparations would be the suitable choice. A great attention is focused on such herbal formulations as on now. Herbs are safe and highly efficacious natural remedies for better health. The traditionally followed folkloric reputed plants for antifertility have been described in detail[3]. Several plants of Indian origin have been tested for their antifertility activities individually, through modern techniques and proved as well[4]. Many polyherbal formulations are also available in the traditional system of medicine. But polyherbal formulations have not been subjected to validate their potentials through scientific methods. Maavilingathy Mathirai is one among the polyherbal formulation said in the literatures as an effective contraceptive medicine comprising eight herbs each possessing anti-fertility activity of it's own by inhibiting ovulation or preventing fertilization or by exerting anti-implantation activity or by causing early abortion or acting as an anti-implantation agent. Hence an attempt was made in identifying such effective polyherbal formulation and detailing the anti-ovulatory effect in female Wistar albino rats by which the medicine works.

MATERIALS AND METHODS

Collection of raw ingredients

The drug "Maavilingathy Mathirai" was prepared as per the steps mentioned in the classical Siddha literatures[5]. The eight ingredients integrated in the preparation were stem bark of *Crataeva*

magna, leaves of *Bambusa arundinaceae*, rhizomes of *Alpinia officinarum*, *Zingiber officinale*, dried fruits of *Piper nigrum*, *Piper longum*, seeds of *Anethum graveolens*, root bark of *Plumbago zeylanica* and lemon juice as an associate drug. The individual raw drugs were procured from various parts of Tirunelveli, Nagercoil districts and Chennai in Tamilnadu. All the individual drugs were recognized and authenticated by experts of Pharmacognosy Department of Siddha Central Research Institute, Chennai and faculties of Department of Gunapadam, Govt. Siddha Medical College, Chennai. A sample specimen of each ingredient was labeled individually and retained in the department for future reference.

Purification and Preparation of Maavilingathy Mathirai

All the ingredients were cleaned by removing dust, sand, mud and other impurities. They were dried in shade for complete removal of moisture. On complete drying, all the ingredients were purified as per the procedures mentioned in the classical Siddha texts[6]. 70 gm of each ingredient was taken and finely pulverized, sieved through a white cloth[7], filtered, weighed and 50 gms of each Chooranam was taken and triturated with lemon juice using stone mortar and made into pills weighing 130mg, dried, weighed, packed and preserved in bottles labeled as MLM.

Animal Selection

Healthy virgin adult female Wistar albino rats weighing 120-200 gm were procured from The King Institute of Preventive Medicine, Guindy and they were maintained under standard conditions of humidity, temperature (20- 24°C) and light (12 h light: 12 h dark cycle) with rodent pelleted diet of Sai meera foods with free access to water in polypropylene cages and were acclimatized for one week under laboratory conditions of the research centre, Sairam Advanced Centre For Research, Tambaram, Chennai after obtaining Institutional Animal Ethical Committee clearance bearing the number 1545/po/a11/CPCSEA/1-14/2013.

Acute toxicity study

Acute toxicity study of Maavilingathy Mathirai was carried out in Wistar Albino rats according to OECD guidelines 423[8]. Different doses up to 2000 mg/kg, p.o. was administered and the rats were observed for behavioral changes, toxicity and mortality up to 48 hrs. There was no toxic reaction or mortality observed. Based on the results of acute toxicity we have chosen 100 mg/kg and 200 mg/kg as test doses for the evaluation of antifertility activity.

Evaluation of Anti-Ovulatory Activity in Female Albino Wistar Rats

The anti-ovulatory method was described as per the procedure mentioned by Koneri et al [9]. In this procedure, healthy adult female Wistar rats were administered the drug and the effect of the drug

on estrous cycle was studied along with the estimation of ovarian weight and cholesterol to rule out whether they are estrous cycle distruptors.

Preparation of vaginal smear

Vaginal smear was prepared in order to sort out the phase of estrous cycle of rats by introducing a drop of distilled water into the vagina with the help of a dropper, collecting back and placing it on a clean slide after adding a drop of glycerin. The prepared smear was examined microscopically under low power for different types of cells. There are four phases in estrous cycle of rat.

- **Proestrous phase** is defined by the presence of large number of rounded nucleated cells.
- **Estrous phase** indicated the presence of more than 50% non-nucleated cornified epithelial cells.
- **Metestrous** phase was indicated by the presence of many neutrophils and scattered squamous epithelial cells with a lot of cell debris in the smear.
- **Diestrous phase** indicated the presence of majority of leucocytes.

Methodology

The female Wistar Albino rats exhibiting three regular menstrual cycles were divided into 3 groups of 6 animals each in estrous phase at the start of the study were fasted over night with free access to water. The drug was administered orally, daily for 15 days in such a way to complete three regular estrous cycles.

The suitable sensitive rats were divided into three groups of six each as follows:

- Group I Normal Control animals were given 2ml/kg of CMC solution.
- Group II animals were administered 100 mg/kg of MLM for 15 days,
- Group III animals received a dose of 200mg/kg of MLM for 15 days

The vaginal smear of each rat was observed every day between 9-10 A.M. The rats were sacrificed under ether anesthesia on 16^{th} day and the ovaries and uterus of the rats were dissected out and weighed on a Precisa, XB series sensitive balance. Biochemical analysis was done in one processed ovary from each rat. Right ovarian tissue homogenate was prepared in cold saline in the ratio of 1:20 (20µLof saline added per mg of tissue). Cholesterol estimation was done using Aspen Laboratory Pvt. Ltd., Delhi supplied kit. Values are in the units of mcg/mgm.

Statistical analysis

Statistical significance of data was assessed by analysis of variance (one-way ANOVA) followed by a comparison between groups using Dunnet's test. Values were expressed as mean ± S.E.M indicating *P<0.05 as significant **P<0.01 as highly significant and ***P<0.001as extremely significant values.

RESULTS AND DISCUSSION

The results of antiovulatory activity were tabulated in the table.1 and figure.1 below.

Table 1: Effect on MLM on the duration of phases on the estrous cycle in rats

Groups	Treatment	Dose	No. of days spent in each phase			
		mg/kg	Proestrous	Estrous	Metestrous	Diestrous
I	Control	(2%CMC)	2.06±0.53	3.37±0.34	4.00±0.28	5.58±0.37
II	MLM	100	6.50±0.43**	1.26±0.28**	1.41±0.2**	5.16±0.47
III	MLM	200	7.42±0.8**	0.75±0.6**	0.75±0.35**	4.86±0.32

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control;n=6

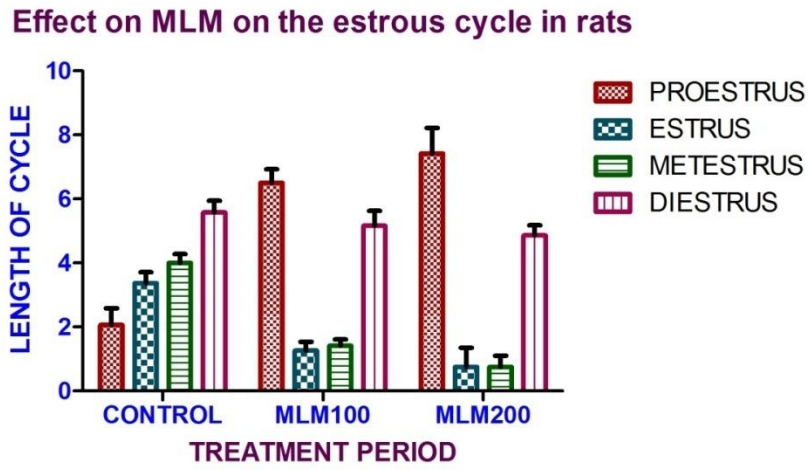

Figure.1. Effect on MLM on the duration of phases on the estrous cycle in rats

Effect on the estrous cycle

The proestrous phase of the rats at dose levels of 100, 200mg/kg were found to be highly significant ($P < 0.01$) when compared with the control. This signifies a remarked increase in proestrous phase. The decrease in median duration of the estrous phase of the rats at dose levels of 100, 200mg/kg were found to be highly significant ($P < 0.01$) when compared with the control. Metestrous phase was also significantly reduced at dose levels of 100, 200mg/kg as compared to the control and the p value was found to be extremely significant.However, there was no significant change in the duration of the diestrous phase between the groups. During the estrous cycle of rats, many morphological, histological, physiological, biochemical changes occur in the ovary, corpus luteum and uterus. Ovulation takes place under the united effect of ovarian and uterine hormones during the maturation of pre-ovulatory follicles. Imbalance in these hormones leads to irregularity in the ovarian functions and duration of the estrous cycle [10]. The estrous cycle in the rats treated with MLM (100 and 200mg/kg) showed a decrease in the duration of estrous and

the metestrous phases and increase in the duration of the proestrous phase. Absence of maturation of graffian follicle was indicated by the prolongation of the proestrous phase. Non-availability of matured graffian follicle was indicated by reduction in the estrous and the metestrous phases. It was evident that, the extract of plumbago leaves given to albino rats distrupted the estrous cycle by inhibiting the ovulation with prolonged diestrous phase[11]. The extract of *Anethum graveolens* also prolonged the diestrous phase according to a recent research[12]. Hydroalcoholic extract of *Bambusa arundinacea* leave also showed reduced duration of estrous and metestrous phases, characterized by a prolongation of the diestrous phase[13]. Since the formulation contained certain drugs, it was hypothesized that the drug might exert certain action. Therefore the above facts supported inhibition of ovulation and the results strongly supported the fact that the drug exerts antiovulatory activity. The results (figure.2, table.2 showed that the p value of ovarian weight at dose levels of 200mg/kg was found to be highly significant. The cholesterol value of the ovarian tissue at the dose of 100,200 mg/kg were found to be significant($p<0.05$) when compared with the control. Phytochemical analysis of the extract of MLM revealed the presence of alkaloids, glycosides, saponins, tannins and phenolic compounds. These phytochemicals are responsible for estrous cycle disruption[14]. Net weight of the ovary includes aggregate weight of three endocrine tissues, the stroma, the follicle and corpus luteum. During the estrous cycle the weight of the ovarian tissue increases under the influence of gonadotrophic and steroidal hormones [15].

Table 2: Effect of MLM on Ovarian weight and Cholesterol level in the body

Group	Treatment	Dose(mg/kg)	Rt. Ovarian weight mg/100mg	Cholesterol level mcg/mg
I	Control	2%CMC	41.34±2.49	3.02±0.68
II	MLM-100	100	39.13±2.08	5.55±0.57*
III	MLM-200	200	31.18±1.8**	5.38±0.54*

Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control**;** n=6

The decrease in the weight of ovaries of the rats treated with MLM indicates a decrease in the activity of the stroma, the follicle, and the corpus luteum in the ovary which may be due to non-availability of gonadotrophic or steroidal hormones or both. Atretic follicles are degenerating preovulatory follicles which were increased in number corresponding with the decrease in the number of graffian follicles. The degeneration of atretic follicles is due to the need of steroidal hormones, non-availability of local estrogen produced by granulosa cells or imbalance in endogenous steroids, protein and hormones. The presence of increased atretic follicles in the rats treated with MLM, compared with control rats, indicates that the MLM promotes the degeneration

of preovulatory follicles. Since cholesterol is the precursor for the steroidogenesis of ovarian endocrine tissues, the significant increase in ovarian cholesterol in the treated groups 100 and 200 mg/kg indicates that cholesterol was not used for steroidogenesis. Hence this drug was proved to have a significant effect on the ovaries of the rats.

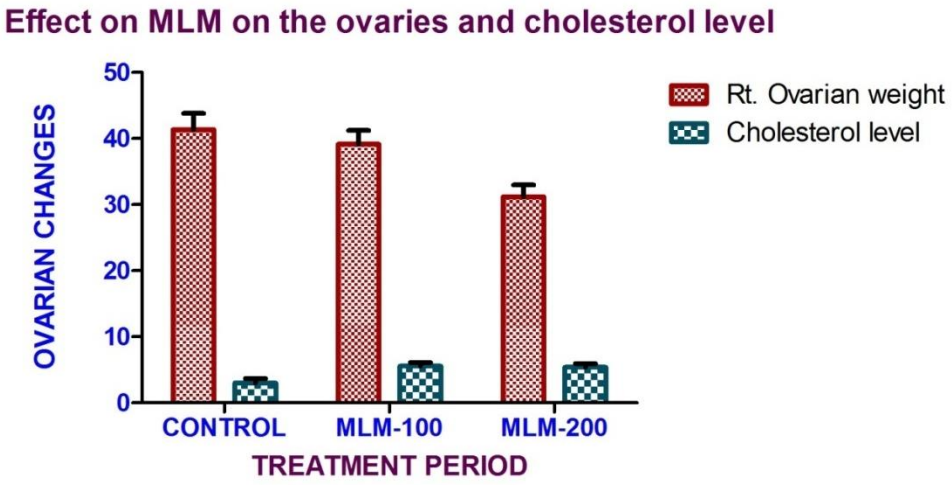

Figure.2. Effect of MLM on Ovarian weight and Cholesterol level in the body

CONCLUSION

The pharmacological activities like antiovulatory activity was studied thoroughly to prove the potency of the drug. In antiovulatory activity, the effect of MLM on estrous cycle was studied. The increase in duration of the proestrous phase, decrease in the duration of the estrous and metestrous phase of the cycle lucidly explains inhibition of ovulation and hence the activity is strongly supported by the data. The antiovulatory activity of the drug was also assessed through estimation of the ovarian weights and the cholesterol estimation of the ovaries. The reduction in weight of ovaries was based on dose dependent manner and the drug at the dose level of 200mg/kg remarkedly reduced the weight of the ovaries. This clearly indicates the atrophy of the ovarian tissue and favours the activity. The cholesterol values of the ovaries significantly increased at both the dose levels of the drug indicating the accumulation of cholesterol in the ovaries and not used for ovulation, conception and pregnancy. Further research should be done for the analysis of the specific phytochemical agent and for specific effect on target tissue so as to develop a foolproof contraceptive. Also the distruptors of estrous cycle either hormonal changes or by other means should be confirmed. The drug Maavilingathy Mathirai had beneficiary values for the therapeutic efficacy for antifertility and hence it can be concluded that Maavilingathy Mathirai could be a scientifically validated and proven drug for the antifertility effect. The world and the health community interested in awareness about population explosion would be benefitted by this drug.

ACKNOWLEDGEMENT

The authors were thankful to the Assistant Professor, Sairam Centre for Advanced Research, Tambaram, Chennai for the assistance provided in conducting the pre-clinical studies and also grateful to the students of Department of Gunapadam (Pharmacology) of Govt.Siddha Medical College, Chennai for their valuable support.

REFERENCES

1. Census of India, 2011, Provisional Population Totals, Paper 2, Volume 1 of 2011; Rural-UrbanDistribution. [Cited 2014, May 5]. Available from http://censusindia.gov.in/2011-prov-results/paper2/prov_results_paper2_india.html//.
2. Carcinogenicity of combined hormonal contraceptives and combined menopausal treatment, Department of Reproductive Health and Research, World Health Organization, September 2005. [Cited 2014, April 14]. Available from http://www.who.int/reproductive-health//.
3. Farnsworth N.R, BingelA.S, Cordell G.A, et al. Potential value of plants as sources of new antifertility agents. J. pharm. Sci 1975; 64:535-98.
4. Sharma R, Chopra G, Bhushan G, Munjal K. Use of Indian plants in fertility regulation in mammals. J. Exp. Zool. India, 2003; 6:57-74.
5. Shanmugavelu M, Naidu GD. The Pharmacopoeia of Siddha Research Medicine, ILWA Press, Coimbatore 1973;126-7.
6. Anonymous, Sarakku Suthi Muraigal, Published by Siddha Maruthuva Nool Veliyitu Pirivu, Indian Medicine and Homoeopathy deptt., First edition, 2008;6-13.
7. Kannusamy pillai C, Sikitcha Rathna Deepam Ennum Vaidiya Nool, Published by B. Rathina Nayakker and sons, Vol 1, 3rd edition, 1991;38-40, 30-32.
8. Organization for Economic Cooperation Development (OECD) Guideline, 423, 2000. Guideline Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24. Adopted Sep 2001. [Cited May 7].
9. Koneri R, Balaraman R, Saraswati CD. Antiovulatory and abortifacient potential of ethanolic extract of roots of momordica cymbalaria Fenzl in rats. Indian J Pharmacol 2006; 38(2):111-4.
10. Prakash AO, Mathur R. Studies on oestrous cycle of albino rats: Response to embelia ribes extracts. Planta Medica 1979;36:131-41.
11. Edwin S, Joshi S.B, Jain D.C, Antifertility activity of leaves of Plumbago zeylanica Linn. in female Albino rats, Euro Journal of Contraceptive Reproductive Health Care.2009;14(3): 233-39.

12. Monsefi M, Ghaesemi M, Bahaoddini A, The effects of Anethum graveolens on the reproductive system. Phytotherapy research 2006;14:131-5.
13. Musa TY, Bimbo BB. Abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. Contracep. 2009;80:308–13.
14. Shivalingappa H, Satyanarayan ND, Purohit MG, Sharanabasappa A, Patil SB. Effect of ethanol extract of Rivea hypocrateriformis on the estrous cycle of the rat. J Ethnopharmacol 2002; 82(1):11-7.
15. Patil SJ, Patil SB. Antiovulatory activity of Petroleum ether extract of chromatographic fractions of Citrus medica seeds in Albino rats. Journal of medical Sciences 2013;13:410-417