Original Article

Validation of Abortifacient Potential of Siddha Polyherbal Formulation Maavilingathy Mathirai in Female Wistar Albino Rats

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ABSTRACT

Objective: To study the abortifacient potential of the Siddha polyherbal formulation Maavilingathy Mathirai in Wistar Albino rats. **Materials and Methods:** Male and Female albino rats of Wistar strain weighing about 120-200 gm in the ratio of 1:2 were caged. Pregnancy was confirmed and the rats were divided into three groups of six animals each and the rats were administered the drug through gastric gavage from 6^{th} to 15^{th} day of pregnancy. The 3 groups of animals were administered 2ml/kg of CMC, 100 mg/kg, 200mg/kg of MLM for 10 days. The animals were laparotomised on 19th day of pregnancy and the uterine horns were subjected for the examination of the live and dead fetuses, implantation sites, resorptions. The serum was separated and let down into a vial and then and subjected to ELISA method for the assessment for LH, Estradiol, FSH and Progesterone.
Results and Discussion: The MLM 100 and MLM 200 reduced the no. of implants in the uterus significantly. The percentage of abortion calculated revealed that it was 34% at the dose level of 100mg/kg and 69% at the dose of 200mg/kg and a significant abortifacient effect indicating the reduction of the number of viable fetuses.FSH hormone level is highly significant ($p<0.01$) at both the dose levels of MLM100, 200mg/kg. The results showed significant decrease in LH at a dose level 0f 100mg/kg and highly significant decrease at the dose level of MLM200mg/kg. Estradiol hormone is highly significant ($p<0.01$) at both the dose levels of MLM100, 200mg/kg. Progesterone hormone is also highly significant ($p<0.01$) at both the dose levels of MLM100, 200mg/kg. The reduction in the level of hormones proved to be the prime reason for abortifacient activity.

Keywords: Abortifacient, MLM, Estradiol, Implants, Resorption

sites.

INTRODUCTION

Plants which are the main source of innumerable medicines widely practiced today are the prime agents of healing ailments. The significance of plants as a source of abortifacients has been stressed by many research scientists**.** An abortifacient is an agent causing abortion by meddling with the development of the foetus in the uterus[1]. The U.S. Department of Health, Education and Welfare defined abortion as follows "All the procedures which harm the viability of the fertilized zygote between fertilization and completion of labor is strictly called as abortion[2]. In India abortion is legally accepted only up to twenty weeks of gestation under certain critical circumstances. Those conditions are broadly defined as the risk of continuance of gestation in two conditions such as involving a risk to the life of the pregnant woman or of grave injury of physical or mental health, or there is a considerable risk of suffering of the child from such physical or mental abnormalities as to be seriously handicapped. Such abortifacients are mainly used to prevent the birth of defective child and to prevent the birth of illegal child. Certain conditions like ectopic pregnancy, sexually transmitted diseases, muscular dystrophy and Down Syndrome can be prevented by using effective herbal abortifacients.

Abortifacient herb is an agent inducing termination of pregnancy prematurely causing an abortion. The implanting action of the progesterone is blocked when given, during first five weeks of pregnancy causing sloughing of the embryo from the uterus.

The commonly used chemical abortifacients are Mifepristone and Misoprostol. But the temporary family planning techniques to control unwanted pregnancies like abortifacient pills in which many chemical formulations including hormonal. and synthetic steroids bear many side effects with them. The common side effects are high bleeding, nausea, headache, fatigue, fever, diarrhea, vomiting, hot flushes, cramping, abdominal pain and sepsis[3]. The hormonal contraceptives can also cause prolonged cycles and results in unexplained fertility when they are discontinued.

Hence the society is in great need of an adverse free, safe abortifacient. To overcome this situation herbal preparations would be the suitable choice. Herbal abortifacient agents would be of enormous benefit, especially to residents of developing countries since the cost and safety of these drugs would be within means. 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 abortifacient have been described in detail[4]. Several plants of Indian origin have been tested for their abortifacient activities individually, through modern techniques. 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 abortifacient medicine comprising eight herbs each possessing pharmacological activity of its own by causing early abortion. There has not been any scientific evidence in the literature that substantiated or reputed this claim. Hence an attempt was made in

identifying such effective polyherbal formulation and detailing the abortifacient activity in female Wistar albino rats by which the medicine works.

MATERIALS AND METHODS

Collection of raw ingredients

The drug "Maavilingathy Mathirai" was prepared in steps according to the procedures mentioned in the classical Siddha literatures[5]. The eight ingredients included in the formulation were *Crataeva magna* (stem bark), *Bambusa arundinacea* (stem bark), *Alpinia officinarum* (rhizome), *Zingiber officinale* (rhizome), *Piper nigrum* (dried fruits), *Piper longum* (dried fruits), *Anethum graveolens* (seeds), *Plumbago zeylanica* (root bark) and lemon juice as an associate drug. The individual raw drugs were collected from various parts of Tirunelveli, Nagercoil, and Chennai in Tamilnadu. The drugs were identified individually and authenticated by experts of Pharmacognosy Department of Siddha Central Research Institute, Chennai and faculties of Department of Pharmacology, Govt. Siddha Medical College, Chennai. The specimen sample of each ingredient was labeled individually and kept in the department for future reference.

Steps in the preparation of Maavilingathy Mathirai

The ingredients were cleaned well by hand sorting to remove sand, dust, mud and other impurities and dried in shade. After complete drying, all the ingredients were purified as per the processes mentioned in the classical Siddha texts[6]. The above ingredients each of 70 gm were taken and finely pounded, sieved through a white cloth[7], filtered, weighed and 50 gms of each powder was taken and triturated with lemon juice using stone mortar and pestle and made into pills with weight of 130mg, dried, weighed, packed and labeled as MLM in glass bottles.

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, any toxicity and mortality up to 48 hrs. There was no toxic reaction or mortality. Based on the results of acute toxicity we have chosen 100 mg/kg and 200 mg/kg as test doses for the evaluation of abortifacient activity.

Evaluation of abortifacient activity in female wistar albino rats

Animal selection

The different doses of MLM were tested in female albino rats for abortifacient activity as per Khanna *et al*[9]. Male and Female albino rats of Wistar strain weighing about 120-200 gm in the ratio of 1:2 were used. Colony bred female Wistar albino rats were caged with male rats of known fertility in the ratio of 2:1 in the evening of proestrous. On the next day the rats were examined for the presence of sperms. Pregnancy was confirmed by the presence of thick clumps of spermatozoa in the vaginal smear[10] and the

rats were separated and that day was designated as day one of pregnancy. Routine affirmation of mating in the rat is done usually by checking for the copulatory plug or presence of spermatozoa in the vaginal lavage. The pregnant rats were divided into three groups of six animals each and the rats were administered the drug through gastric gavage from 6th to 15th day of pregnancy.

- Group I Normal Control animals were given 2ml/kg of CMC solution for 10 days.
- Group II animals were administered 100 mg/kg of MLM for 10days.
- Group III animals received a dose of 200mg/kg of MLM for 10 days.

At the end of experiment, the animals were laparotomised under light ether anesthesia on the 19th day of pregnancy for observation of full term of the fetuses[11]. The uterine horns were subjected for the examination of the live and dead fetuses, implantation sites, resorptions. Also the percentage of abortion was calculated as per the formula,

Percentage of abortion = (No. of implantations - No. of live fetuses) x100 / No. of implantations.

Statistical analysis

The statistical significance between groups was analyzed using the one-way ANOVA test followed by a comparison between different groups using Dunnett's test. Values are expressed as mean ± S.E.M. Significant value is indicated by *P<0.05, highly significant value by **P<0.01, extremely significant value indicated by ***P<0.001respectively.

Evaluation of hormonal assay in female albino wistar rats

Blood samples of the rats were collected by cardiac puncture technique in centrifuge tubes. The blood was allowed to stand for 10 minutes to clot at room temperature and centrifuged at 3000 r/min for 10 minutes. The serum was separated and let down into a vial and then and subjected to ELISA method for assessment for LH, Estradiol, FSH and Progesterone.

Procedure of ELISA[12]

0.05 ml of the serum was pipetted into the assigned wells of the control and doses. LH enzyme reagent at a quantity of 0.001 ml was added respectively to all the wells. The micro plate was swirled for 20-30 seconds and covered, incubated at room temperature for 1 hour. This process resulted in the formation of the precipitate along with a clear fluid. The decanted fluid was added with 350 ml wash buffer and the decantation process was repeated for 3 times. 100µl working substrate solution was added to the wells and incubated for fifteen minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm within 30mins in a micro plate reader. A standard curve was obtained by plotting the mean absorbance values of the control, test drug against its concentration from the standard curve. The same procedure was repeated for estimation of Progesterone, Estradiol and FSH using Progesterone - enzyme reagent, Estradiol-enzyme reagent, FSH –Enzyme reagents.

Statistical analysis

The statistical significance between groups was analyzed using the one-way ANOVA test followed by a comparison between different groups using Dunnett's test. Values are expressed as mean ± S.E.M denoted by *P<0.05-significant, **P<0.01-highly significant and ***P<0.001-extremely significant.

RESULTS AND DISCUSSION

The results of abortifacient activity and hormonal assay were described below in the table 1. At 100 mg/kg the drug showed

significant value of p<0.05 and 200mg/kg the drug MLM showed a highly significant (P< 0.01) abortifacient effect indicating the reduction of the number of viable fetuses and it was shown in the figure 1. Abortion refers to the premature expulsion of the products of conception from the uterus. However, it reduced the number of viable fetuses.

Insufficient progesterone secretion by the corpus luteum or placenta, also termed a luteal phase defect, might be a reason to cause abortion. The drug MLM showed significant abortifacient activity at the dose of 100 and highly significant at a dose of 200 mg/kg and showed a dose dependant activity. Therefore, this could be a possible cause for the significant increase in percentage of abortion and abortifacient effect.

Significant reduction in the hormonal levels of FSH, LH, Estradiol, Progesterone was recorded statistically as shown in the table 2. FSH hormone level is highly significant (p<0.01) at both the dose levels of MLM-100, 200mg/kg and it is shown in the figure 2. The results showed significant decrease in LH at a dose level of 100mg/kg and highly significant decrease at the dose level of MLM 200mg/kg and it is shown statistically in the figure.2. Estradiol hormone is highly significant (p<0.01) at both the dose levels of MLM-100, 200mg/kg and it is shown in the figure 3. Progesterone hormone is also highly significant (p<0.01) at both the dose levels of MLM-100, 200mg/kg and it is shown in the figure 4.

Follicle stimulating hormone

Follicle stimulating hormone is the prime hormone of reproduction in mammalian groups. FSH is mainly responsible for the development of male and female reproductive gonads, maturation at the time of puberty and for the genesis of mature graafian follicles during the fertile period[13]. The hormone acts directly on the receptors located on the granulosa cells.

The reduction in the levels of FSH by the MLM-100 and MLM-200 may impede folliculogenesis and interrupt the maturation of the follicle in the preovulatory phase[14]. It may be due to the fact that the drug might exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus. The reduction in the levels of the hormone may adversely affect conception in the female rats.

Luteinizing Hormone

Luteinizing hormone stimulates secretion of sex hormones from the gonads. In rats, Luteinizing hormone during the pre-ovulatory period suddenly surges and stimulates ovulation of mature follicles in the ovary to rupture. LH release surges at the proestrous stage are responsible for ovulation. Any substance capable of inhibiting this release could rouse disruption of ovulation and cause a reduction in the number of mature follicles or induce an estrous cycle. Therefore, the sudden decrease in the serum LH levels may be explained by an inhibitory effect of the drug on the release of LH which may produce disruption of ovulation[15]. This may result in impairment of estrous cycle and hinder conception and normal reproduction in the females.

Progesterone

Progesterone is produced in the ovaries, placenta, and adrenal glands, helps in regulating the menstrual cycle and preparing the uterus for conception and pregnancy. The hormone promotes the growth of lactation glands in the breast during pregnancy. High progesterone levels are supposed to be partly responsible for symptoms of premenstrual syndrome (PMS), such as hot flushes, breast tenderness, abdominal bloat and mood swings.

The feedback inhibition of GnRH secretion by estrogens and progesterone is the

basic principle for the contraception most widely-used. Such feedback inhibition of GnRH prevents ovulation by causing surge of LH in middle of the cycle[16]. The reduction in the levels of serum progesterone by MLM-100 and MLM-200 may have substantial effect on conception in females; hamper ovulation which may result in absence of ovulation and further sequential actions.

Estradiol

Estradiol stimulates the proliferation of the uterine lining and increase the thickness during the pre-ovulatory phase of the cycle. Estradiol is directly responsible for the growth and development of reproductive organs. In synergy with FSH, Estradiol stimulates granulosa cell proliferation during follicular development. Plants with estrogenic property blocks ovulation by directly acting on the pituitary gland and influence peripheral modulation of LH and FSH, thereby decreasing secretion of these hormones. Thus, the reduction in the serum concentration of Estradiol observed in this study may be attributed to estrogen synthesis. Consequently, such decrease in Estradiol levels may hamper ovulation, preparation of the reproductive tract for zygote implantation, and the subsequent maintenance of the pregnancy state[17]. Therefore, the decrease in serum levels of reproductive hormones strongly supported the abortifacient activity.

CONCLUSION

The abortifacient activity of the drug was assessed through the estimation of number of implants, resorption sites and the no. of live fetuses. The MLM 100 and MLM 200 reduced the no. of implants in the uterus remarkedly. Also the no. of live fetuses greatly reduced and showed significant values at dose level of 100mg/kg and highly significant value at the dosage of 200mg/kg. The percentage of abortion calculated revealed that it was 34% at the dose level of 100mg/kg and 69% at the dose of 200mg/kg and a significant abortifacient effect indicating the reduction of the number of viable fetuses.

The hormonal assay of the rats was evaluated using the estimation of parameters like FSH, LH, Estradiol, and Progesterone. Significant reduction in the hormonal levels of FSH, LH, Estradiol, Progesterone was recorded statistically. FSH hormone level is highly significant ($p<0.01$) at both the dose levels of MLM100, 200mg/kg. The results showed significant decrease in LH at a dose level 0f 100mg/kg and highly significant decrease at the dose level of MLM200mg/kg. Estradiol hormone is highly significant ($p<0.01$) at both the dose levels of MLM100, 200mg/kg. Progesterone hormone is also highly significant ($p<0.01$) at both the dose levels of MLM100, 200mg/kg. The reduction in the level of hormones proved to be the prime reason for abortifacient activity.

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Table 1. Abortifacient effects of MLM in rats when fed orally between days 6 and 15 days of pregnancy

Group	Treatment	Dose Mg/kg	Total No. of implantations	No. of live fetuses	Abortion in %
I	Control	2%CMC	12.5±0.43	12.5 ±0.43	0
II	MLM-100	100	13±0.57	8.83±0.94	34.08±4.47*
III	MLM-200	200	13.6±0.60	2.5±0.42	68.69±14.01**

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

Table 2. Effect of serum levels of reproductive hormones in wistar rats

Groups	Treatment (Mg/kg)	FSH (mIU/ml)	LH (mIU/ml)	Estradiol (Pg/ml)	Progesterone (Pg/ml)
I	2%CMC	1.56±0.06	0.31±0.04	152.25±1.24	16.94±0.86
II	MLM-100	1.12±0.02**	0.13±0.07*	93.50±0.18**	8.48±0.76**
III	MLM-200	0.59±0.08**	0.06±0.02**	81.75±0.06**	7.14±0.34**

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

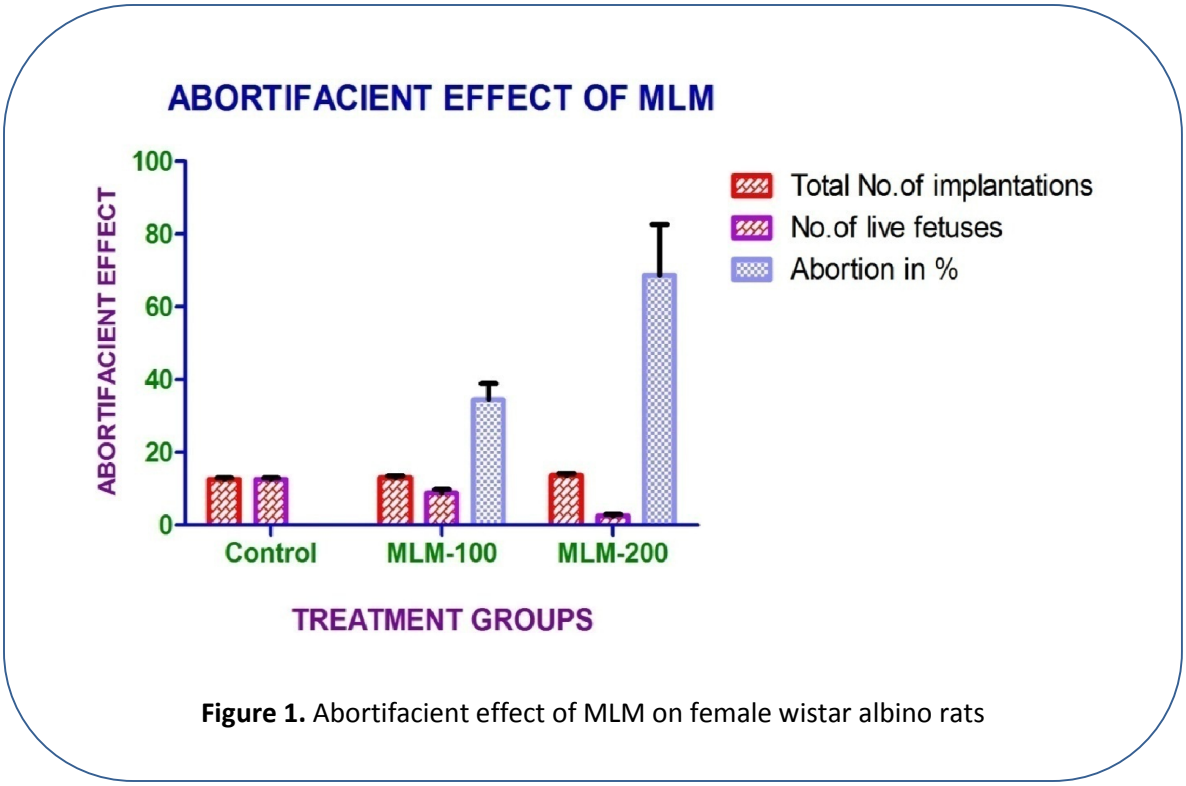

Figure 1. Abortifacient effect of MLM on female wistar albino rats

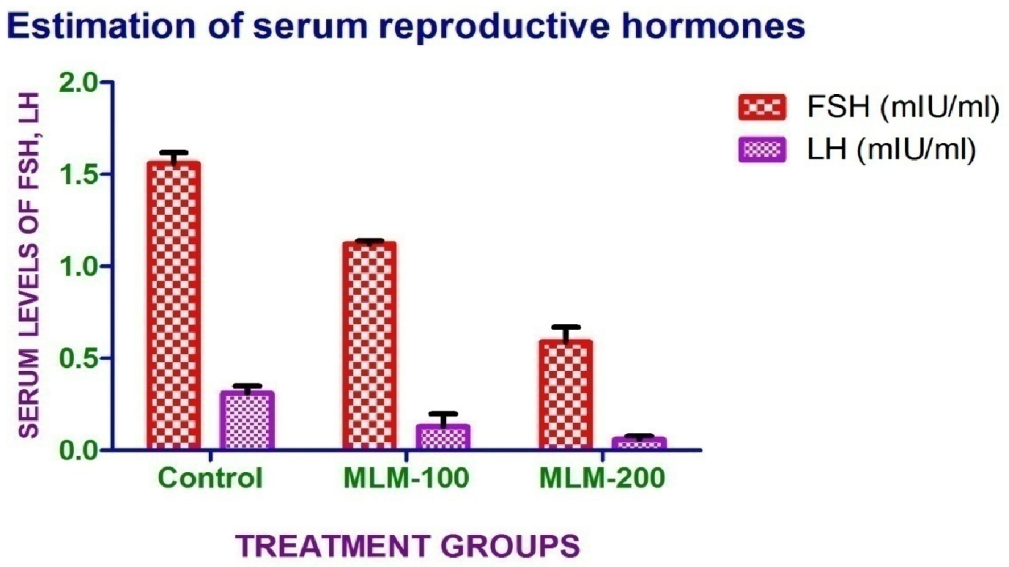

Figure 2. Effect of serum levels of FSH and LH in wistar rats

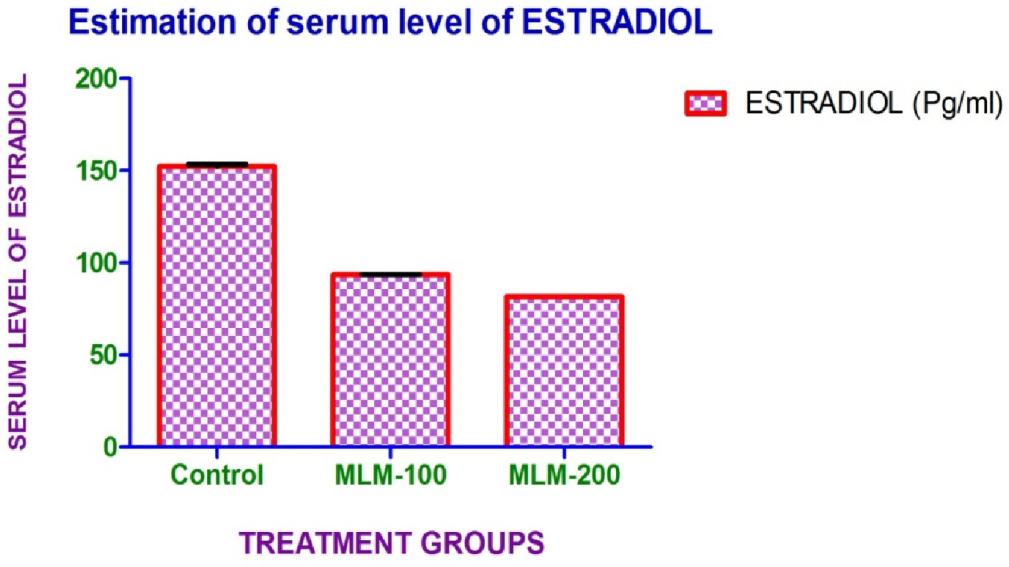

Figure 3. Effect of serum level of estradiol in Wistar Rats

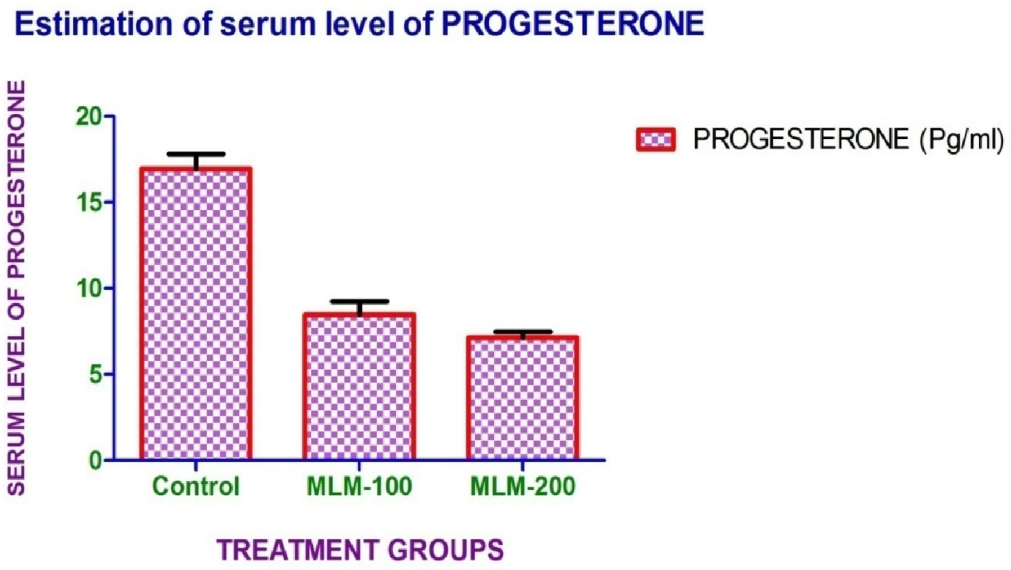

Figure 4. Effect of serum levels of progesterone in wistar rats