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## IN VIVO ANTI-OVULATORY ACTIVITY OF TALISADI YOGA: A HERBOMINERAL COMBINATION

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### ABSTRACT

In this study, female Wistar rats are used to test anti-ovulatory and contraceptive effects of talisadi yoga containing equal quantity of talisapatra (*Taxus baccata*) and Gairika (red oxide) mentioned in ancient ayurvedic classics yogaratnakara. After acclimating to conventional laboratory conditions, the healthy Wistar albino rats weighing 200±20 g were chosen for the study and placed into three groups, with six animals in each group. Group I animals were treated with distilled water (10 ml/kg body weight) and were regarded as Controls. Group II received the test substance at a dose level of 1000 mg/kg b. wt. whereas Group III was treated with Primolut-N as a positive control. For 21 days in a row, the animals in each group received treatment with the appropriate chemical or vehicle. Every day prior to the compounds being administered, the vaginal smears of all the animals were produced. At the conclusion of the trial, body weight, blood serum levels of cholesterol, triglycerides, and total cholesterol were measured in each group. The ovary and uterus histopathology analyses of each group were also performed. At the end of study it is found that the oral administration of the test compound i.e. Talisadi yoga resulted in the inhibition of normal Estrous cycle of female Wistar albino rats suggesting its anti-ovulatory activity.

**Keywords:** Antifertility; Contraceptive; Talisadi yoga; Yogratnakar; Ayurveda

## 1. INTRODUCTION:

Oral contraceptives, commonly known as antifertility medicines, are medications that regulate fertility [1]. These medications have an impact on and are connected to the female menstrual cycle and ovulation. Antifertility drug stops ovulation, implantation, fertilization, zygote destruction, or abortion in females. Population control is crucial to the welfare of the individual and the country. While there are many different synthetic contraceptive drugs on the market, consuming them has been associated to significant negative effects [2]. As a result, a strategy was used to find novel natural sources of antifertility drugs. Given Ayurveda's advancement in contraceptive practice, Yogaratnakara listed several medication combinations for both oral and local contraceptive use. Talisadi yoga (TY) is one of the listed medications in yogratnakara which is a mixture of the Talispatra (*Taxcus baccata*) and Gairika (red oxide) in equal quantity. The anti-fertility activity of the combination was not so far evaluated earlier so the present study was conducted to evaluate anti-ovulatory activity of TY on female wistar rats. Female rats go through four distinct phases in their reproductive cycle, called the estrous cycle: proestrous, estrous, metaestrous (also known as diestrous I), and diestrous (also known as diestrous II). The female rat's

cycle lasts an average of four days [3, 4], with ovulation occurring between the start of proestrous and the end of diestrous [5]. Rats are an excellent model to study alterations in the reproductive cycle because of their short cycle length.

## 2. MATERIAL AND METHODS:

**2.1 Test Drug:** The test drug components purchased from a reliable supplier in Gurugram, Haryana and after receiving accreditation from SGT University in Gurugram, Delhi, NCR, drug was prepared.

**2.2 Animals:** Female Wistar albino rats (nulliparous and non-pregnant) weighing  $200 \pm 20$  g and aged 8–12 weeks were obtained from the Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar. They were kept in standard circumstances with a temperature of  $22 \pm 3$  °C and a relative humidity of 30–70%. Throughout the trial, the 12-hour light and 12-hour dark cycles were manually maintained. The experimental room's floor was swept and twice scrubbed with a disinfectant solution every day. Standard polypropylene cages with a stainless steel grill top, equipped with space for pelleted food storage and a stainless steel sipper tube for a water bottle, could accommodate up to three animals. Urine and excrement were collected in sterilised paddy husks, which were replaced daily. San Biotech Pvt. Ltd., 27 Street No. 2, Madhu Vihar, IP Extn.

110092, Delhi, India provided the pelleted feed for the animals. Animals received the transportable, purified water in autoclavable polypropylene bottles.

IAEC: The IIRT Institutional Animal Ethical Committee has approved this technique, and the committee reviews it at least one a year.

**2.3 Toxicity study:** AOT of the drug was carried out on the according to Organisation for Economic Cooperation and Development (OECD) criteria 425 in albino Wistar rats. The LD50s of the TY sample, which contains an equal amount of Talispatra (*Taxcus baccata*) and Gairik (Red oxide), are more than 2000 mg/kg, suggesting that they are safe for therapeutic use.

#### 2.4 Anti-ovulatory activity:

The study was performed on healthy Wistar albino rats, weighing  $200 \pm 20$  g, were

divided into three groups of six animals each under standard laboratory circumstances. The animals in Group I were considered Controls and received distilled water (10 ml/kg body weight), Group II received the TY at a dose level of 1000 mg/kg b. wt. while Group III was treated with Primolut-N as a positive control, and the animals in each group were treated with the relevant chemical or vehicle for 21 consecutive days. The study design is described in **Table 1**. Every day before the compounds were given, all of the animals' vaginal smears were made. Each group's body weight, blood serum cholesterol, triglyceride, and total cholesterol levels were tested at the end of the experiment. Each group's uterine and ovarian histopathology analyses were also carried out.

Table 1: Study Design

Groups	Dose (mg/kg)	No. of animal per dose level	Animal ID
Control	0 mg/kg b. wt.	6	2023-085-01 to 06
Treated	1000 mg/kg b. wt.	6	2023-085-07 to 12
Positive Control	600 µg/kg b. wt.	6	2023-085-13 to 18

#### 2.4.1 Dosage preparation

A few minutes before dosing, the test article TY was prepared freshly. A sufficient quantity of the material was weighed, and then a vehicle was added. To get the appropriate volume, additional vehicle was added after the sample had been thoroughly triturated.

In a similar manner, Primolut-N dosages were prepared.

**2.4.2 Administration of the test compound** using an oral cannula, the test chemical was given orally at a maximum volume of 10 ml/kg body weight. 10 ml/kg dose of distilled water were

given to the control group of animals.

#### 2.4.3 Preparation of vaginal smear

Every animal's vaginal smear was prepared the day before dosage. In a nutshell, the animals were gently handled, and a drop of sterile saline was given via the vagina using a dropper. Afterwards, the liquid was sucked onto a sanitised slide using the same dropper. After being covered with a sterile cover slip, the liquid drop was examined under a microscope. Before mounting the cover slip, a drop of saline was added if the vaginal drop was thick or turbid.

**2.4.4.** Body weight Every week on day 0 (pretreatment), 7, 14, and 21 (post treatment), the body weight of each animal was measured.

**2.4.5** Evaluation of the estrous cycle all the animals' estrous cycle stages were monitored every day until the study's 21<sup>st</sup> day.

**2.4.6.** Biochemical Parameters on the 22nd day of the trial, the levels of serum triglycerides and total cholesterol were assessed.

**2.4.7.** Hormonal study on the 2nd day of the trial, the serum levels of the hormones progesterone, FSH, and estradiol were measured in all of the animals.

**2.4.8.** Necropsy At the conclusion of the study, all of the animals that had been evaluated was sacrificed, and a necropsy was performed to look for any obvious pathological abnormalities.

**2.4.9** Histopathological evaluation all three groups' ovaries and uteruses were maintained in buffered formaldehyde and processed for staining, slide preparation, and observation.

### 3. RESULTS:

#### 3.1 Body weight

Wistar albino rats treated with the test compound TY at the dose level of 1000 mg/kg body weight showed normal gain in body weight on day 7th, 14th, and 21st day (post treatment). Body weight detail data is shown in **Table 2**. No negative effect on the body weight of treated animals was observed.

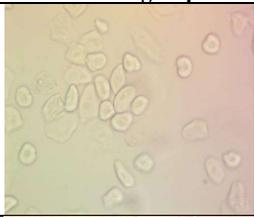
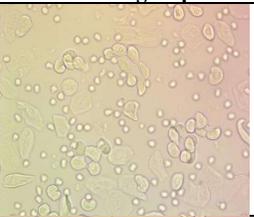
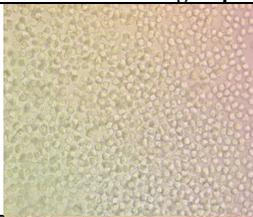
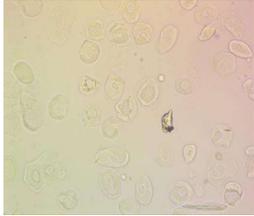
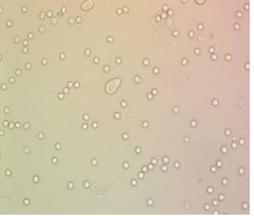
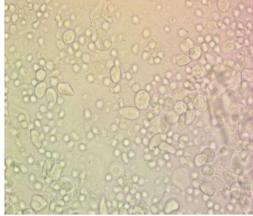
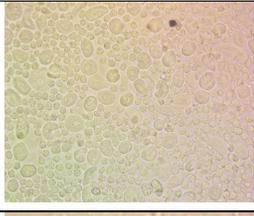
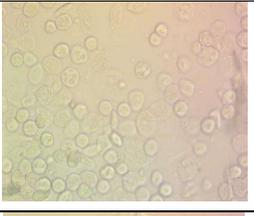
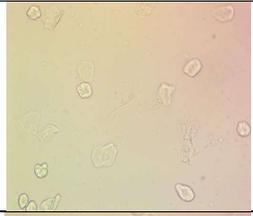
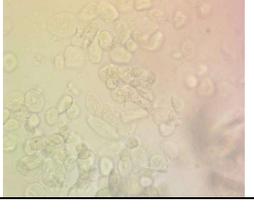
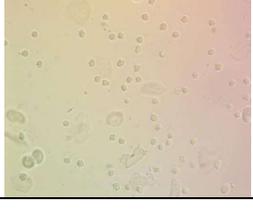
#### 3.2 Evaluation of estrous cycle:

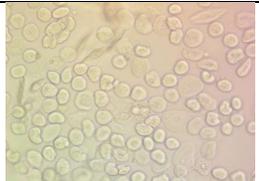
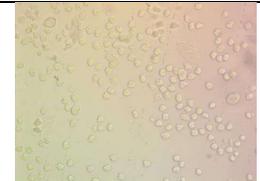
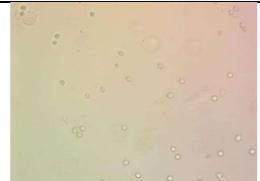
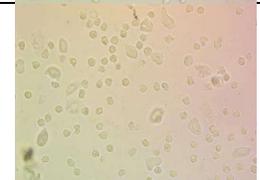
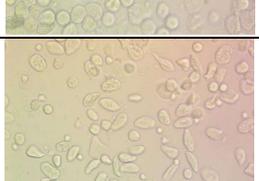
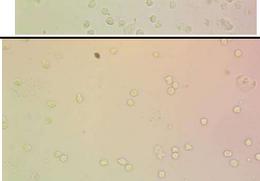
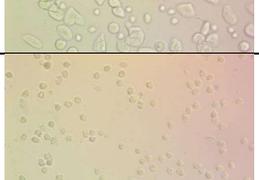
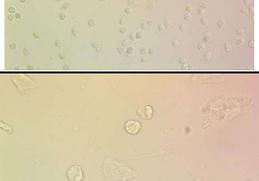
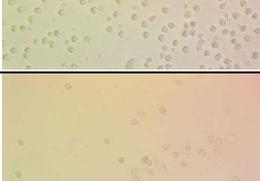
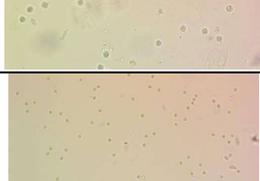
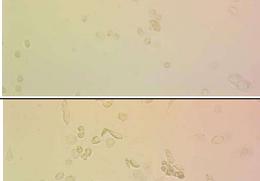
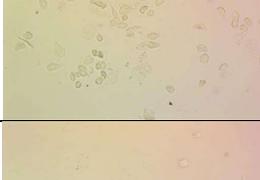
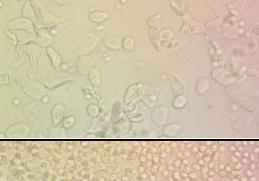
The analysis of estrous cycle of the animals of all the three groups suggest that in the animals of control group the estrous cycle was running normally. However, in case of Talisadi Yoga and Primolut-N the estrous cycle was likely to be halted at the stage of Metestrus after dosing of 5 days. The data is presented in following **Table 3**:

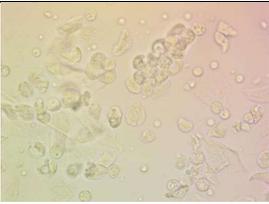
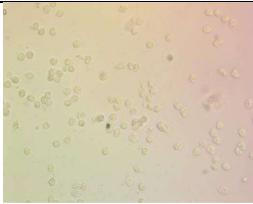
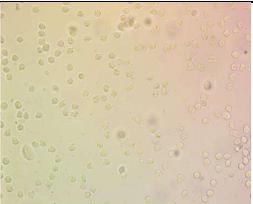
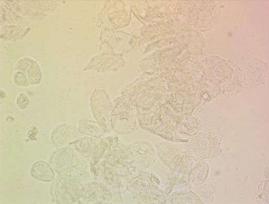
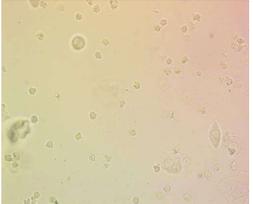
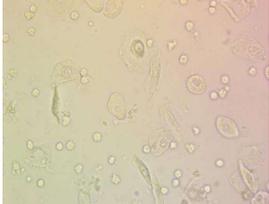
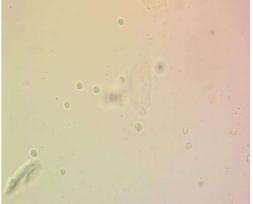
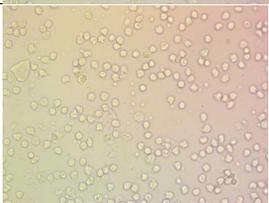
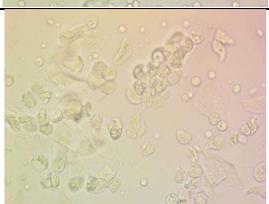
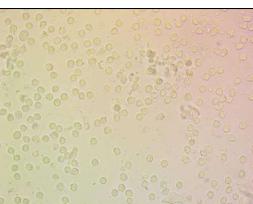
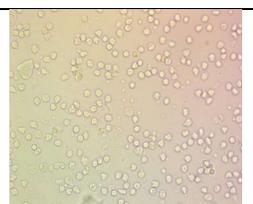
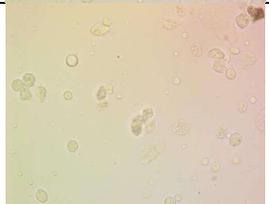
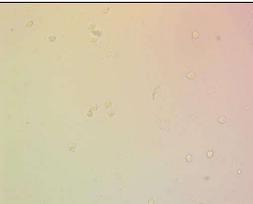
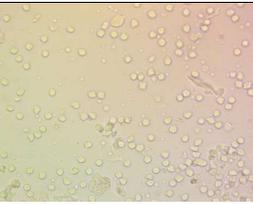
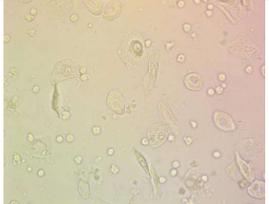
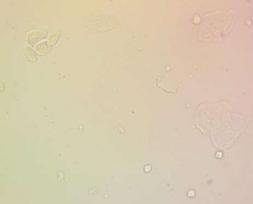
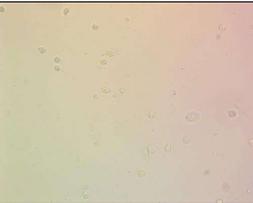
Table 2: Body weight details of all animals

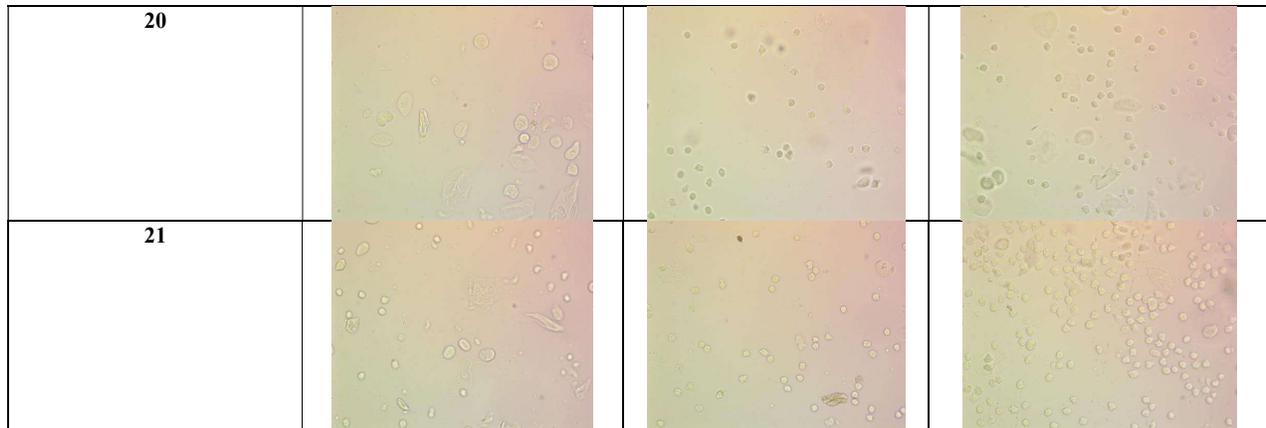
Group	Animal ID	Bodyweight (g)				Percentage Bodyweight Change			
		Day-0	Day-7	Day-14	Day-21	Day 0 to Day 7	Day 7 to Day 14	Day 7 to Day 21	Day 0 to Day21
Group I	2023-085-01	204.09	216.48	223.78	233.45	6.07	3.37	4.30	14.36
	2023-085-02	204.47	215.09	224.85	236.13	5.19	4.54	5.02	15.48
	2023-085-03	199.59	210.59	217.59	226.83	5.51	3.32	4.25	13.65
	2023-085-04	185.58	194.78	206.42	214.63	4.96	5.98	3.98	15.65
	2023-085-05	198.25	210.48	218.28	229.69	6.17	3.71	5.23	15.86
	2023-085-06	195.69	207.59	216.89	225.02	6.08	4.48	3.75	14.99
	Mean	197.95	209.17	217.97	227.62	5.59	3.74	4.52	14.50
	SD	2.71	3.08	3.92	4.78	0.44	0.69	0.43	0.93
Group II	2023-085-07	191.97	202.04	213.94	225.13	5.25	5.89	5.23	17.27
	2023-085-08	182.03	193.02	200.93	208.01	6.04	4.10	3.52	14.27
	2023-085-09	186.19	194.03	207.21	214.41	4.21	6.79	3.47	15.16
	2023-085-10	189.2	193.03	199.7	208.64	2.02	3.46	4.48	10.27
	2023-085-11	194.71	199.23	206.71	214.52	2.32	3.75	3.78	10.17
	2023-085-12	192.93	204.92	213.23	218.22	6.21	4.06	2.34	13.11
	Mean	189.51	197.71	206.95	214.82	5.16	5.59	4.08	15.57
	SD	4.99	4.94	6.51	8.65	0.92	1.37	1.00	1.54
Group III	2023-085-13	194.97	202.04	207.94	212.88	3.63	2.92	2.38	9.19
	2023-085-14	183.03	193.02	198.93	206.01	5.46	3.06	3.56	12.56
	2023-085-15	181.19	190.03	197.21	206.59	4.88	3.78	4.76	14.02
	2023-085-16	194.03	204.84	212.73	220.19	5.57	3.85	3.51	13.48
	2023-085-17	199.24	209.92	218	225.4	5.36	3.85	3.39	13.13
	2023-085-18	187.34	195.83	205.34	214.07	4.53	4.86	4.25	14.27
	Mean	189.967	199.28	206.69	214.19	4.90	3.72	3.56	12.77
	SD	7.21	7.59	7.97	7.59	0.74	0.69	1.19	1.86

Table 3: Estrous cycle analysis of animals treated with test items

Day	Control group	Treated group	Positive control group
0			
1			
2			
3			

4			
5			
6			
7			
8			
9			
10			
11			

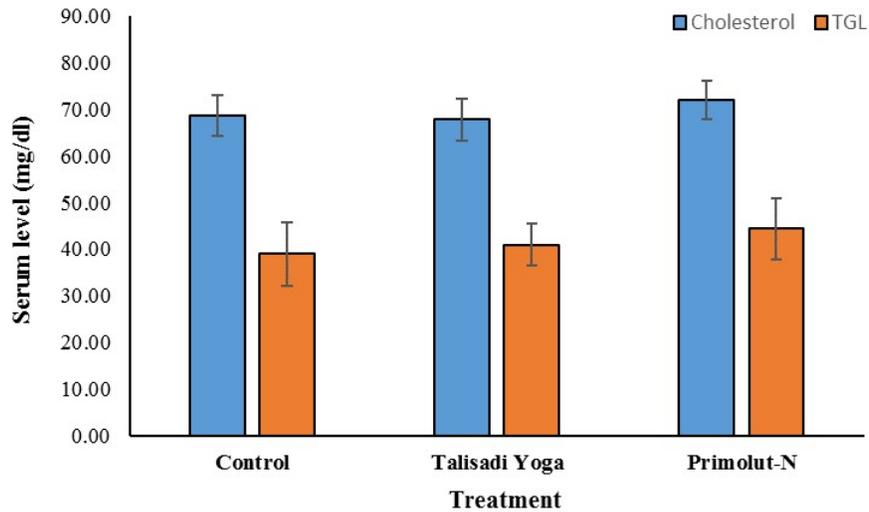
12			
13			
14			
15			
16			
17			
18			
19			



**3.3 Biochemical Parameters:**

No significant variation in the serum cholesterol and triglyceride was

observed in the G II animals in comparison to control. The data is presented in **Graph 1**.

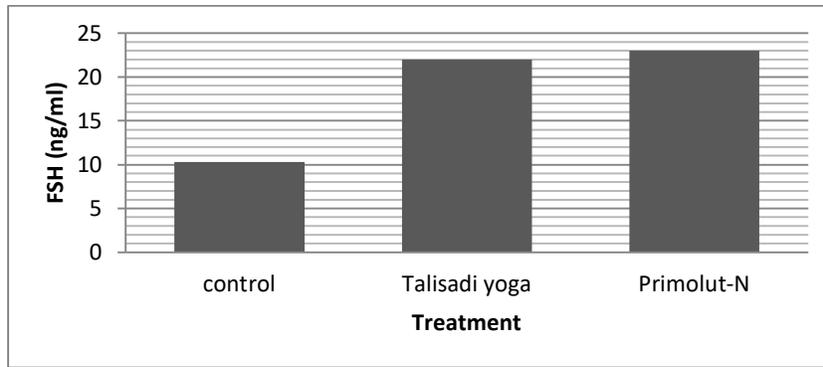


Graph 1: Serum levels of cholesterol and triglycerides in different groups

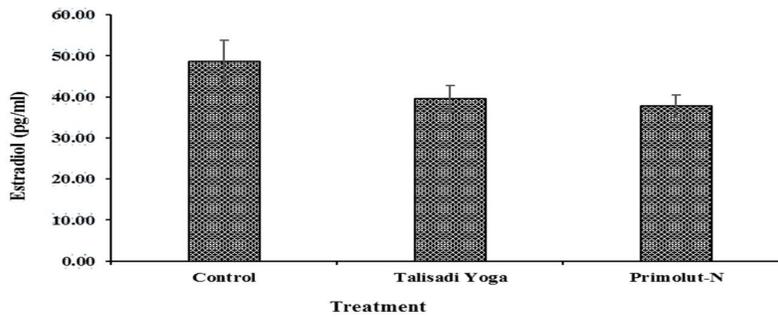
**3.4 Hormonal study:**

Progesterone, FSH, and estradiol hormone levels in the serum were measured in each group and compared with the control. At the conclusion of the study, it was discovered that the animals receiving TY had higher serum levels of

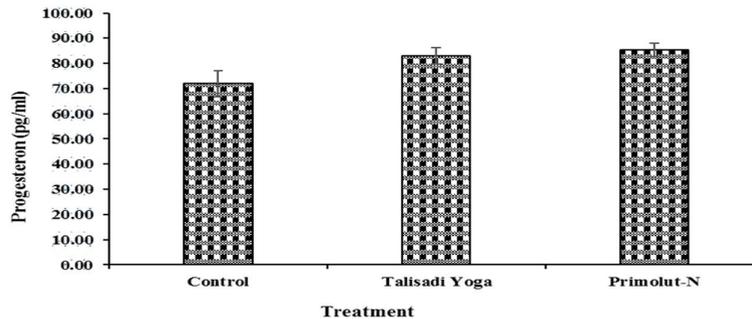
FSH (**Graph 2**). In addition, animals treated with Primolut-N had somewhat greater levels of FSH than those with TY. When it came to estradiol and progesterone, the TY and Primolut-N treated animals had lower serum levels of both hormones (**Graph 3 and 4**).



Graph 2: Serum level of FSH in different groups



Graph 3: Serum level of Estradiol in different groups



Graph 4: Serum level of Progesterone in different groups

**3.5 Necropsy Finding:**

The necropsy of all the animals of control, treated and positive control

groups suggested normal necropsy finding.

Table 4: Summary of necropsy findings

S. No.	Fate	Group/Dose		
		GI/0 mg/kg	GII/1000 mg/kg	GIII/600 mg/kg
1	Terminal sacrifice	6/6	6/6	6/6
2	Found Dead	0/6	0/6	0/6
3.	Abnormalities detected	NAD	NAD	NAD

**3.6 Histopathological evaluation**

Following study completion, the histopathology of each group's uterus

and ovary was examined, and the results were compared with those of the control group. The findings imply that the

application of Talisadi yoga to female rats did not alter the ovary and uterine morphology in any way. Rats given Primolut-N as a positive control showed similar results, with no morphological changes seen in their tissues (Plate 2). It was also investigated that Talisadi yoga

dosage affected the thickness of the endometrium. According to the observations, the test item-treated animals showed a decrease in endometrial thickness. However, the endometrial thickness of the control animals was normal.

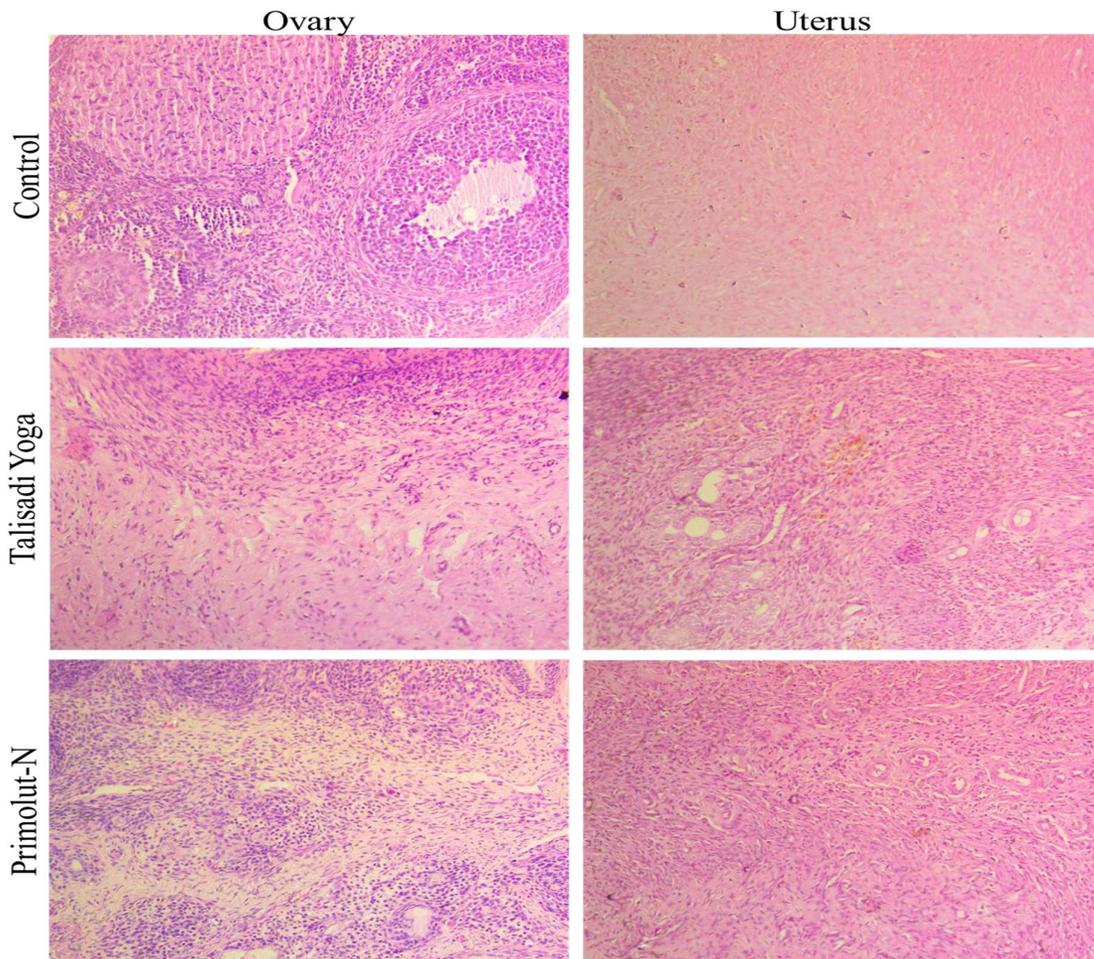


Figure 1: Histopathological representative images of ovary and uterus of study groups

## DISCUSSION:

Growing populations are one of the main issues facing emerging nations like India. Our economic policies will be negatively impacted by this population explosion, which could concurrently throw off the

equilibrium of our socioeconomic system. Controlling human fertility in the sense of limiting it is therefore the most significant and pressing issue facing modern medicine and biosociology. Despite the wide variety of synthetic contraception medications

available, using them has been linked to serious side effects. Consequently, a method was developed to locate new natural sources of medications that treat infertility. Yogaratnakara identified a number of drug combinations for both oral and local contraceptive use, taking into account Ayurveda's advances in contraceptive treatment. One of the prescribed drugs in Yogaratnakara is Talisadi Yoga (TY), which is a combination of Talispatra (*Taxcus baccata*) and Gairika (red oxide) in equal proportion [6]. The current investigation was carried out to assess the anti-ovulatory activity of TY on female wistar rats because the anti-fertility activity of the combination had not yet been assessed. Wistar albino rats have been utilized as test subjects for this research because of their reproductive system's resemblance to that of humans, making them an excellent model for examining anti-ovulatory effects. There are similarities between their ovarian structure, hormone regulation, and estrous cycle with that of humans. In rats, the estrous cycle typically lasts 4-5 days. During a typical rat estrous cycle, the vaginal smear contains three different types of cells. The stages of the estrous cycle are determined by the presence or absence of certain cell types as well as the proportion of each type [7]. The low chance of the rats being pregnant due to nonviability of mature graffian follicle is explained by the extension of the diestrous

period and reduction of the Metestrus phase [8]. Consequently, ovulation is prevented. The Talisadi yoga treated group of animal estrous cycle shows halted at the stage of Metestrus after dosing of 5 days indicating its anti-ovulatory activity. An important determinant of an animal's health is variations in its body weight. Losing weight is frequently the first indication that anything bad is happening [9]. As the TY is safe No negative effect on the body weight of treated animals was observed. Serum triglyceride and total cholesterol shows no any difference. The hormones like Progesterone, FSH, and Estradiol are related to fertility. Progesterone and estradiol work together to prepare the uterine lining for the fertilised egg. TY decreases the levels of Progesterone and estradiol also the histopathological evaluation exhibited decrease in the thickness of endometrium confirming its antifertility action. Follicle-stimulating hormone (FSH) levels above normal indicate a problem with the ovaries might lead to anovulatory cycle. In the study, it was discovered that the animals receiving Talisadi yoga had higher serum levels of FSH again indicating its anti-ovulatory activity.

#### **CONCLUSION:**

It can be concluded that talisadi yoga has potential Anti-ovulatory and eventually antifertility activity.

When administered at a dosage level of 1000 mg/kg b. wt, the test substance Talisadi yoga has the ability to stop the Wistar albino female rats' estrous cycle at the stage of metestrus. Furthermore, the animals' body weight, serum triglyceride, and cholesterol levels were unaffected by the repeated administration at the aforementioned amount. The levels of estradiol and progesterone, however, were altered. While there is decrease in the thickness of endometrium. Furthermore, the test substance did not result in any abnormalities in any of the body's organs.

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