

## THE ROLE OF ETHANOLIC EXTRACT OF SYZYGIUM CUMINI STEM BARK ON FEMALE REPRODUCTIVE SYSTEM IN WISTAR RATS

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### ABSTRACT

**Background:** The medicinal plant's role is well established in folklore medicine in India. Among numerous health complications, infertility is a crucial condition that bothers modern society. The traditional practitioners are using various parts of plants to overcome infertility in women. One such plant is *Syzygium cumini* (stem bark), used by traditional practitioners for pro-fertility in females without the scientific literature endorsing the fact.

**Material and Methods:** Wistar rats approximately six months age and weighing 190-210 g were used for the experimental study. The rats were treated with 500 mg/kg body weight of *Syzygium cumini* stem bark ethanol extract with a vehicle through oral administration. The ether anaesthesia was given on the day of sacrifice and noted the body weight. The ovaries were carefully dissected, cleaned, weighed and processed for histological studies.

**Results:** The body and ovarian weights were slightly increased in the treated group but not statistically significant. The microscopic structure of the ovary showed a normal histo-architecture. The ovarian corpora lutea number increased and was statistically significant in the treated group when compared to control.

**Conclusions:** So, from the present study, it can be concluded that the ethanolic extract of stem bark of *Syzygium cumini* brings about the pro-fertility effect on the female reproductive system in Wistar rats.

**KEY WORDS:** Estrous cycle, Hormones, Reproductive system, *Syzygium cumini*.

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### INTRODUCTION

It is a well-known fact that the plants are used as medicine from ancient time, and the role of medicinal plants is well established in folklore medicine in India. Herbal medicines usage is overwhelming as it has minimal or no side effects [1]. According to the World Health Organization survey in primary healthcare sectors, around 80% of the world population of developing nations relies on traditional

medication [2].

Among numerous health complications, infertility is a necessary condition that bothers modern society. Infertility is a worldwide health complication with one in six couples suffering from this condition and with a primary economic burden on the global healthcare industry. Infertility is believed to be a painful and heart-breaking problem

for couples and naturally for their family members [3]. The infertility is generally defined as the failure of a couple to achieve pregnancy after 12 months of regular unprotected sexual intercourse [4]. Infertility has been estimated worldwide to affect around 8 to 12% of couples [5].

To overcome infertility women have used the herbal remedies to affect fertility, numerous herbal medicines are used as contraceptives in the traditional system [6]. Now the plant extracts used as a fertility enhancer in animals are gaining importance since the shifting of attention from synthetic medicine to natural compounds [7]. Several plants are used regularly by the local practitioners as fertility regulating agents, but many of them have not been tested for such effects in the developing countries [8]. *Syzygium cumini* (stem bark) is the one used by traditional practitioners in the rural areas for pro-fertility in females without the scientific literature endorsing the fact.

The *Syzygium cumini* L. Skeels (family Myrtaceae) is a widespread tropical tree native to India. It is also known as Indian java plum or black plum, jambolan, jamun, jambul, jamman, jambolao, jamelao. The synonyms are *Eugenia cumini* (L) Druce and *Eugenia jambolana* Lamarck [9]. In the scientific literature, it is mentioned that almost all parts of the *Syzygium cumini*, including stem bark, are having tremendous medicinal properties. Some authors have noted that the bark of *Syzygium cumini* with milk is used to treat excessive menstruation [10]. The juice obtained from the bark is given orally for the treatment of women with a history of repeated abortion [11]. The dried green bark powder of *Syzygium cumini* is administered orally with a small amount of goat or cow milk twice a day to treat leucorrhoea [12].

Even though a great deal of scientific literature is available on the *Syzygium cumini* as whole plant, leaves, flowers, aerial parts, seeds, roots and stem bark, but there is no literature on the exact scientific role of these on the reproductive system, fertility in general and in particular on the female reproductive system. Thus, the present study

is planned to evaluate a scientific validation for the claims of the traditional practitioners of the usage of stem bark of *Syzygium cumini* as pro-fertility or fertility enhancer activity in the reproductive system of female Wistar rats.

## MATERIALS AND METHODS

**Plant material:** The stem bark of *Syzygium cumini* used in the present study.

**Plant Extraction:** The ethanol extraction of the *Syzygium cumini* stem bark was used for the present study. After identification of the plant, the stem bark of *Syzygium cumini* was collected, washed thoroughly in tap water and subjected to the drying for 20 days in the shade under controlled temperature ( $25 \pm 2$  °C). The material was powdered in crude form, passed through a sieve of 40 mesh and stored in a well-closed container for further usage. The coarsely powdered stem bark was successively Soxhleted using petroleum ether, ethanol and chloroform for 72 hours. The extracts were filtered, and the solvents were evaporated to dryness under reduced pressure, at 40 to 45 °C in an Eyela rotary evaporator [13]. The yield of ethanol extracts of the stem bark of *Syzygium cumini* was 8%.

**Animals:** Inbred twenty-five adult nulliparous and non-pregnant Wistar rats approximately six months age and weighing 190-210 g were used for the experimental study. The experimental study procedures involving the handling and treatment of animals were approved by the Institutional ethical committee Teena Biolabs Pvt. Ltd. Reg. No. 177/PO/cb/99/CPCSEA. Project No: TBLSTPRJ0032014. Before experimentation, all the rats were examined for the standard regular oestrous cycle. The rats with normal, regular oestrous cycle were selected for the study. The rat oestrous cycle was determined by vaginal smear. Samples of vaginal smears were collected by swab smear (cotton buds) technique [14].

**Experimental design:** A total of twenty-five rats in the oestrous phase were randomly divided into five groups, each group consisting of five rats. The control group (C), the sham control one day (SC1), sham control four to six days (SC4), *Syzygium cumini* stem bark ethanol extraction one day (SE1) and four to six days (SE4).

**Treatment:** The C group rats were given the regular standard diet. SC1 group rats treated with 5 ml/kg body weight of the vehicle, twice a day for 1 day. SC4 group rats treated with 5 ml/kg body weight of the vehicle, twice a day for 4 to 6 days. SE1 and SE4 group rats were treated with 500 mg/kg body weight of *Syzygium cumini* stem bark ethanol extract with a vehicle through oral administration, twice a day for 1 day and twice a day for 4 to 6 days respectively. All the 1-day treatments started on the oestrus phase of the oestrous cycle, whereas all 4 to 6 days treatments started on oestrus phase oestrous cycle and continued to oestrus phase of the next oestrous cycle. The vehicle contains a mixture of 100 ml of water, 25 ml of curd, 0.5 g pepper and 0.5 g *Allium sativum*.

**Animal Sacrifice:** The animals were sacrificed on the day of the oestrus phase of the next oestrous cycle by euthanasia. The ether anaesthesia was given to all the control and treated group animals. The bodyweight of control, sham control and treated group rats were weighed on the day of sacrifice. After recording body weight, the control, sham control and treated group rats were euthanized and carefully dissected for the ovaries, cleaned for connective tissue and fat, examined macroscopically and immediately weighed.

**Histological study:** After collection, the ovaries were processed for histological studies by standard methods [15]. The stained ovarian sections were analyzed in Labomed Vision 2000 binocular microscope with a low power objective lens 4X, 10X and a high power 40X. The Digi Eye digital microscope camera was used for the photomicrographs of stained sections. The stained ovarian sections were analyzed for the number of different follicle and corpora lutea by using Labomed Vision 2000 binocular microscope [16].

**Statistical analysis:** All the data was collected and tabulated by using the Microsoft excels worksheet. The mean, standard error (SE) and t-test were performed by using statistical software Sigma Plot 10, to find out the significance level between control and treated groups. The P-value < 0.05 is considered as

statistically significant.

## RESULTS

**Rat oestrous cycle length:** The oestrous cycle length of both control and treated groups was 4 to 6 days. There is no statistical significance between control and treated groups.

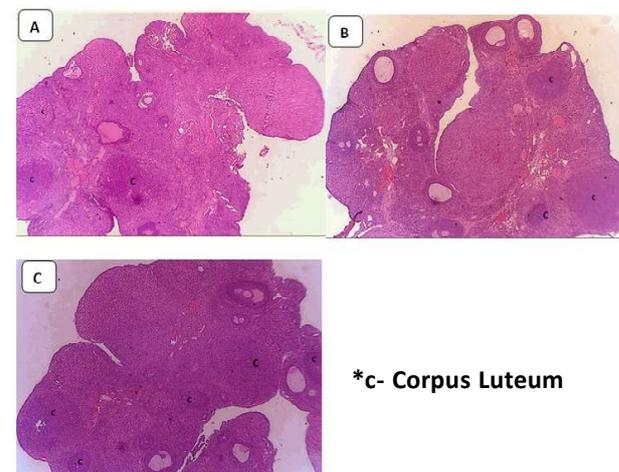
**Body, and ovarian weight changes:** The body, and ovarian weights of stem bark of *Syzygium cumini* treated group rats were slightly increased, and the increase was statistically not significant when compared with the control and sham control group rats (Table 1).

**Table 1:** Effect of ethanolic extract of stem bark of *Syzygium cumini* on body, and ovarian weight in Wistar rats.

Group	Weight (g) ( $\bar{x} \pm SE$ )	
	Body	Ovary
Control	190.1 $\pm$ 17.0	0.045 $\pm$ 1.2
Sham Control 1day (SC1)	190.2 $\pm$ 17.7	0.045 $\pm$ 1.3
Sham Control 4 days (SC4)	190.3 $\pm$ 16.5	0.047 $\pm$ 1.1
<i>Syzygium cumini</i> 1 day (SE1)	190.7 $\pm$ 15.3	0.045 $\pm$ 1.2
<i>Syzygium cumini</i> 4 days (SE4)	191.9 $\pm$ 12.7	0.047 $\pm$ 0.6

All the data were expressed as  $x \pm SE$ , n = 5 in each group. x = mean, SE = standard error.

**Ovarian histology and morphometry:** The microscopic structure of the ovary revealed the normal structure with outer cortex and inner medulla. The cortex composed of different stages of ovarian follicles and corpora lutea, the medulla composed of connective tissue stroma with blood vessels. This was seen both in control as well as treated groups. But the number of follicles and corpora lutea differed in treated group rats (Figure 1).



**Fig. 1:** A. Photomicrograph of control ovary. B. Photomicrograph of sham control ovary. C. Photomicrograph of *Syzygium cumini* ethanol extract treated rat ovary showing the increased number of corpora lutea.

**Table 2:** Effect of ethanolic extract of stem bark of *Syzygium cumini* on the number of corpora lutea and ovarian follicles in the ovary.

Group	Corpora lutea	Follicles		
		Primary	Secondary	Tertiary
Control	7.6 ± 0.67	8.6 ± 1.6	8.0 ± 1.5	2.8 ± 0.5
Sham Control 1day (SC1)	7.8 ± 0.37	8.8 ± 1.2	8.2 ± 1.8	3.0 ± 0.7
Sham Control 4 days (SC4)	8.0 ± 0.54	9.0 ± 1.7	8.6 ± 1.9	2.6 ± 0.5
Syzygium cumini 1 day (SE1)	10.8 ± 0.58** #	15.2 ± 1.0** ##	8.4 ± 0.4	0.4 ± 0.2** ##
Syzygium cumini 4 days (SE4)	10.4 ± 0.67** #	9.2 ± 0.3	4.2 ± 0.8	1.8 ± 0.9

All the data were expressed as  $x \pm SE$ ,  $n = 5$  in each group.

$x$  = mean, SE = standard error.

\* Indicates the significance level between control and treated. \*  $P < 0.05$ , \*\*  $P < 0.01$

# Indicates the significance level between sham controls and treated. #  $P < 0.05$ , ##  $P < 0.01$ .

The number of corpora lutea in control and sham control groups was similar. In the SE1 and SE4 groups, the number increased, and the increase was statistically significant ( $P < 0.01$ ) when compared with control and sham control groups. The number of corpora lutea in C, SC1 and SC4 group rats was 4 to 6, SE1 group it was 6 to 8, SE4 group it was 7 to 8 (Table 2).

The microscopic anatomy of ovarian follicles showed the normal histo-architecture. The number of primary follicles in control and sham control group was similar. In treated group SE4 the number was increased, and the increase was statistically non-significant, only in the SE1 group the increase was statistically significant ( $P < 0.01$ ) when compared to the control and sham control groups. The number of secondary follicles in control, sham control and treated groups remained the same. The number of tertiary follicles in control and sham control groups was similar. In the treated group SE4 the number was decreased, and the decrease was statistically insignificant, only in the SE1 group, the decrease was statistically significant ( $P < 0.01$ ) when compared to the control and sham control groups (Table 2).

## DISCUSSION

**Estrous cycle length:** The present study indicates the normal length of the oestrous cycle in control, sham control and also in treated group of rats. The oestrous cycle has been familiar to be disrupted by endocrine, nutritional and genetic factors. Numerous drugs influence oestrous cycles by acting at various levels of the hypothalamic-pituitary

axis [17]. The results of the length of the oestrous cycle in present study correlate with the previous study by Long and Evans [18], also within the normal range and similar with the previous research by the Mandal [19]. So, the stem bark of *Syzygium cumini* did not alter the normal oestrous cycle. It indicates that the *Syzygium cumini* did not exert any toxic effect on the reproductive system. Further, it may be considered as favorable for the normal functioning of the female reproductive system.

**Bodyweight changes:** The body weight is an essential factor to monitor the health of an individual. The decrease in the bodyweight is usually the earliest sign of the onset of an adverse effect. In the present study bodyweight results of stem bark of *Syzygium cumini* treated group rats showed a slight increase, proposing that the treatment had no adverse effects. Further, the previous studies well established that the weight and composition of the body are an essential link for the sexual maturation [20]. So, from the present study, it is clear that the ethanolic extract of the stem bark of *Syzygium cumini* may exert a pro-fertility effect by increasing the body weight, thereby maintaining the health of the animal.

**Ovarian weight changes:** In the present study, the ovarian weights were slightly increased in the treated groups SE1 and SE4. But the increase was not statistically significant when compared with control and sham control groups. The increased ovarian weight in the treated rats may be due to the effect of the stem bark *Syzygium cumini*, which also resulted in a slight increase in body weight. So, the light increased body weight may be responsible for

the rise in the ovarian weights in treated groups, and vice versa also is a possibility. So, in the current study, we also found that the body weight, ovarian weights have increased in the treated group, which is a positive factor and may improve the fertility in-toto. Thus, *Syzygium cumini* can be considered as a pro-fertility agent.

**Microscopic structure of the ovary:** The histological evaluation of the ovary revealed the presence of corpora lutea, primary, secondary and tertiary follicles in both the control and treated groups. But their number varied in treated and control groups. The corpus luteum is a small endocrine gland derived from secretory cells of ovarian follicles after ovulation. The primary function of corpus luteum is the production of progesterone, which regulates several reproductive functions [21]. The corpora lutea number increased in the treated group (SE1 and SE4) rats was significantly increased. The increased number of corpora lutea may be due to the inductive effect of stem bark of *Syzygium cumini* on ovarian follicle growth, and well-differentiated follicles are converted into corpus luteum after ovulation. These results are in line within a study which reported that the oral administration of the *Justicia insularis* aqueous extract showed an inductive effect on ovarian follicle growth, follicular cell growth peak up to the ovulation and then the well-differentiated ones are converted into the corpus luteum.

Further, it is also reported that the corpus luteum number in the ovary is a well-recognized and excellent parameter to analyze the ovarian folliculogenesis induction and improve fertility [22]. The enhanced folliculogenesis and ovulation lead to female fertility-enhancing activity, while the decreased number of corpora lutea leads to antifertility in female rats [23]. So, with the above facts, it is clear that the increased number of corpora lutea saw in the present study with respect to the treated group of rats is facilitating the fertility factor in female rats. Further, it proves that the *Syzygium cumini* promotes the folliculogenesis in the ovary, which in turn leads to the maturation of the follicle and ovulation, which are fertility-

enhancing factors. So, *Syzygium cumini* stem bark is a pro-fertility agent and may bring about this action by acting on the reproductive system through its phytochemicals either at the organ level or by acting centrally through the hypothalamic-pituitary-gonadal axis.

Ovarian folliculogenesis is one of the best parameters to assess female fertility. In the present study, there is an increase in primary follicles in treated group rats. The increase in primary follicles indicates that the stem bark of *Syzygium cumini* induces the folliculogenesis and promotes fertility. There is a slight decrease in the secondary follicle and tertiary follicle number in the treated group rats. The reduction in the number of follicles in the treated group rats may occur because of a more significant number of follicles that have matured and ovulated, resulting in the increased number of corpora lutea. It has been reported that the induction of folliculogenesis improves fertility [22]. It is said that decreased folliculogenesis leads to antifertility, while the increased folliculogenesis leads to female fertility-enhancing activity [23]. So, it is clear that the increased primary follicle is an indication of increased folliculogenesis and increased corpora lutea is an indication of ovulation by the maturation of a more significant number of follicles. Both these factors are pro-fertility factors of the treating agent. So, the present study results confirm the pro-fertility character of stem bark of *Syzygium cumini*.

## CONCLUSION

So, from the present study, it can be concluded that the stem bark of *Syzygium cumini* brings about the pro-fertility effect on the female reproductive system in Wistar rats. This effect is brought about by improving the gross parameters, increasing the histological architecture positively.

**Conflicts of Interests: None**

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