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RESEARCH ARTICLE

THE EFFECT OF *SYZYGIUM CUMINI* STEM BARK FRESH EXTRACT ON FEMALE REPRODUCTIVE SYSTEM IN WISTAR RATS

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ABSTRACT

In India there is a practice of using plants in traditional medicines from the time immemorial. Among many other problems, infertility is a major problem taunting the modern society. With increasing pollution at all levels and stress factors in the present day situations, infertility is mushrooming like an epidemic and creating havoc for couples and their families causing lot of turmoil in their lives. To overcome the problem of infertility the traditional practitioners are using various plant products to treat infertility one among them is stem bark of *Syzygium cumini* (jamun). Though it is administered to treat infertility by traditional practitioners, the scientific literature about its effect on the fertility is lacking and less research work is done on this aspect. The female Wistar rats weighing about 190 – 210 grams were used for the study. The oral administration of fresh extract of stem bark was done by utilizing a curved ball-tipped intubation needle affixed to a 2 ml syringe. In the present study treated group showed slight increase in body and uterine weight. The microscopic structure of uterus showed unaltered histo-architecture with respect to myometrial and perimetrial thickness in treated and control groups while the endometrial thickness was found to be more in the treated group. We can conclude from the present study that the fresh extract of stem bark of *Syzygium cumini* exerted a profertility effect. Further studies are required with *Syzygium cumini* stem bark to determine the mechanism of action that leads to endometrial thickness increase.

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INTRODUCTION

In India, plants have been used in traditional medicines from the time immemorial. Rigveda which is one of the historic storage of human knowledge mentions the use of plants in medicine. Recently around 20,000 medicinal plant species have been recorded in India, further more than 500 traditional communities utilize about 800 plant species for curing new diseases (Rekha Sharanappa and Vidyasagar, 2014). According to the World Health Organization survey close to 80% of the world's population of developing nations rely on traditional medication in primary healthcare sectors (Geneva, 2002). Among other problems, infertility is a major problem taunting the modern society. With increasing pollution at all levels and stress factors in the present day situations, infertility is mushrooming like an epidemic and creating havoc for couples and their families causing lot turmoil in the society.

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The female infertility incidence is advancing worlwide and varies from 10 to 20% (Romero Ramos *et al*., 2008). Infertility is typically defined as inability of a couple to achieve pregnancy after 12 months of regular unprotected sexual intercourse (Aflatoonian *et al*., 2011). Worldwide 15 % all women facing either primary infertility or secondary infertility, the primary infertility is defined as a couple never achieved pregnancies for a period of 2 years and the secondary infertility is defined as a couple failed to achieve pregnancy following previous pregnancy problems for a period of 2 years (Kumar, 2007). To overcome the problem of infertility the traditional practitioners are using various plants and their products. One such plant is the stem bark of *Syzygium cumini* (jamun). The species *Syzygium cumini* (Indian Blackberry) is a large evergreen tree of the genera of the myrtle family Myrtaceae. It grows quickly, attains full size by 40 years either in wild or cultivated forms; the height of the tree is up to 30 m and 3.6 m girth, with a bole up to 15 m and grown up to an altitude of 1,800 m found throughout India (Kuncha Jayachandra and Sharmila Devi V, 2012). The stem bark of *Syzygium cumini* is

thick grayish-brown and the scales of wood are exfoliating. The whitish wood closely grained and durable, affords brown dyes and a kind of gum Kino. Internal surface is fibrous in nature. The matured stem bark powder is light brown shows thin walled cork cell fragment, fibers in single or groups, the shape varies from oval to pointed, lengthened, stone cells. Calcium oxalate crystals are rosette and prismatic. Starch grains are simple circle to oval measuring 5-11 µ in diameters (Kuncha Jayachandra and Sharmila Devi V, 2012).

The earlier studies on the *Syzygium cumini* stem bark has been reported several medicinal properties like antidiabetic, carminative, astringent, refrigerant, digestive, stomachic, gastric disorder, anthelmintic, antidiabetic, intrinsic hemorrhage, sore throat, asthma, bronchitis, thirst, diuretic, antibacterial, febrifuge, skin diseases, strangury, wounds, fever, dysentery, chronic diarrhea, leucorrhoea, and menorrhagia (Jadhav VM *et al*., 2009; Salim and Paarakh, 2009). The previous study on the phytochemical screening of the ethanolic extract of the *Syzygium cumini* plant bark showed the presence of tannins, flavonoids, sugars, amino acids, proteins, sterols and absence of alkaloids, steroids, terpenoids, saponins (Venugopal *et al*., 2016). Even though scientific literature is available on the phytochemical constituent, toxicity and medicinal activities of *Syzygium cumini*, the role of *Syzygium cumini* stem bark on female reproductive system is completely lacking in spite its wide usage in traditional practice of medicine for treating infertility. So the present study is planned to evaluate the effect of fresh extract of stem bark of *Syzygium cumini* on female reproductive system.

MATERIALS AND METHODS

Plant material

The *Syzygium cumini* stem bark was used for the study. Plant material used for the study was collected in the month of june and july from Hyderabad-17 ° 22' 31" North, 78 ° 28' 28" East, Telangana, India, Asia and authenticated by Head of Botany department, Osmania University, Hyderabad, Telangana, India. The plant specimen is identified as *Syzygium cumini* (L) Skeels it belongs to the family Myrtaceae. The voucher No.0271 specimen sample of the plant was deposited in the department herbarium for future reference.

Fresh extraction

After identification of the plant, around 0.5 kg of the stem bark was collected and washed thoroughly and grounded by adding 100 ml of water, 25 ml of curd, 0.5 g pepper and 0.5 g Alium sativum. Finally the extraction was filtered and the filtrate was used for the study.

Animals

Inbred twenty five adult nulliparous and non-pregnant female Wistar rats of age around six months and weighing 190-210 g were used for the experimental study. The rats used were procured from Teena Biolabs Pvt. Ltd, Hyderabad. The rats were acclimatized to the testing conditions for a week before the beginning of the experiment. The rats were maintained as per the Institutional ethical committee (IAEC) norms, with 12 h dark and light cycle with food and water at ad libitum. The 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. Prior to experimentation all the rats were examined for the normal regular estrous cycle. The rats with a normal regular estrous cycle were selected for the study. The rat estrous cycle was determined by vaginal smear. Samples of vaginal smears were collected by swab smear (cotton buds) technique.

Experimental design

A total of twenty five estrous phase rats were randomly separated into five groups, each group consisting of five rats. The control group (C), the sham control A one day (SCA1) and four to six days (SCA4), *Syzygium cumini* stem bark fresh extract treated for one day (SF1) and four to six days (SF4) groups (Table 1).

Table 1. Experimental Design

Groups	Treatment	Duration
Control	Normal standard diet	
SCA1	5 ml/kg body weight of vehicle	Twice a day for 1 day
SCA4	5 ml/kg body weight of vehicle	Twice a day for 4 to 6 days
SF1	5 ml/kg body weight of *Syzygium cumini* stem bark fresh extract	Twice a day for 1day
SF4	5 ml/kg body weight of *Syzygium cumini* stem bark fresh extract	Twice a day for 4 to 6 days

Treatment

The oral administration was done by utilizing a curved ball-tipped intubation needle affixed to a 2 ml syringe.

Vehicle - Mixture of 100 ml of water, 25 ml of curd, 0.5 g pepper and 0.5 g Alium sativum.

C - The control group was given the normal standard diet.

SCA1 - The sham control A treated with 5 ml/kg body weight of vehicle, twice a day for 1 day on the estrus phase of the estrous cycle.

SCA4 - The sham control A treated with 5 ml/kg body weight of vehicle, twice a day for 4 to 6 days starting on estrus phase of the estrous cycle to the estrus phase of the next estrous cycle.

SF1 - Experimental rats were treated with 5 ml/kg body weight of *Syzygium cumini* stem bark fresh extract through oral administration, twice for 1 day on the estrus phase of the estrous cycle.

SF4 - Experimental rats were treated with 5 ml/kg body weight of *Syzygium cumini* stem bark fresh extract through oral administration, twice a day for 4 to 6 days starting on the estrus phase of the estrous cycle to the estrus phase of the next estrous cycle.

Sacrifice: On the day of sacrifice, all the animals were anesthetized with ether anesthesia.

Body weight

The body weight of control, sham control and treated group rats were weighed on the day of sacrifice with the Wensar top table balance model no TTB 3 and the weights were noted.

Uterine horn weight

The control, sham control and treated group rats were euthanized and carefully dissected for the uterine horns, cleaned for connective tissue and fat, examined macroscopically and immediately weighed by using Shimadzu AX-200 analytical balance and the weights were recorded.

Histological Study

After collection and weighing, the uterine horns were labeled for identification and transferred to cassettes and fixed at 10% buffered formalin for around 48 h, followed by automatic tissue processing which was carried on the Yorco automatic tissue processor (YSI-3). The automatic tissue processing included dehydration in graded isopropyl alcohol, clearing in xylene I & II, impregnation in Fisher Scientific paraffin wax I & II. Finally, tissue paraffin blocks were prepared by using Thermo Shandon paraffin dispenser and L modes. After embedding, the Leica RM2125RTS Rotationsmikrotom was used for cutting sections of uterine horn. The sections were fixed to the slide by heat technique followed by staining with the Harris's Haematoxylin and Eosin yellow stain solution- 2% W/V, Nice Chemical Pvt. Ltd (Khalid Ghazanfar., 2015). The stained uterine sections were analyzed in Labomed Vision 2000 binocular microscope using a low power objective lens 4X, 10X and a high power objective lens 40X. The photomicrographs of stained sections were taken with Digi Eye digital microscope camera. The photomicrographs of uterine horns were analyzed for uterine diameter, wall thickness, endometrial thickness, myometrial thickness and epithelial thickness by using image analysis software Digimizer version 4.5.2.

Statistical data analysis

All the data was compiled and tabulated by using Microsoft excel worksheet. The mean, standard deviation (SD), standard error (SE) and t-test were performed by using statistical software Sigma Plot 10 to find out the significance level. The P value < 0.05 is considered as statistically significant. All the values were shown as $\bar{x} \pm SE$.

RESULTS

Body weight

The stem bark of *Syzygium cumini* treated rats showed slightly increased body weight, but the increase was statistically insignificant when compared with the control and sham control group (Table 2). The mean body weight of C group showed 1901 mg (1880 to 1951 mg), where as SCA1 group showed 1902 mg (1890 to 1960 mg), SCA4 group showed 1903 mg (1850 to 1950 mg), SF1 group showed 1916 mg (1878 to 1952 mg), SF4 group showed 1919 mg (1893 to 1958 mg) – which were more than control but statistically insignificant.

Uterine weight

The uterine weight of stem bark of *Syzygium cumini* treated groups did not show variation when compared with the control and sham control groups (Table 2). The mean uterine weight of C group showed 297 mg (288 to 303 mg), where as SCA1 group showed 298 mg (280 to 323 mg), SCA4 group showed 297 mg (263 to 329 mg), SF1 group showed 298 mg (270 to 342 mg), SF4 showed 303 mg (287 to 310 mg). The SF4 showed a slight increase in uterine weight but it was statistically insignificant.

Table 2. Effect of stem bark of *Syzygium cumini* on body and uterine weight in rats

Group	Weight (mg)		
	Initial body	Final body	Uterine horn weight
Control (C)	1897.2 ± 17.0	1901.6 ± 17.0	297.2 ± 2.7
Sham control A 1 day (SCA1)	1899.0 ± 16.8	1902.0 ± 17.7	298.7 ± 9.8
Sham control A 4 days (SCA4)	1897.6 ± 16.7	1903.8 ± 16.5	297.9 ± 12.5
Syzygium cumini stem bark fresh extract 1 day (SF1)	1909.6 ± 12.7	1916.8 ± 12.5	298.5 ± 12.1
Syzygium cumini stem bark fresh extract 4 days (SF4)	1911.6 ± 14.0	1919.6 ± 13.6	303.0 ± 4.0

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

Histological Parameters

Uterine horn

The microscopic structure of treated uterine horn showed normal wall with epithelium, endometrium, myometrium and perimetrium. The microscopic structure further revealed normal columnar epithelium, numerous uterine glands in the endometrium, various muscle layers with connective tissue in the myometrium and normal perimetrium.

Wall thickness

The wall thickness of control and sham control groups were similar where as in the treated groups slight increase in thickness was noticed but it was statistically insignificant when compared with control and sham control groups (Table 3).

Table 3. Effect of stem bark of *Syzygium cumini* on wall thickness, myometrial and perimetrial thickness of uterine horns (µm) in rats

Group	WT ($\bar{x} \pm SE$)	MT & PT ($\bar{x} \pm SE$)
Control (C)	338.1 ± 10.9	144.6 ± 8.2
Sham control A 1 day (SCA1)	331.7 ± 19.0	135.8 ± 10.8
Sham control A 4 to 6 days days (SCA4)	337.6 ± 21.8	145.5 ± 18.3
Syzygium cumini stem bark fresh extract 1 day (SF1)	382.0 ± 22.9	155.3 ± 10.1
Syzygium cumini stem bark fresh extract 4 to 6 days (SF4)	413.6 ± 40.9	154.9 ± 19.0

All the data were expressed as $\bar{x} \pm SE$, n = 5 in each group, $\bar{x}$ = mean, SE = standard error, WT-Wall thickness, MT-Myometrial thickness, PT-Perimetrial thickness.

Myometrial and perimetrial thickness

The microscopic structure of myometrium and perimetrium showed a normal architecture. No change in the myometrial and perimetrial thickness of control and sham control groups were noticed while the thickness in the treated group showed a slight increase when compared to other groups (Table 3).

Endometrial thickness

The microscopic structure of the endometrium showed a normal architecture. The endometrial thickness of control group was more than the sham control group but it was statistically insignificant. Further, treated groups showed an increased endometrial thickness which is statistically significant (P< 0.05) when compared with control and sham control animals (Table 4 & Fig 1).

Epithelial thickness

The epithelial thickness of the treated group was more than the control and sham control groups. But the increased thickness was statistically insignificant (Table 4).

Table 4. Effect of the stem bark of *Syzygium cumini* on endometrial and epithelial thickness of uterine horns (µm) in rats

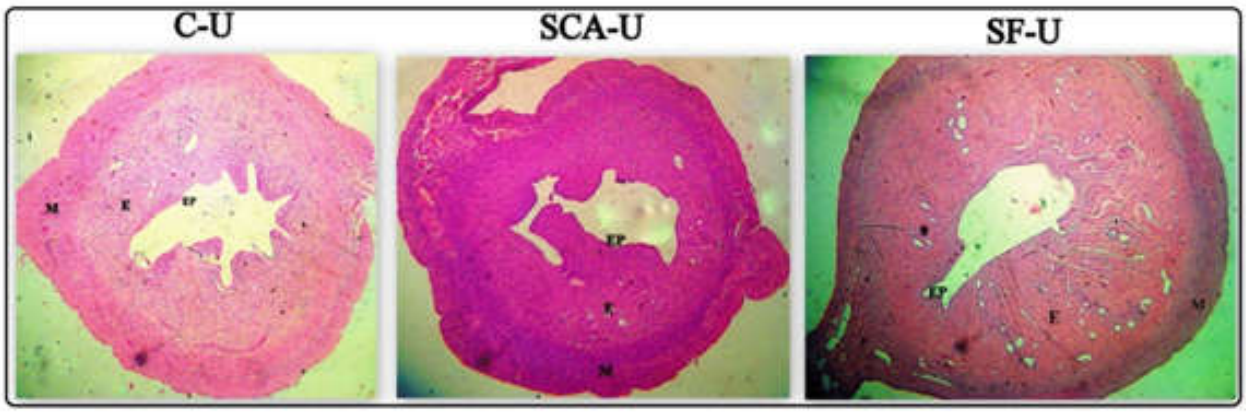
Group	ET ($\bar{x}$ ± SE)	EPT ($\bar{x}$ ± SE)
Control (C)	188.5 ± 9.0	18.7 ± 1.1
Sham control A 1 day (SCA1)	195.2 ± 9.0	18.8 ± 0.8
Sham control A 4 days (SCA4)	193.1 ± 10.8	18.1 ± 2.0
Syzygium cumini stem bark fresh extract 1 day (SF1)	231.4 ± 12.4* #	21.3 ± 2.7
Syzygium cumini stem bark fresh extract 4 days (SF4)	258.7 ± 22.8* #	19.7 ± 3.9

All the data were expressed as $\bar{x}$ ± SE, n = 5 in each group.
$\bar{x}$ = mean, SE = standard error.
* Indicates significance level between control and treated.
# Indicates significance level between sham controls and treated.
* : P<0.05, # : P<0.05.
ET- Endometrial thickness, EPT- Epithelial thickness.

DISCUSSION

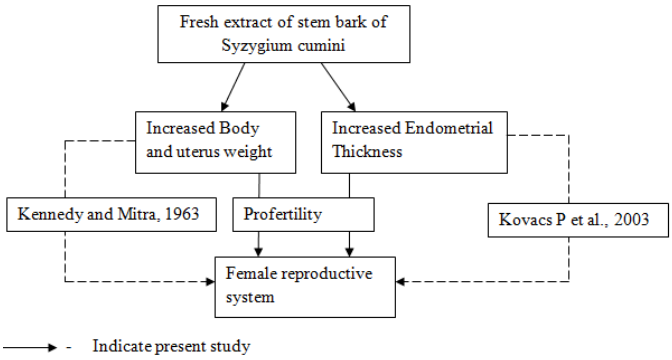
The results of the present study show an increased body weight, uterus weight but were not statistically significant. The histoarchitecture revealed no cytotoxic effect but suggested a profertility effect by increasing the morphometrical parameters. Among the morphometric parameters the increase in thickness of endometrium was statistically significant. Most of the indigenous plants used in traditional system of medicine had an anti-fertility activity (Dabhadkar *et al*., 2015; Jain *et al*. 2015; Pawan Kumar *et al*., 2015; Daniyal and Akram, 2015) and only few of them had pro-fertility activity (Oladimeji *et al*., 2014; Ugwah-Oguejiofor *et al*., 2011) one such plant is *Syzygium cumini*, the stem bark of it showed pro-fertility effect in the current study. There were no previous studies regarding the effect of the stem bark of *Syzygium cumini* on female reproductive system. The body weight is an essential factor to monitor the health of an individual. The decrease in the body weight is usually an early sign of the onset of an adverse effect. The present study results show an increase in bodyweight which is even though statistically non-significant it proves the fact that it is not resulting in any adverse effect but rather indicative of positive effect. It is reported that the weight and composition of body is well linked with the sexual maturation (Kennedy and Mitra, 1963).

So from the present study it is clear that the fresh extract of stem bark of *Syzygium cumini* is exerting a profertility effect. The fresh extract treated for 4 days showed an increased uterine weight but there was no difference between control and fresh extract treated for 1 day. This suggests that probably prolonged use may increase the weight of the organ which will strongly substantiate the profertility property of the stem bark. The mean thickness of uterine wall of treated groups was increased when compared with control and sham control groups this may be due to the increased thickness of endometrial, myometrial and perimetrial thickness. The previous studies stated that the endometrial thickness determines the pregnancy outcome and the higher pregnancy rates are significantly associated with the increased thickness of endometrium (Kovacs *et al*., 2003).



H & E stain (4 x magnifications)
C- U (Control uterus)
SCA-U (sham control uterus)
SF-U (*Syzygium cumini* fresh extract treated uterus)
EP – Epithelium, E – Endometrium and M – Myometrium.

Fig. 1. Photomicrograph of uterus



Possible Mechanism of Action of fresh extract of stem bark of *Syzygium cumini*

Some authors also have demonstrated a greater chance of pregnancy once the endometrium reaches a threshold thickness (Rinaldi *et al*., 1996; Noyes *et al*., 1995; Check *et al*., 1993). In the present study endometrial thickness of fresh extract treated groups was increased and statistically significant when compared with control and sham control groups. So the *Syzygium cumini* stem bark fresh extract may increase the pregnancy rate and outcome thereby may act as a profertility agent. The increase in the endometrial thickness may be due to the phytochemicals present in the stem bark of *Syzygium cumini* which in turn may probably exert the profertility effect by their antioxidant property.

Conclusion

We conclude from the present study that the fresh extract of stem bark of *Syzygium cumini* is having a profertility effect on the female reproductive system. Further, future studies are required on *Syzygium cumini* fresh and other extracts to evaluate the exact mechanism of action on the female reproductive system.

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