

Research Article**In vivo assessment of antifertility potential of Pteridophytic plants: *Actiniopteris radiata* (SW.) L. and *Selaginella bryopteris* (L.) Baker in swiss albino mice****Jyothi Chandrakant^{1,2}, Arjun N. Shetty^{1,2}, Sundar Mety², Md. Liyakat Ali³, Pratima Mathad^{2*}**¹Department of P. G. Studies in Botany, Sharnbasva University, Kalaburagi, Karnataka, India²Department of P. G. Studies and Research in Botany, Gulbarga University, Kalaburagi, Karnataka, India³Luqman College of Pharmacy, Kalaburagi, Karnataka, India

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Abstract

Objective: Population explosion is a foremost problem and its control in colonized countries may have an effect on the economic growth, in this regard family planning has been promoted through several methods of contraception, however current methods of contraception result in undesirable side effect. Thus there is a need to replace these agents with potent drugs with least or zero side effects. Hence, the present study was undertaken to evaluate the antifertility properties of *Actiniopteris radiata* and *Selaginella bryopteris* on female albino mice. **Material and methods:** Experimental mice were orally administered with methanol extract of *Actiniopteris radiata* (175 and 300 mg/kg body weight) and *Selaginella bryopteris* (250 and 500 mg/kg body weight) for 30 days. Effect of the treatment on female reproductive organ was investigated and level of oestrogen was determined by ECLIA oestrogen standard kit. Quantitative estimations of protein, cholesterol and alkaline phosphatase of blood serum were carried out using Biosystems Diagnostics standard kit. **Results and conclusion:** The treatment showed decrease in weight of the ovary and decrease in oestrogen level and on the other hand histological changes were also observed. The overall results of the present study revealed that the low dose of (175 mg/kg body weight) methanolic extract of *A. radiata* induced infertility whereas in high dose (300 mg/kg body weight) it is not observed. While *S. bryopteris* had not shown much significant variation in female albino mice when compared to control.

Keywords: Antifertility, *Actiniopteris radiata*, *Selaginella bryopteris*, oestrogen, Pteridophytes

Introduction

Anti-fertility is a relative term to sterility which is the process of inducing or making inability to produce the offspring. Causes for infertility in females are ovulation disorder, infections and tubal blockage, uterine tumour, abnormal uterus, and endometriosis. Ovulation disorder may be due to a hormonal imbalance, sometimes the hypothalamus or pituitary gland fail to produce hormones normally due to which either no follicles develop (absence of FSH) or ovulation is absent (absence of LH). Further, the ovaries may not be producing oestrogen or progesterone normally. In some cases there may be physical damage to the ovaries and oviducts due to infections which may

result in the partially or complete blockage of tubes. Uterine tumour and polyps also serve as barrier for anti-fertility foremost caused due to benign growth composed of fibrous and muscle tissue and second one due to tiny bud like growths in the uterus, however these could be removed by surgery. The problem of infertility is common and having children is one of the most important aims of the every couples. If infertility strikes a couple, it is personally divesting and can cause emotional pain, and to overcome this disaster peoples will be ready to pay large money for treatment to have children. And on the other hand growth and control of population is an important issue because it has direct proportionality to country's life supporting systems (Chandrakant, 2018).

Since immemorial plants are being used as contraceptive agents, and many plants and their products are used as antifertility agents, which were later experimentally proved in laboratory using modern techniques (Sharma et al., 2003).

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Research work on antifertility activity from Indian plants has been reviewed by Kamboj (1988) and Satyavati (1984). Different researchers have reported antifertility activities of different plant extracts viz., methanolic extract of *Abutilon indicum* (Maurya et al., 2004), Aqueous extract of *Aloe barbadensis* (Maharjan et al., 2010), Acetone extract of *Plumbago rosea* (Edwin et al., 2009), Ethanolic extract of *Cichorium intybus* (Danial and Akram, 2015), *Urtica dioica* and *Cuscutare flexa* (Sharma et al., 1983), petroleum ether extract of *Curcuma longa* (Chattopadhyay et al., 2004) and *Abroma augusta* (Kumar et al., 2012).

Acalypha indica and *Moringa oleifera* plants showed oestrogenic and abortifacient activity respectively (Hiremath et al., 1999; Nath et al., 1992), *Abrus precatorius* and *Aegle marmelos* showed antifertility efficacy in male rats (Bhatt et al., 2007; Chauhan et al., 2008). Antispermatic activity of *Enicostemma axillare*, *Morinda whitei*, *Bacopa monnieri*, *Cannabis sativa* (Dhanapal et al., 2012). Antispermatic activity is also reported from many plants viz., *Enicostemma axillare*, *Morinda whitei*, *Bacopa monnieri* and *Cannabis sativa* by Dhanapa et al. (2012), Watcho et al. (2001), Singh et al. (2009) and Sailani et al. (2007) respectively. Apart from herbal remedies for fertility related disorders many synthetic contraceptive agents are marketed though they have side effects. Thus there is need to identify a prominent herbal drug which is have zero or least side effect. Therefore an attempt is made to examine the antifertility activity of two pteridophytic plants by considering the ethnobotanical information on *A. radiata*. As per recent review of literature and best of our knowledge there were no antifertility studies on female albino mice using either of plants used in the present investigation but only two reports stating the antifertility effect of *A. radiata* is available on male rats reported by Sharma et al. (1999) and Dixit and Bhat (1975) have reported the same activity in combination with *Ocimum americanum*.

Pteridophytes are the second largest group of plants occupying next place to angiosperms in numbers. As a generalized opinion is that Pteridophytes have very limited uses compare to higher plants even though they have given sufficient benefits to mankind since ancient time. The diversity of Pteridophytes in India is remarkable due to variation in eco-climate, soil types and altitude conditions. Distribution of Pteridophytes in Karnataka is also interesting. In Northestern part of Karnataka especially in the Kalaburagi made us to think about the usefulness of this nature's gift for the benefit of society. Pteridophytes show medicinal utility and many of them are being used medicinally from ancient time, even though they have been unfortunately ignored. Hence, this available earlier evidence made to carry out the present work on *Actiniopteris radiata* and *Selaginella bryopteris* which are found distributed

in specific and limited area and recognized as rare available to this region.

Actiniopteris radiata is used as a styptic, anthelmintic also used in bronchitis and gynecological disorders (Upreti et al., 2009). Decoction of leaves is used in tuberculosis in the Mt. Abu area by Bheels tribe (Sharma and Vyas, 1985). The juice extracted from the stem is taken orally twice a day to treat diarrhoe and fever (Karthik et al., 2011). And the plant is used for enhancing the birth rate as well as for birth control (Parihar and Parihar, 2006).

Single teaspoon of paste of leaves with water is given twice a day in gonorrhoea and other venereal diseases (Spermatorrhoea and Leucorrhoea) and also used as diuretic. Dried plant with tobacco is smoked for hallucination. Paste of young leaves with sugar is taken in stomachache, urinary tract inflammation in children. It is a popular strength tonic amongst local people/dried plants along with tobacco are smoked by tribal people for inducing hallucination used as witch craft and worship. (Shweta et al., 2005; Pratibha et al., 2011). Relief from heat stroke and the burning sensation during urination, restoring menstrual irregularities to normal, helping in easy delivery of pregnant women (in minimizing the labour pain), treatment of jaundice (Antony and Thomas, 2011).

Materials and methods

Plant material

In the present study methanolic extract of shade dried whole plant material of *Actiniopteris radiata* and *Selaginella bryopteris* is used.

Experiment animals

Adult (age 12-14weeks) Swiss albino mice weighing 25-30g of either sex were (one month female offspring's were isolated for study and kept under observation, after 2 months animals were used for study) selected for Antifertility activity was housed and maintained under hygienic conditions. All the animals were fed with standard pellet food from VRK nutrition Ltd, Pune. Extracts were prepared in gum *Acacia* (1%) administered orally with the help of oral feeding needle and distilled water was provided as control. Body weight of the animals was recorded regularly during the entire experiment. Animals were maintained according to the guidelines of institutional animal ethic committee.

Experimental design

The present antifertility study comprises of randomly selected animals (n=6+2) which included of virgin females and males and they were divided into six groups, as follows,

- Group-1 Control (only distilled water and food)
 Group-2 Standard (ethylene estradiol) 0.468 mg/kg
 Group-3 Methanolic extract of *Actinopterys radiata* (MEAR) 175 mg/kg
 Group-4 Methanolic extract of *Actinopterys radiata* (MEAR) 300 mg/kg
 Group-5 Methanolic extract of *Selaginella bryopteris* (MESB) 250 mg/kg
 Group-6 Methanolic extract of *Selaginella bryopteris* (MESB) 500 mg/kg

Group-1 served as control which receives only distilled water and food and Group-2 served as standard and administered with ethylene estradiol 0.468 mg/kg. Group-3 and 4 animals administered with methanolic extract of *Actinopterys radiata* 175 mg/kg and 300 mg/kg respectively. Group-5 and 6 animals administered with methanolic extract of *Selaginella bryopteris* 250 mg/kg and 500 mg/kg respectively.

The two doses of methanolic extract of *Actinopterys radiata* (175 and 300 mg/kg), and *Selaginella bryopteris* (250 and 500 mg/kg) were administered for 30 days. Body weight of the animals was recorded before and after the treatment, the animals from each group were sacrificed 24 hr after the last treatment. The ovaries were dissected out and adhering tissues were removed and weighed. Blood was collected and serum was prepared and stored at -20 °C for further use (Oestrogen, and also for biochemical estimations such as protein, cholesterol, and alkaline Phosphatase). Ovary from one side of each animals were used for histological studies

Weight of the mice

Initial and Final body weight of the animals was recorded i.e., before and after the treatment.

Weight of the mice ovary

After dissection of the mice, ovaries were dissected out and adhering tissues were removed, blotted free of blood and weight of the ovary were recorded.

Table 1. Weight of the animals after treatment

Weight of the animals	Group-1 Control	Group-2 Standard	Group-3 MEAR 175mg/kg	Group-4 MEAR 300mg/kg	Group-5 MESB 250mg/kg	Group-6 MESB 500mg/kg
Initial weight (g)	25.2±0.30 ^{ns}	25.96±0.31 ^{ns}	24.90±0.20 ^{ns}	25.76±0.14 ^{ns}	25.13±0.24 ^{ns}	25.86±0.40 ^{ns}
Weight after 1 week	25.77±0.39 ^{ns}	24.57±0.78 ^{ns}	23.70±0.30*	26.25±0.77*	25.96±0.26 ^{ns}	25.86±0.40 ^{ns}
Weight after 2 week	27.1±0.43*	25.22±0.77*	24.11±0.67**	27.83±0.21 ^{ns}	26.93±0.46 ^{ns}	27.78±0.34
Weight after 3 week	26.33±0.88*	26.88±0.48 ^{ns}	25.80±0.37*	27.68±0.50*	27.96±0.48 ^{ns}	28.86±0.18
Weight after 4 week	28.37±0.48 ^{ns}	27.42±0.71 ^{ns}	26.11±0.67*	28.88±0.48*	28.83±0.21*	28.90±0.55
Weight after 5 week	29.00±0.57 ^{ns}	28.02±0.36 ^{ns}	26.91±0.27*	29.41±0.30*	28.70±0.65 ^{ns}	28.70±0.65 ^{ns}

MEAR: Methanolic extract of *Actinopterys radiata*, MESB: Methanolic extract of *Selaginella bryopteris*. Values are expressed as Mean ± SE for three trials, a values are significantly different compared to control when p* > 0.05 ** > 0.01 *** > 0.00

Blood serum analysis of mice

Estimation of Proteins, Cholesterol and Alkaline phosphatase

The level of proteins, cholesterol and alkaline phosphatase were measured by using standard kit obtained from Biosystems Diagnostics Pvt. Ltd.

Oestrogen hormonal assay

The level of oestrogen in serum was measured by ECLIA oestrogen standard kit. The serum was extracted from the blood samples, collected through retro orbital vein puncture method.

Histological studies

Randomly selected mice were dissected for histological studies of ovaries portion of the ovary were fixed in fixative and dehydrated in graded ethanol series. Cleared in benzene and embedded in paraffin, then these were sectioned at 5 µm using rotator microtome. Then these sections were stained with haemotoxyline-eosine, and photographed under microscope (x200).

Statistical analysis

Each treated group consisted of 6 animals and quantitative estimation were analysed by Duncan's Multiple Range test, one way analysis of variance (ANOVA) using online software <http://shiny.stat.tamu.edu:3838/hassad/SumAOV1/>. Values are the mean of three replicates.

Results and discussions

A number of contraceptive methods were available worldwide for women, such as oral contraceptive pills, injectable, intrauterine and natural family planning methods but continuous usage of these drugs may lead to side effects as a generalized opinion. Thus there is a need of drug with zero or lesser side effects.

Body and ovary weight of mice

Body and ovary weights of the animals were recorded at an interval of a week and the results were presented in the table 1 and 2. Initial weights of all (Group 1-6) experimental mice

Table 2. Weight of the organ

Group	Group-1 Control	Group-2 Standard	Group-3 MEAR 175 mg/kg	Group-4 MEAR 300 mg/kg	Group-5 MESB 250 mg/kg	Group-6 MESB 500 mg/kg
Weight of the Ovary	1.38±0.34*	0.71±0.01	0.19±0.00*	0.91±0.05	1.35±0.20	1.37±0.30

Data represents average of three replicates. Values (mean±SD) in a column followed by the same letter are not significantly different (P>0.05). MEAR: Methanolic extract of *Actinopterys radiata*, MESB: methanolic extract of *Selaginella bryopteris*

are nearly same with a variation of 0.5-1.0 g. After five weeks of experiment period also there was no significant variation in the body weight of the all 6 grouped mice but Group-1 mice showed normal growth rate throughout the experiment period. But there was a significant decrease in the weight of the ovary of Group-2 and 3 compared to the Group-1 (Control). It is well documented that FSH is essential for follicular growth and LH is necessary for ovulation and corpora lutea formation, which are responsible for the growth and increase in the weight of ovary (Richards, 1980). Therefore from the present result, it can be explained that reduction in the ovary weight after the treatment (Group-3) may be due to reduction in the follicular growth and ovulation, which are dependent on availability of gonadotrophins.

Estimation of Protein, Carbohydrates, Alkaline phosphatase and Oestrogen

The results of the Proteins, Cholesterols, Alkaline phosphatase and hormonal (oestrogen) assay were presented in table 3 respectively.

It is observed that protein content in all groups of mice is similar with negligible variations, which indicates no effects occurred on normal physiology of mice. Whereas, blood cholesterol level is slightly increased in Group-3 and slightly decreased in Group-1, 4, 5 and 6 animals, when compared to Group-2 (standard). Generally cholesterol is an important precursor in the synthesis of steroid hormone. Similar work were carried out by Anju et al., (2008) on *Nelumbo nucifera* in female mice and found that significant decrease in the cholesterol level. In

contrary, *Citrus medica* seeds (Sharangouda and Saraswati, 2009) showed increase in ovarian cholesterol level. Alkaline phosphatase activity is increased in Group-3, slightly decreased in Group-1, 4, 5 and 6 when compared to standard (Group-2). It is reported that increase in the Alkaline phosphatase in granulosa and theca cells precedes histological changes which leads to the degeneration of follicles (Stansfield and Flint, 1967). The main part of the study was the quantification of steroidal hormones (Oestrogen) was done by using ECLIA estrogen standard kit. Significant decline in the estrogen level in Group-3 (12.40±0.29) and increase in Group-1 (18.27±0.18) and Group-4 (18.11±0.05), Group-5 (17.24± 0.15) and Group-6 (18.37± 0.27) when compared to Group-2 (13.40±0.28). Similar work were carried out by Mishra et al., (2009) on *Bougainvillea spectabilis* in Swiss male and female mice and reported the significant decrease in oestrogen levels in female mice and significant decrease in testosterone in male mice, which shows antifertility effect of *Bougainvillea spectabilis*.

Histological studies

The results of the histological studies were presented in figure 1. From the results of the present study it is observed that the histology of the Group-1 animals ovary were normal and divisible into outer cortex and an inner medulla. In normal condition the ovary is covered by tough connective tissue capsule called the tunica albuginea, which

Table 3. Biochemical changes in mice due to administration of MEAR and MESB

Groups	Protein (µg/100mg)	Cholesterol (µg/mg)	Alkaline phosphatase (ALP) (µg/100mg)	Estradiol
Group- 1	14.29±0.19*	302.66±1.45	179.52±2.34	18.27±0.18
Group-2	11.17±0.09*	380±2.88*	191.88±1.56*	13.4±0.28
Group-3	11.32±0.19*	391±4.09*	274.33±2.33*	12.40±0.29
Group-4	15.14±0.09**	249±2.08***	142.84±1.52	18.11±0.05
Group-5	13.10±0.06**	315±2.88	152.1±1.53	17.24±0.15
Group-6	14.17±0.12*	305±2.88	136.43±0.66	18.37±0.27

MEAR: Methanolic extract of *Actinopterys radiata*, MESB: methanolic extract of *Selaginella bryopteris*. Values are expressed as Mean ± SE for three trials, a values are significantly different compared to control when p>0.05 **>0.01 ***>0.0001.

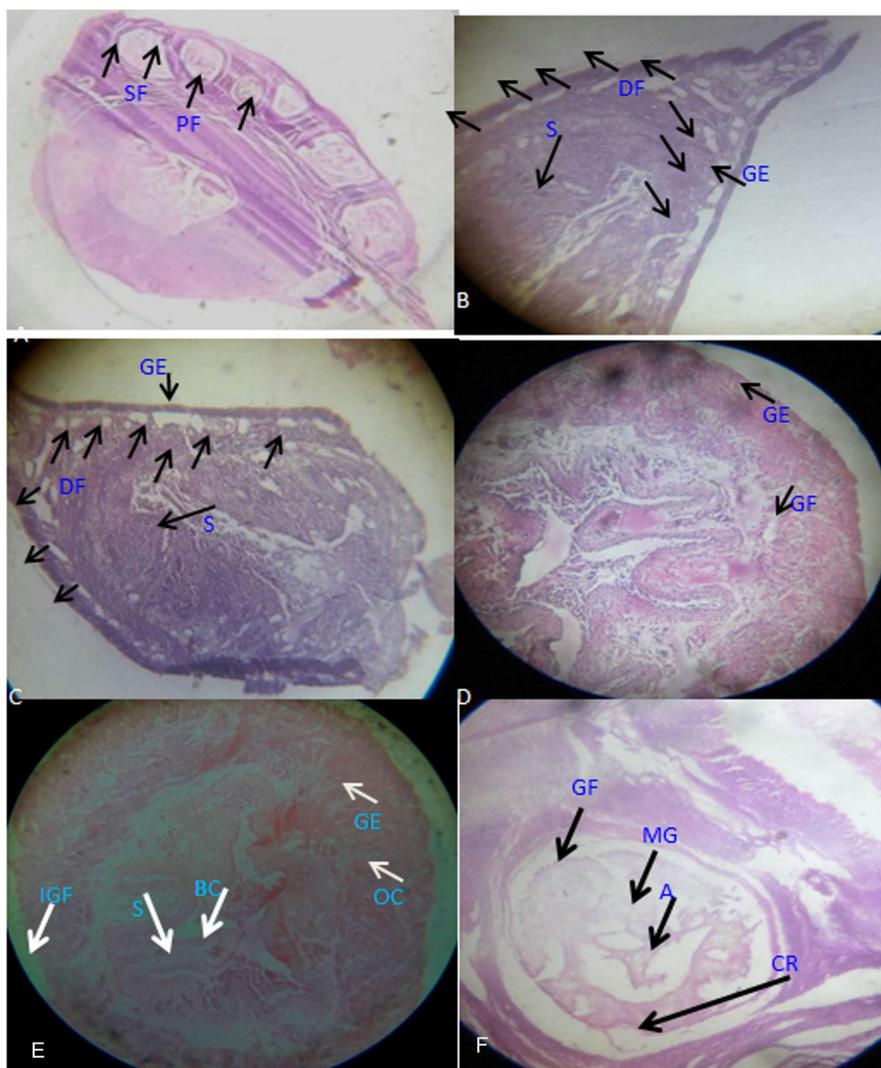


Figure 1. Photomicrograph of histology: SF: Secondary Follicle, PF: Primary Follicle, S: Stroma, DF: Degenerating Follicle, GF: Graffian follicle, GE: Graffian IGF: Initiation of Graffian Follicle, BC: Blood Cells, CO: Cumulus oophorus, MG: Membrane granulosa, A: Antrum, CR: Corona radiata

has an epithelial covering called germinal epithelium and which was observed in Group-1 animals, and there was no degenerating follicle, hemorrhagic follicle or any other abnormal features were observed. But various developmental stages of ovarian follicles viz., primary and secondary follicles were observed (Figure 1A). In Group-2 germinal epithelium is observed and a number of degenerating follicles were also observed, degeneration of follicles may be due the effect of the plant extract (Figure 1B). In Group-3 degenerating follicles were observed, the degeneration of the follicle may be due the slight increase in Alkaline phosphatases in ovary which may be leading degeneration of follicle (Figure 1C), the result is evidenced by the similar observation by Lobel et al., (1961). In Group-4 germinal epithelium is observed and initiation of graffian follicle formation was also observed (Figure 1D). The Group-5 and 6 animals administered with *S. bryopteris* methanolic extract showed normal histology which is almost near to the histology of control grouped animals (Group-1). Histology of Group-5 animals showed germinal epithelium,

normal stroma and also initiation of graffian follicle and in Group-6 a fully developed graffian follicle was observed with theca externa, theca interna, membrane granulosa but antrum, corona radiata and cumulus oophorus was not clearly differentiated (Figure 1E and F). Similar work were carried out by Mishra et al., 2009 on *Bougainvillea spectabilis* and from the results it is found that male mice have shown more degeneration of their gonads in comparison to the female mice and histologically there were no significant changes were observed. Increase in the alkaline phosphatase in granulosa and theca cells precedes histological changes leading degeneration of follicles (Friedrich et al., 1975).

Conclusion

It is believed that only angiospermic plants are having more medicinal property but lower plants are also have medicinal property but are least concerned due to lack of proximate knowledge. Two important medicinal pteridophytes, *Actinopteris radiata* and *Selaginella bryopteris* have shown

significant variation during antifertility studies. From the results of the antifertility study it can be concluded that, *Actioniopteris radiata* exhibited antifertility at lower dose (175 mg/kg), whereas at higher dose (350 mg/kg) results were complete contradictory to the lower dose i.e., at higher dose the plant extract exhibited fertility, this dual properties in the same plant enhanced the scope of further research at molecular level to evaluate active bio-compounds which could help in laying down standards for authentication of original drug, and *Selaginella bryopteris* shown potent productiveness, the present study can serve valuable source of information and provide suitable diagnostic tool for the further standardization of medicinal plant also in future investigation or applications, because drugs obtained from plants is no doubt cheaper but crude extract intake must be recommended with care, as it may control one ailment but due accumulation of variety of biomolecules which may affect other physiological phenomenon in the body, therefore it is advisable to go for single novel compound which is a need of the present generation.

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