IJPSR (2016), Vol. 7, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 May, 2016; received in revised form, 11 June, 2016; accepted, 29 June, 2016; published 01 September, 2016

EVALUATION OF ANTIFERTILITY ACTIVITY OF *ACHYRANTHUS ASPERA* LEAVES IN FEMALE MICE

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Keywords:

Antifertility, *Achyranthus aspera*, Leaves, Antiovulatory, Estrogenic, Teratogenic

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ABSTRACT: Present study was conducted to know the antifertility potential of *Achyranthus aspera* leaves in female mice and has shown promising results of antiovulatory activity, estrogenic and constant teratogenic activities. Administrations of *A. aspera* leaves methanolic extract at the dosage 25 mg/kg and 50 mg/kg body weight were subjected for testing their antiovulatory, estrogenic and teratogenic activity in female mice by applying statistical analysis in gravimetric, biochemical and histopathological parameters of ovary and uterus. At autopsy on day 31st, both the dose treated mice revealed increase in ovarian weight, histological changes like increasing number of atretic follicle and decreases in healthy developing follicles, *Graafian* follicles and corpora lutea. Total cholesterol content and alkaline phosphatase of the ovary were increased and protein, glycogen, acid phosphatase content were decreased. Teratogenic activity did not alter any changes in the morphological and physiological behavior of experimental period. Results suggested that, methanolic extract of *A. aspera* leaves are more active at low dose compared to high dose and might be used as an herbal contraceptive in females.

INTRODUCTION: Fertility control is a unique medical endeavour to find an agent that interferes with normal reproductive functions by creating a non-physiological situation for the control of fertility. Surgical, chemical and physical approaches are available, of which chemical contraception isomer convenient and widely used. The search for oral chemical agent that can control human fertility is as old as recorded in history and was in practice even in pre-historic times [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(9).3794-01

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (9).3794-01

Currently, population control has assumed greater significance. In this context search for harmless, inexpensive and effective agents for fertility control in human beings has gained tremendous importance. Investigation on search for synthesizing new antifertility agents is under process. Past two decades has witnessed a number of developments in finding of newer drugs for contraception [2].

In recent years much attention has been paid by many international bodies for birth control. There was always a hope that, research on traditional medicinal plants may provide reliable contraceptive agents. Significant steps have been taken by world Health Organization (WHO) to carry out research aimed at finding out new and effective fertility regulating agents from plants and their products [3].

Presently available steroid steroidal contraceptive have severe side effects and are not being used by the developing countries due to some social and economic reasons.

India has tremendous wealth of medicinal plants. Systematic efforts to explore and to exploit this valuable potential are lacking. Although a large number of highly active pure compounds have been isolated from plants, but there are few reports to establish their usefulness [4]. Hundreds of medicinal plants have been reported to possess antifertility activity and are compiled in books such as [5], 75 % fold population in India still use herbal preparations in the form of crude powder, extract or decoction because these are easily available and rural folk have strong faith in traditional medicines and natural products [5].

According to ancient ayurvedic concepts, systematic contraceptives had to be administered during menstrual blood flow period as this was supposed to be accompanied by ovulation [6]. In our laboratory previous findings were reported on antiovulatory acivity on these plant extracts, they are *Hibiscus rosa sinesis* flowers [7] *Momordica charantia* seeds [8] *Rivea hypocraterformis* aerial parts [9], *Crotalaria juncea* seeds [10,] *Citrus meidca* seeds [11, 12] and *Oxalis corniculata* whole plant [13].

MATERIALS AND METHODS:

Current study aimed to assess the evaluation of antiovulatory potential, estrogenic and teratogenic activity using standard bioassays.

Plant material:

Fresh leaves of *Achyranthus aspera* were collected from the fields in and around Gulbarga, Karnataka, India during August-October 2015 and authenticated at the herbarium, Department of Botany, Gulbarga University, Gulbarga, where voucher specimens are deposited.

Extraction of plant material:

Leaves were shade dried, chopped into small pieces, powdered and subjected to soxhlet extraction of methanol for 18-20 hours. The decoction so obtained was evaporated under reduced pressure and controlled temperature (50-60˚C). Dried mass considered as the extract, preserved at 6˚C in refrigerator until used and diluted as required for experimental studies.

Animals:

Mature, healthy, virgin adult female mice of 6-8 weeks old weighing (20-25 gm) with normal estrous cycle were kept under controlled conditions of light (12 Hour) and temperature (24±3 ºC) having free access to mice chow pellets (CFTRI, Mysore, India) and water ad libitum. Extracts were prepared in Tween-80 (1%), suspended in distilled water, administered orally to animals with the help of intragastric catheter at selected doses and control animals received an equivalent amount of vehicle only.

Experimental protocol:

Methanol extract of *Achyranthus aspera* leaves was subjected to antifertility studies, on a colony bred adult female albino mice weighing 20-25 gm were used for this experiment. They were divided into three groups, each group consisting of 5 mice. First group served as control (no treatment), second group of mice treated 25mg/kg body weight and third group mice were received 50mg/kg body weight of *A. aspera* leaves methanol extract. All the above treatments were given for 30 days continuously.

On 31st day all mice were sacrificed by cervical dislocation and the ovary & uterus were dissected out, the surrounding unwanted tissues were adhered, organs washed in 0.9% NaCl, blotted in filter paper and weighed quickly on sensitive electronic balance (Anamed Electronic Balance, India). Wet weights of these organs were taken and also subjected for biochemical analysis. Tissues from one side of each animal were fixed in Bouin's fluid, processed for histological preparation and Haematoxylin- Eosin stained slides examined in microscopically. Number of developing follicles, *Graafian* follicles, corpora lutea and atretic follicles were observed from stained serial sections of the ovary from each rat [14].

Micrometric measurements like diameter of uterus, thickness of endometrium, myometrium and height of epithelial cells were calculated by the method described by Deb et al., [12]. Organs from other side were used for biochemical estimations like protein

[15], glycogen [16], cholesterol [17], acid and alkaline phosphatase [18]. Statistical analyses were carried out using Students '*t*' test to find significance. Results were judged significant if $p<0.05$ [19].

Teratogenic Test:

Embryotoxic and malformation of fetus inducing chemicals called as terata. Chemical agents that produce a terata are called as teratogen or teratogenic agent.

Experimental study on mice was done to assess the methanolic extract of *A. aspera* leaves to know adverse effects on fertility and pre-natal development. Adult female mice weighing between 20-30 gm showing regular 4-5 day estrous cycle were divided into 4 groups of 5 mice. Each cage containing 5 mice, young males of proven fertility were admitted to each of the cages, so that ratio between females and males are 3:1, these males were kept overnight and separated every morning. Vaginal smears were taken daily at 09.00 AM for the cornification of successful mating. Day on that thick clumps of spermatozoa were detected in vaginal smear, it was termed as the first day of pregnancy.

First group of mice was treated as control (untreated). Second group of mice were given 0.2 ml/distilled water/mice, third group of mice received 25 mg/kg/day body weight of the extract and fourth group of mice received 50 mg/kg/day body weight of the methanolic extract of *A. aspera* leaves for 18 days continuously from the first day of pregnancy throughout gestation period. All experimental mice were under observation for one month in postnatal periods, for the growth of mice and their litters.

RESULTS AND DISCUSSION:

Administration of methanolic extract of *A. aspera* leaves after 30 days resulted that, did not show any changes in general behavior and morphology in mice. But changes were observed in gravimetric studies, biochemical and histological studies.

Changes in the ovary:

Gravimetric changes: Results are detailed in **Table 1**. Administration of *A. aspera* methanolic extract leaves at both the doses to adult female mice decreased ovarian weight significantly ($p<0.05$).

TABLE 1: GRAVIMERTIC CHANGES OF THE SEX ORGANS DUE TO THE INTRAPERITONEAL ADMINISTRATON OF METHANOLIC EXTRACT OF *ACHYRANTHUS ASPERA* (MEAa) LEAVES FOR 30 DAYS

Treatment	Doses (mg/kg body weight)	Ovary Weight (mg/100g body weight)	Uterus (mg/100g body weight)
Control	Dw	0.089±0.0826	0.250±0.042
MEAa	25	0.067±0.0056*	0.456±0.0319*
MEAa	50	0.054±0.0045*	0.445±0.0015*

Values are mean ± SEM of five animals * significant compared to control ($p<0.05$)

Biochemical studies:

There was slight decrease in the level of protein when observed at 50mg /kg extract treated mice but statistically not significant and significant ($p<0.05$) increase in the amount of protein in mice was observed at 25 mg/kg treated group. Lowered protein content of gonads indicates the retarded ovarian growth which is dependent on availability of pituitary FSH, FSH is essential for protein synthesis in gonads [20].

No significant decrease in amount of glycogen was seen in mice treated with 25 mg/kg, but significant decrease in the amount of glycogen in mice treated with 50mg/kg of extract. Therefore, decrease in glycogen content indicates the utilization of carbohydrates for energy in disintegration process of carbohydrates. Ovarian glycogen is an energy source for various processes like ovulation, transformation, survival of egg and implantation [21]. Decreased ovarian cholesterol could be attributable to a probable alteration in its synthesis of steroids or transport of gonads. It is evident that biosynthetic capacity of ovary is influenced by FSH, LH and prolactin [22, 23].

There was non-significant increase in alkaline phosphates level in 25 mg/kg treated group and increase in level of alkaline phosphates only at 50mg/kg treated mice. There was significant increase in activity of acid phosphates indicates at both treated groups. Increase in the activity of acid phosphates was noticeable at both treated groups. Increase in activity of acid phosphates indicates the disintegration of lysosomes and liberation of more of hydrolytic enzymes. Increase in acid and

alkaline phosphatases in granulose and thecal cells precede histological changes leading degeneration of follicles [24]. Glycogen is a source of readymade energy for various metabolic processes, its increase or decreasing amount under the effect of extract certainly reflects the disturbances in regular supply of energy to vital organs [25]. (**Tab. 2; Fig.1** to **3**).

TABLE 2: BIOCHEMICAL CHANGES IN THE OVARY OF MICE AFTER TREATMENT WITH MEAa LEAVES FOR 30 DAYS

Treatment	Dose (mg/kg body wt)	Protein (µg/100mg)	Cholesterol (µg/100mg)	Glycogen (µg/100mg)	ALP (µg/100mg)	ACP (µg/100mg)
Control	Dw	11.07 ± 0.159	2.205±0.075	3.2±0.212	11.5±1.092	8.16±0.321
MEAa	25	13.5 ± 0.691*	1.07±0.007*	2.8±0.457	12.1±0.869	12.0±0.031*
MEAa	50	11.4±0.129	0.89±0.078*	2.51±0.231*	11.1±0.149	13.1 ±0.102*

Values are mean ± SEM of five animals * significant compared to control ($p < 0.05$)

Histopathological studies:

This study revealed that there was antiovulatory activity after 30 days of extract treated mice. There was reduction in the number of *Graffian* follicles, complete disintegration of primary and secondary follicles and corpus luteum was noticed at 25 mg/kg treated mice. But there was 100% disintegration of the follicles, such as *Graffian* follicles, primary and secondary follicles and corpus luteum at 50 mg/kg treated mice (**Fig. 3; Tab. 3**). However, absence of corpus luteum and other follicle in ovaries of experimental mice indicated the absence of ovulation, suggesting that critical balance of pituitary gonadotrophins are disturbed after the extract treatment [26, 27]. Present study also revealed that, there was reduction in diameter of ovary at 25 mg/kg, but further more decrease in diameter was observed at 50 mg/kg treated mice. Decreases in measurements are statistically non-significant (**Tab.3**).

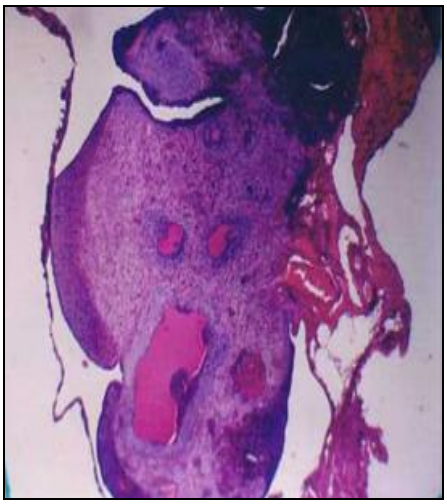

FIG. 1: NORMAL

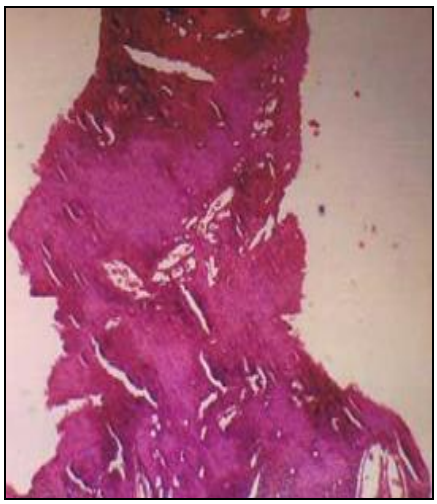

FIG. 2: TREATED WITH 25 MG

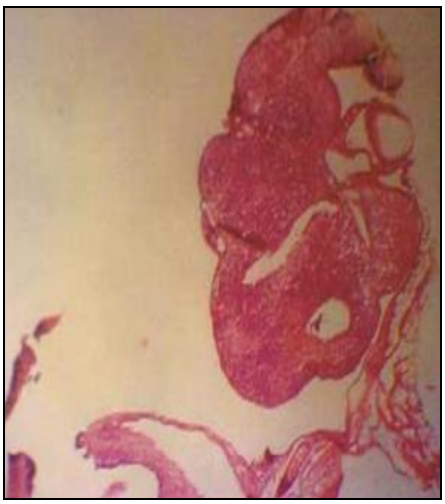

FIG. 3: TREATED WITH 50 MG

PHOTOMICROGRAPHS OF OVARIAN HISTOLOGY OF: FIG. 1: CONTROL RAT SHOWING NORMAL FULLY DEVELOPED GRAAFIAN FOLLICLE AND HEALTHY FOLLICES (X 100). FIG. 2: RAT TREATED WITH MEAa (25 MG/KG B.W.) OVARY SHOWING UNDER DEVELOPED AND DEGENERATING FOLLICLES (X 100). FIG. 3: RAT TREATED WITH MEAa (50MG/KG B.W.) OVARY SHOWING DEGENERATIVE FOLLICLES (X 100).

TABLE 3: HISTOPATHOLOGICAL CHANGES IN THE OVARY AFTER THE ADMINISTRATION OF THE (MEAa) LEAVES FOR 30 DAYS.

Treatment	Diameter of the ovary (µm)	No. of griffin Follicles	No. of corpus Latium
Control	1026.76± 11.210	4.5 ± 0.275	1
MEAa (25mg/kg)	1016.568± 13.302	2.7 ± 0.295	0
MEAa (50mg/kg)	1009.479± 17.865	0	0

Values are mean ± SEM of five animals

It is well documented that FSH is essential for follicular growth and LH is necessary for ovulation and corpora lutea formation [28] and also responsible for the growth and weight of ovary. Therefore, reduction in ovarian weight after the treatment of methanolic extract of *A. aspera* leaves may be due to reduction in the follicular growth, ovulation and also they are dependent on availability of gonadotrophins. Though, follicular atresia is common in mice ovary, increased number of atretic follicles of experimental animals indicates non-availability of gonadotrophins in required amount for follicular growth and ovulation [29].

Changes in the Uterus:

Gravimetric changes:

Results are detailed in **Table 1**. Administration of both doses of *A. aspera* methanolic extract leaves to adult female mice increased in weight of uterus significantly ($p < 0.05$).

Biochemical studies:

There was a significant increase in amount of protein and glycogen in both treated groups of mice. Protein is considered to be the building material, is involved in alteration of almost every physiological function and cellular functions, but protein synthesis in uterus is considered to be regulated by estrogen and progesterone. Increase in protein concentration generally led to increase in uterine weight. Increase in uterine glycogen due to treatment of extract may be increase in production of more estrogens are consequently and also increases rate of hexokinase reaction are determining factor for increased glycogen formation [30].

Different concentration of *A. aspera* leaves reduced significant amount of cholesterol in both group of treated mice. Decrease in cholesterol amount affects the induction of estrogen and progesterone hormone in females. There was an increase in alkaline and acid phosphates level is statistically non-significant. Uterine growth depends on the availability of ovarian steroid hormones, particularly estrogens [31]. Possibility of these changes may be due to progestogenic and estrogenic effect of *A. aspera* leaves extract as uterine growth and secretion depends on availability of ovarian steroid hormones [32]. Increase in protein concentration, glycogen content, cholesterol level, acid and alkaline phosphatases of uterus are responsible for changing uterine milieu [33] which are unfavorable for implantation and maintenance of pregnancy is presented in **Tab. 4** and **Fig. 4** to **6**.

TABLE 4: BIOCHEMICAL CHANGES IN THE UTERUS OF MICE AFTER TREATMENT WITH MEAA LEAVES FOR 30 DAYS.

Treatment	Dose (mg/kg)	Protein (µg/100mg)	Cholesterol (µg/100mg)	Glycogen (µg/100mg)	ALP (µg/100mg)	ACP (µg/100mg)
Control	_	7.67 ± 0.1079	3.252 ± 0.012	1.78 ± 0.059	7.69± 1.278	4.51 ± 1.34
MEAa	25	10.75 ± 0.405*	2.989 ±0.02*	1.9 ±0.018*	8.28 ± 0.112	5.78 ±0.89
MEAa	50	9.89 ± 0.040*	2.567±0.213*	2.5 ± 0.115*	8.766 ±0.097	6.95 ± 1.75

Values are mean ± SEM of five animals * significant compared to control ($p < 0.05$)

Histopathological studies:

Histometric changes in uterus showed that there was significant increase in musculature, well developed endometrial glands, increase in luminal epithelial height and loosely arranged stroma in endometrium and also increase in total diameter of uterus. It also showed increase in thickness of endometrium and myometrium significantly. These changes occur in uterus only due to estrogenic influence of extract treatment. Increase in uterine weight and thickness further supports to the estrogenic activity [34] (**Tab. 6**).

TABLE 5: HISTOPATHOLOGICAL CHANGES IN THE UTERUS AFTER TREATMENT WITH THE MEAa LEAVES FOR 30 DAYS.

Treatment	Dose (mg/kg)	Diameter of the uterus (µm)	Height of the epithelial cells (µm)	Myomerium (µm)	Thickness of the endometrum (µm)
Control	No	1821.386± 1.431	38.027± 0.520	43.75± 1.09	90.54± 4.81
MEAa	25	2981.219± 0.231*	49.51± 0.203*	47.145± 0.461*	231.071± 8.144*
MEAa	50	3312.180± 9.120*	51.12±1.890*	55.126± 0.781*	261.085± 1.013*

Values are mean ± SEM of five animals * significant compared to control ($p < 0.05$)

Methanolic extract of *A. aspera* leaves treated with both doses showed opening of vagina, while all the control mice were shown closed vagina (**Tab. 6**). Examination of vaginal smears of treated mice revealed predominantly cornified, nucleated epithelial cells and also showed irregular estrous cycle. There are early reports were suggested that treated rats showed opening of vagina and control rats shown closed vagina indicating that drug has estrogenic activity [35].

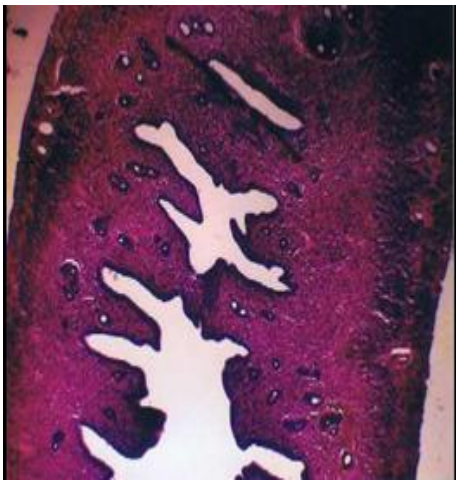

FIG.4: NORMAL

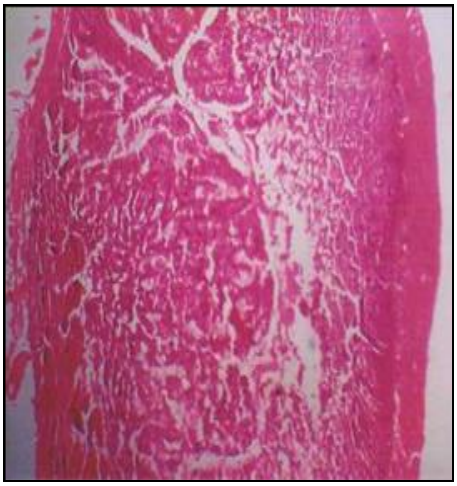

FIG. 5: TREATED WITH 25 MG.

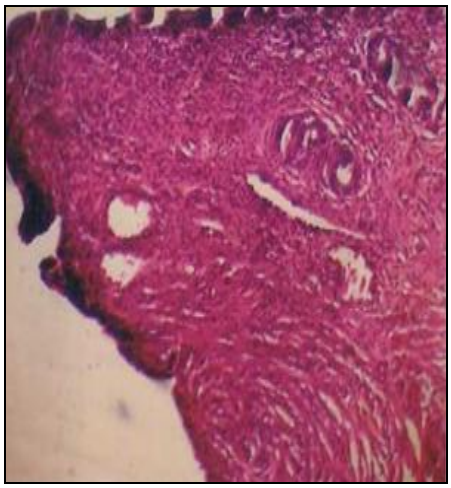

FIG. 6: TREATED WITH 50 MG.

PHOTOMICROGRAPHS OF UTERUS HISTOLOGY: FIG.4: CONTROL RAT SHOWING NORMAL ENDOMETRIUM WITH ENDOMETRIAL GLANDS AND LUMINAL EPITHELIAL CELLS (X 100). FIG. 5: RAT TREATED WITH MEAa (25 MG/KG B.W) EXTRACT SLIGHTLY HYPERTROPHIED ENDOMETRIUM, ENDOMETRIAL GLANDS AND LUMINAL EPITHELIUM (X 100). FIG. 6: RAT TREATED WITH MEAa (50 MG/KG B.W) EXTRACT SHOWING HYPERTROPHIED ENDOMETRIUM, ENDOMETRIAL GLANDS AND LUMINAL EPITHELIUM (X 100).

TABLE 6: ESTROGENIC ACTIVITY OF MEAa LEAVES.

Group	Treatment (mg/kg)	Vaginal opening	State of estrous cycle on autopsy
1	Control (No)	Not opened	--
2	MEAa (25)	Opened	Estrous (cornified cells)
3	MEAa (50)	Opened	Estrous (cornified cells)

Values are mean ± SEM of five animals

Teratogenic Test:

No adverse effects on pregnancy and growth of fetus in the prenatal and postnatal periods were encountered. The data further confirmed the complete absence of any teratogenicity of *A. aspera* leaves in these doses and established its safety even during the pregnancy periods and mice did not show any changes after post natal periods like morphological and physiological behavior of litters examined upto one month period compared with untreated mice. Shibeshi et al., reported methanolic extract at dose level of 1 gm/kg and 1.6gm/kg body weight of *A. aspera* levaes has antifertility effect and is safer contraceptive doses and also reported recently abortifacient activity with hormonal profile at dose level of 3gm/kg and 5.5gm/kg body weight [36, 37]. All the treated and control groups of mice delivered normally after full term. There was no reduction in mean number of litters and no defect found in any of the litters in drug treated groups. *A. aspera* leaves is having estrogenic property. Antiprogestational agents are usually preferred over estrogenic compounds in order to regulate fertility as they have less binding capacity with non-target tissues.

CONCLUSION: No death was recorded during the treatment period either in control or in drug treated groups. The mice did not show any changes in the general behavior and other physiological activities, but in treated groups, significant decrease in weight of the ovary and significant increase in uterus weight was noticed. The results of biochemical and histopathological studies revealed that extracts at the dose of 25 mg/kg and 50mg/kg body weight both have antiovulatory and estrogenic activities. Therefore on the basis of these studies, is to conclude that administration of methanolic extract of *Achyranthus aspera* leaves induced

antifertility activity in female mice. At these dose levels the *A. aspera* leaves thus established its safety in efficacy. It did not show any teratogenic activity, antiimplantation and abortifacient activity.

Mice did not show any changes in the morphological and physiological behavior during pregnancy of pre and post-natal periods. Mice were examined continuously after one month of post natal periods of both the treated groups of mice and their litters did not find any changes when compared with control group of mice.

ACKNOLEDGEMNTS: Authors are grateful to Department of Biochemistry, Gulbarga University, Gulbarga, for their infrastructure, laboratory assistance and for providing facilities.

CONFLICT OF INTEREST: None Declared.

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How to cite this article:
Reddy VC, Kamble AK and Patil SJ: Evaluation of Antifertility Activity of *Achyranthus Aspera* Leaves in Female Mice. Int J Pharm Sci Res 2016; 7(9): 3794-01.doi: 10.13040/IJPSR.0975-8232.7(9).3794-01.