ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antifertility Activity of Ethanolic and Aqueous Extracts of *Aloe Vera* Mill on female wistar rats: Rising Approaches of Herbal Contraception

Sunil Kumar Shah[1]*, Deenanath Jhade[2], Rajendra Chouksey[3]

[1]Department of Pharmaceutics,
College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M.P.)
[2]Department of Pharmacognosy,
College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M.P.)
[3]Department of Pharmaceutics,
School of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore (M.P.)

Abstract

Ethanolic and Aqueous extract of Leaf of *Aloe vera* was study, antifertility activity in proven fertile female Wistar Rats at the doses 500mg/kg b.wt./day for 30 days. Different parameters were studied in female wistar rats including effect of Reproductive outcome, Anti-implantation, Abortifacient study and Estrogenic and Anti-estrogenic activity, Phytochemical were observed.**,** *Aloe vera* Leaves shown positive test for Alkaloids, Steroid, Flavonoids, Terpene, Carbohydrates and Tannin. The extract of *Aloe vera* has anti-fertility effect the control rats showed good number of litters. Treatment of animal with different extracts resulted a significant ($P< 0.05$, $P< 0.01$). Antifertility activity (42.2% and 7.8%) was exhibited by AAV and WAV respectively. After 21 days of the extracts free period, the antifertility effect of the extracts was reversed. The extract treatment with AAV, an increase in the percentage of resorption index indicates the failure in development of embryo. The mean percentages of anti-implantation and percent resorption (abortifacient) were found to be highest for AAV-29.46%, WAV -12.17% and AAV-32.96%, WAV-7.78% respectively. The decrement in implantation caused by the extracts may be due to estrogenic or anti-estrogenic activity. However, along with standard AAV exhibiting more potent estrogenic and less potent anti-estrogenic when compared with standard. Clinical assessment of Female antifertility agents should include acceptability, safety and efficacy during and after the treatment. The present study was therefore carried out to evaluate the claimed antifertility effect of *Aloe vera* leaf using different aspects of reproductive physiology in female wistar rats.

Keywords: Herbal Contraceptive, Antifertility, Reproductive outcome, Anti-implantation, Abortifacient, Estrogenic and Anti-estrogenic.

INTRODUCTION

Fertility control is an issue of global and national public health concern. There is a global need to support individuals in family planning due to the increasing growth rate of the world's population with its negative impact on environment, economic growth and poverty reduction in underdeveloped countries. About 90% of the world's contraceptive users are women. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception in females, progress and possibilities on males are still slow and limited [1]. Aware of this responsibility, health organizations and pharmaceutical companies continue to financially support or actively pursue research towards new contraceptive approaches [2, 3]. Current methods of contraception result in an unacceptable rate of unintended pregnancies and many side effects also [4].

Herbal drugs are the therapeutic herbs used to prevent and treat diseases and ailments or to support health and healing. Aloe Vera (also known as *Aloe Barbadensis*) contains over 200 active components including vitamins, minerals, amino acids, enzymes, polysaccharide, and fatty acids-no wonder it's used for such a wide range of remedies Herbal drugs are the oldest form of health care known to mankind. Fertility regulation with plant preparations in indigenous systems of medicine has been reported in ancient literature4. A number of plants species have been tested for fertility regulation years ago and were subsequently fortified by national and international agencies [5, 6].

Therefore, in the present investigation, herbalism and its efficacy as antifertility activity will open new avenues to scrutinize rich natural resources for further analysis in order to develop the potential of herbal medicine6. Such screening and scientific validation may provide the basics for developing novel antifertility without possible side effect [7].

METHODS AND MATERIALS

Collection of Plant Material

The Plant specimens for the study were collected from the Satpura region of Madhya Pradesh, India, identified and authenticated by NISCAIR (National Institute of Science Communication and Information resources, New Delhi, A voucher specimen no. is (NISCAIR/RHMD/Consult/2015/2903/102-1). Care was taken to select healthy fully grown plant and normal parts. The samples of different parts were cut suitably and removed from the plant and thoroughly washed with water to remove the adherent impurities and dried in sunlight [8].

Determination of physicochemical parameters
Physicochemical parameters of *Aloe vera* Leaf were determined and reported as total ash, water-soluble ash, acid-insoluble ash (Jarald, Edwin E, 2010). Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content and pH was also determined (Jarald, Edwin E, 2010).

Successive solvent extraction
The method is based on the extraction of active constituents present in the drug, using various solvents ranging from non-polar to polar. The solvents used were Petroleum Ether, Ethanol and Aqueous. Crude drug was subjected to soxhlet extraction with 1.5 litters of each solvent depending on their polarity. Each time before extraction with next solvents the marc was air-dried [9]. All the extracts were concentrated by distilling the solvent at low temperature. They were then weighed and percentages of different extractive values were calculated with respect to air-dried substance [10].

Phytochemical screening
Identification of the chemical constituents were carried out on the powdered Bark of *Aloe Vera* and the extract was concentrated and subjected to various chemical tests to detect the presence of different Phytoconstituents [11].

Animals
Anti-fertility test was performed on adult female wistar rats weighing between 180-200g and Mice. They were housed in polypropylene cages and fed with standard chow diet and water ad labium [12]. The institutional ethical committee for animal cares and use approved all experimental procedures. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 hours. The experimental protocols were subjected to the securitization of the Institutional Animal Ethics Committee and were cleared by the same (1587/PO/Re/S/11/CCSEA).

Acute oral Toxicity
The Acute oral toxicity studies were carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD-423), Ministry of Social Justice and Empowerment, Government of India [13].

Antifertility Study
Antifertility activity of plant extracts were evaluated with the help of reproductive outcome, anti-implantation, abortifacient, estrogenic and anti-estrogenic study was also performed, which further supported by the hormonal analysis [14,15].

Reproductive outcome in rats
Three groups of mature female rates (five rat/group) were selected for received extracts for 8 days and control group received vehicle for the same period. All the experimental rats were then allowed to mate with mature fertile male rat and the treatment continued for 21 days. The number of litters was determined after the completion of one gestation period in all-experimental groups. The litters were allowed to grow and the growth of litters produced from the extract-administered group was compared with those of control group. The reversibility of antifertility effect of the extracts was also studied in the treated groups. For this study, the extracts were administered continuously for 21 days and then the extract was withdrawn. After 21 days of extracts withdrawal, animals were allowed to mate with male rate. The number of litters was determined after the completion of one gestation period (Salhad *et al*., 1997).

Anti-implantation study
(Jain et al. 2012) with help of estrous cycle studies, proven fertile female wistar rats, weighing between 150 and 200 g were selected and left overnight with male of proven fertile in the ratio of 3:1. The extracts were administered orally to separated group rats at the dose level of 500 mg/kg from day 1 to day 7 of pregnancy. Control animal received the vehicle (CMC 0.5%). The animals were then laparotomised on day 10 of the pregnancy under excess dose of thiopentone sodium and uteri were examined to determine the number of implantation sites (Salhad *et al*., 1997).

Abortifacient study
Female rats at first day of pregnancy were divided into three groups, consisting of (5-6) animals in each group. The animals were laparotomised under light ether anesthesia and semi-sterile conditions on 10^{th} day of pregnancy. Both horns of the uterus were observed for the number of implants. The rats were sutured and allowed to recover. The first group served as control and received vehicle only (Tween-80, 1%) and group second and third received suspension of extract at a dose of 500 mg/kg b. wt. in 1% Tween-80, respectively, from day 10 to 18 of pregnancy. During the experiment, animals were observed for vaginal bleeding. On 21st day, animals were laparotomised under light ether anesthesia and observed for number of litters and percentage of resorption compared with initial number of implantation observed on 10th day of pregnancy (Khanna and Chaudhury, 1968).

Estrogenic and antiestrogenic study
Colony breed immature ovariectomised female rats (21–23 days) weighing between 25 and 30 g were used. They were divided into experimental and control groups, consisting of six animals each group. The extracts were suspended in 0.5% CMC and administered orally for 7 days at the dose level of 500 mg/kg body weight. Ethinyl Estradiol (Unicure Remedies Pvt. Ltd., Baroda, India) in olive oil 1 µg/rat per day was injected subcutaneously for 7 days in another group to induce estrous. CMC 0.5% was administered orally to the control animals. The extract at the dose level of 500 mg/kg was also administered orally along with Ethinyl Estradiol in olive oil at 1µg/rat per day subcutaneously to different groups of rat for the same period (Sharma, 2003).
On the 8th day of the experiment, all the animals were sacrificed by decapitation under light ether anesthesia and the uteri were dissected out, surrounding tissues removed, blotted on filter paper and weighed quickly on balance sensitive to 0.0001 g. A portion of the uterine tissues and

adrenal glands from the control and treated animals were fixed in Bouin's fluid for 24 h, dehydrated in alcohol and then embedded in paraffin. The paraffin blocks were sectioned at 6 mm intervals and stained with haematoxylin-eosin for histological examinations (Pal, 1990).

Hormonal Analysis

Hormonal Analysis were determined by Merck Kit method (Merck cat # 15891) by using microlab-300 IX Merck apparatus (Autoanalyser). Blood (2ml) was drawn by retro-orbital puncture and was immediately transferred into EDTA coated vacationer. The samples were mixed gently and were left for more than half an hour at room temperature, and finally centrifuged at 3000 rpm for 15 minutes. Serum was separated and assayed for FSH, LH, 17β- estradiol, and prolactin and 17-OH progesterone using enzyme linked immunoassay (EIA) technique. [Elisa reader (BIORAD 680 Microplate Reader [16]

Statistical Data

All values are expressed as mean±sem. Means were statistically analyzed by one-way analysis of variance (ANOVA) and values of $P<0.05$ were considered statistically significant [17].

RESULT AND DISCUSSION

Physicochemical Parameters

Physicochemical parameters of *Aloe vera* leaf were determined. In physicochemical parameter total ash is approximately seven times and four times more than acid insoluble ash and water soluble ash respectively [18]. Ethanol soluble extractive is approximately two times higher than water soluble extractive. Moisture content was less than 7.6 % and pH was 6.8. ware shoe in Table 1.

Table 1: Various Physicochemical Parameters

Physicochemical Parameter	Value % w/w* Mean± SD.
Total Ash	14.5 ±0.5% w/w
Acid insoluble ash	1.90±0.1% w/w
Water soluble ash	3.75±0.2% w/w
Water soluble extract	3.75±0.3% w/w
Ethyl alcohol soluble extract	7.0±0.3% w/w
Moisture content	7.6%
pH	6.8

w/w*- weight/weight, Value (%) Mean±S.D

Preliminary phytochemical investigation

A number of Phytoconstituent from natural sources have been proved efficacy to prevent the pregnancy. Many scientific reports were published for antifertility activity of Flavonoids (Hiremath *et al.*, 2000), Glycosides, Alkaloids, Steroid (Sadik *et al.*, 2001). Phytochemical investigation of *Aloe vera showed* (Table 2) the preliminary phytochemical study of *Aloe vera* leaf showed that Alkaloid, Steroid, Flavonoid Glycoside, Tannin, was present in Alcoholic Extract. Whereas, Steroid, Terpene, Tannin were present in Petroleum Ether Extract and Terpene, Glycoside and Tannins were present in Aqueous Extract. The successive solvent extraction with Petroleum Ether, Alcohol and Aqueous ater gave 3.5%, 7.4% and 5.4% Practical Yield.

Table 2: preliminary phytochemical study of *Aloe vera* Leaf

Test for Constituent	*Aloe vera* leaf		
	Petroleum Ether Extract (P.E.E)	Alcoholic Extract (A.E).	Aqueous Extract (W.E.)
Alkaloid	-ve	+ve	-ve
Steroid	+ve	+ve	-ve
Terpene	+ve	-ve	+ve
Flavonoid	-ve	+ve	-ve
Glycoside	+ve	+ve	+ve
Sugars	-ve	+ve	+ve
Saponins	+ve	-ve	-ve
Tannin	+ve	+ve	+ve
Carbohydrate	+ve	+ve	-ve
Colour and Consistency	Green colour	Brownish green colour	Dark green
Yield	3.5%	7.4%	5.4%
Code	PAV	AAV	WAV

Whereas code **PAV**-Petroleum Ether *Aole vera* Extract, **AAV**-Alcoholic *Aole vera* Extract and **WAV**-Aqueous *Aole vera* Extract

Acute oral toxicity

Acute toxicity studies were carried out to evaluate toxicity and to determine the minimum lethal dose of the drug extracts, using Wistar Rats. No clinical signs were evident in any animal during treatment period. (Clinical observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma). No mortality as well as any clinical sign of toxicity has been observed at a dose level of 2000 mg/kg indicating that all the extracts comes under category 5 and hence, LD50 cut-off was found to be 2000 mg/kg body weight. Hence, one-five of this dose, i.e. up to 500 mg/kg body weight, was used for antifertility investigation.

Heamatological and biochemical parameters were also performed before and after treatment and no significant changes were observed [19, 20].

Reproductive outcome study

Table 3 shows the effect of different extracts on the fertility of female rats. The control rats showed good number of litters. Treatment of animal with different extracts resulted a significant ($P< 0.05$, $P< 0.01$). A significant antifertility activity (42.2% and 7.8%) was exhibited by AAV and WAV respectively. It was also found that the litters of the extract treated rats did not show any physical deformity. All litters grew up to normal adult stage, which indicates that the extracts do not have teratogenic effect and the absence of teratogenic effect of extracts at a given dose justifies the safety of the plant. The present observations agree with Salhad *et al.*, (1997) who reported the reversible antifertility effect of *Ricinus communis* (castor beans) on female rabbits, and also supported by Endalk *et al.*, (2005) who reported the same effect of the methanolic root extract of *Rumex steudelii* on female rats.

After 21 days of the extracts free period, the antifertility effect of the extracts was reversed for all animals. An increase in the number of litters observed in all the post treatment groups may indicate the reversible antifertility effect of all extracts. These observations correlate the findings of Ganguly *et al.* (2007) and Gebrie *et al.* (2005) who reported the reversible antifertility effect with similar observations on the treatment with methanolic extract of *Cissampelos pareira* leaves in mice and methanolic root extract of *Rumex steudelii* in rats respectively. The animal groups gave 9.06 ± 0.15 litters at an average. This showed that there was no statistically significant change from the control group (10.00 ± 0.03).

Table 3: Effect of Extracts on Reproductive Outcome

Group	Oestrous Cycle	Fertility	Litters Present
Control	Regular	100 % + Ve	10.00 ± 0.03
AAV	Irregular	42.2% - Ve	5.78 ± 0.10 [a]
WAV	Irregular	7.8% - ve	9.22 ± 0.15[b]
W.D-AAV	Regular	80.5 % + Ve	8.05 ± 0.05 [a]
W.D-WAV	Regular	84.5 % + Ve	8.45 ± 0.25 [b]

Whereas AAV: Alcoholic *Aloe vera* Extract, WAV: Aqueous *Aloe vera* Extract, W.D-AAV: withdrawal Alcoholic *Aloe vera* Extract and W.D-WAV: withdrawal Aqueous *Aloe vera* Extract. Values are expressed as mean ± S.D.
P values a = P < 0.05, b= P < 0.01, when compared with normal control

Anti-implantation and Abortifacient activities

Postcoital antifertility study showed the anti-implantation activity in the treated animals. Treated animals delivered litters which, was significantly less than control (Table 4). The extract treatments with AAV, significantly (P<0.001) reduced the number of litters born (Table 4). This indicates the abortifacient nature of extracts. An increase in the percentage resorption index by the extract is an indication of failure in the development of the embryo (Dhanwad *et al.*, 2005). Such occurrence of foetal resorption suggests that interruption of pregnancy also occurred after implantation (Elbetieha, 2000). These observations indicate the pregnancy terminating potential of the extract. Embryonal resorption could be due to modifications of uterine lining function or maternal toxicity which consequently may increase early resorption and late fetal death (Chaves, 1985; Khera, 1987). Hence, the present investigation clearly reveals that the extracts are effective before and after the implantation occurs (Vasudeva and Sharma, 2006).

Both these activities were calculated on the basis of number of implants and number of litters. The mean percentage of anti-implantation and percent resorption (abortifacient) were found to be highest for AAV-29.46% and AAV 12.17%, whereas in the case of percent resorption; AAV-32.96% and AAV-7.78%, These results (show Table 4 and Table 5) indicated that all the extracts inhibited the conversion or development of implants into litters. The decrement in implantation caused by the extracts may be due to estrogenic or anti-estrogenic activity as described by Hafez (1970).

Table 4: Effect of Extracts on Anti-Implantation Activity

Treatment (Dose)	ANTI-IMPLANTATION ACTIVITY		
	No. of implants	No. of litters	Mean % anti-implantation
Control	7.23 ± 0.52	7.20 ± 0.65	Nil
AAV	5.10 ± 0.89	5.08 ± 0.22	29.46
WAV	6.35 ± 0.43[c]	6.05 ± 0.10 [a]	12.17

Values are expressed as mean ± S.D.
P values a = P < 0.05, b= P < 0.01,

Table 5: Effect of Extracts on Abortifacient Activity

Treatment (Dose)	Abortifacient Activity		
	No. of implants	No. of litters	% Resorption
Control	7.32 ± 0.62	7.10 ± 0.30	3.00
AAV	5.40 ± 0.52	4.95 ± 0.42	32.96
WAV	6.85 ± 0.20	6.75 ± 0.42	7.78

Values are expressed as mean ± S.D.
P values a = P < 0.05, b= P< 0.01, when compared with normal control

Estrogenic and anti-estrogenic study

Antifertility activity of all the extracts were finally evaluated with the help of estrogenic and anti-estrogenic activity associated with hormonal level and histological parameter like uterine weight, diameter of uterus, thickness of endometrium and height of endometrium epithelium [21]. The stages of estrous cycle and its duration were determined as described by Makonnen et al. (1997). The detail data has given in Table 6 and Table 7. The uterotropic potency, in terms of the weight of uterus, AAV (Alcoholic Extract of *Aloe Vera*) were found to be 61.25%, and WAV (Aqueous Extract of *Aloe vera)* were found to be 43.24%, when compared with standard (ethinyl estradiol). The Number of cornified cells in vaginal smears was considerably higher (+ to ++) than that of controls (0 to +), but notably less than that of Ethinyl Estradiol treated rats (+++). All the treated rats showed open vagina. Oral administration of AAV and WAV alone at a dose level 500 mg/kg body weight, AAV show highly significant (P < 0.001) change in uterine weight, thickness of endometrial epithelium and height of endometrial epithelium when WAV show less significant (P < 0.001) as compared with that of control.

However, along with standard AAV exhibiting strong estrogenic property, increase in uterine weight, diameter of uterus, thickness of endometrium and height of endometrial epithelium and WAV exhibiting strong anti-estrogenic property, decrease in uterine weight, diameter of uterus, thickness of endometrium and height of endometrial epithelium when compared with standard. These observations are similar to the finding of Ravichandran *et al.* (2007) and Vishnukant and Rana (2010) on the effect of hydroalcoholic extract of *Ailanthus excels* (Roxb.) stem bark and *Plumbago zeylanica* leaves on uterus of female Wistar rats. These observations revealed that these extracts acted as competitive antagonist to Ethinyl Estradiol. Hence, the anti-implantation activity of these extract may be due to their anti-estrogenic nature, which antagonise the action of estrogen and cause structural and functional changes in uterus and finally decreases the implantation [22, 23].

Table 6: Effect of Extracts on Estrogenic and Anti- Estrogenic Study

Treatment (Dose)	Uterine weight (mg/100 g body weight; mean ± S.D)	Vaginal cornification
Control	70.24 ± 5.35[a]	NIL
Ethinyl Estradiol (1 µg/rat per day)	335.40 ± 7.56[a]	+++
AAV (500 mg/kg)	205.45 ± 5.13	+ to ++
WAV (500 mg/kg)	145.05±0.25 [b]	+ +
Ethinyl Estradiol (1 µg/rat per day) +AAV (500 mg/kg)	386.83 ± 5.87 [a]	+++
Ethinyl Estradiol (1 µg/rat per day) +WAV (500 mg/kg)	185.02 ± 4.40	+

Values are expressed as mean ± S.D.
P values a = P < 0.05, b = P < 0.01, c = P < 0.001 when compared with normal control
Whereas, +: Nucleated Epithelial Cells, ++: Nucleated Epithelial Cells and Cornified Cells, +++: Cornified cells.

Table 7: Histological Changes in the Uterus and Endometrium after Treatment with Extracts

Treatment (Dose)	Diameter of uterus (µm ± S.D)	Thickness of endometrium (µm ± S.D)	Height of endometrial epithelium (µm ± S.D)
Control	330.54 ± 5.25 [a]	54.14 ± 2.12	17.4 ± 0.25[a]
Ethinyl Estradiol (1 µg/rat per day)	821.25 ± 6.25 [c]	245.45± 15.15 [c]	45.10 ± 4.18[c]
AAV (500 mg/kg)	465.17± 4.66	194.01 ± 5.50[c]	25.00 ± 4.43[b]
WAV (500 mg/kg)	217.24 ± 1.05	90.24±4.08	22.45±4.52
Ethinyl Estradiol (1 µg/rat per day) +AAV (500 mg/kg)	825.17 ± 4.66	248.01 ± 5.50[c]	45.02 ± 4.43[b]
Ethinyl Estradiol (1 µg/rat per day) +WAV (500 mg/kg)	425.28± 01.25[c]	95.09± 6.24[b]	25.02± 4.14[a]

Values are expressed as mean ± S.D.
P values a = P < 0.05, b = P < 0.01, c = P < 0.001 when compared with normal control

Hormonal analysis

Sex hormones were assayed based on their roles in maintaining pregnancy, since a failing pregnancy could be correlated to the levels of these hormones in the body fluids (Yakubu and Bukoye, 2009). The reduction in the concentration of FSH is an indication of disturbance of estrus cycle and ovulation (Ganguly *et al.*, 2007). LH is required for continued development and normal function of corpora lutea. The significant reduction in the level of serum LH could be associated with the physiological process of luteolysis preceding parturition (Yakubu and Bukoye, 2009). It could possibly be attributed to pregnancy failure resulting from a luteal phase that is not being maintained. The reduced level of hormone may also be due to inactivation of lutenization of ovarian follicles, which could be responsible for the reduction in the concentration of serum progesterone in this study [24, 25]. Elevated level of progesterone during pregnancy plays a key role in maintaining the conditions and is an important factor in the implantation process. Therefore, luteolysis and reduction in the blood levels of progesterone may contribute to abortion and anti-implantation activity of the all extracts [26, 28]. The findings of present study were agreed with previous studies which reported the effect of *Inula viscose* and *Bambusa vulgaris* leaf extract on implantation and abortion in rats and rabbits (Yakubu and Bukoye, 2009). In this study, an increase in prolactin level was observed (Table 8), These findings were also supported by Ganguly *et al.* (2007), who reported that a combination of enhanced prolacin and suppressed LH secretion is due to prolongation of estrus cycle (Ganguly *et al.*, 2007). An imbalance in endogenous estrogen and progesterone levels could be responsible for Anti-implantation activity (Dhanwad *et al.*, 2005).

Table 8: Hormonal Levels in Various Groups of Animals

Treatment 500mg/kg	LH	FSH	Prolactin	17β estradiol	17 OH Progesterone
Control	6.25±2.42	8.64±5.20	40.25±6.10	745.12±45.40	14.54±1.10
AAV	4.10±4.17	5.16±2.05	40.70±3.20	587±14.42	25.14±4.10
WAV	3.20±4.10	4.14±1.14	26.15±2.20	430±02.02	18.02±2.05

N=5, Data representation as Mean±SD
P values a = P < 0.05, b = P < 0.01, c = P < 0.001 when compared with normal control

CONCLUSION

The present findings inferred that the gathering treated with the most noteworthy convergence of plant concentrate indicated great come about as that of the standard medication and was underpinned by histopathological investigations of the antifertility activity on female Wistar rats [29]. Antifertility activity of plant extracts was evaluated with the help of reproductive outcome, anti-implantation, abortifacient, estrogenic and anti-estrogenic study was also performed, which further supported by the hormonal analysis [30]. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility. In our study clearly demonstrates that Extract of *Aloe vera* leaf, the control rats showed good number of litters. Treatment of animal with different extracts resulted a significant (P< 0.05, P< 0.01). A significant antifertility activity (42.2%) was exhibited by AAV. It was also found that the litters of the extract treated

rats did not show any physical deformity. All litters grew up to normal adult stage, which indicates that the extracts do not have teratogenic effect and the absence of teratogenic effect of extracts at a given dose justifies the safety of the plant. After 21 days of the extracts free period, the antifertility effect of the extracts was reversed for all animals. An increase in the number of litters observed in all the post treatment groups may indicate the reversible antifertility effect of all extracts.

Estrogenic in nature at the dose of 500mg kg $^{-1}$ body weight as evident form the along with standard, AAV Alcoholic Extract significance increases in the diameter of uterus, height of endometrial epithelium, and thickness of endometrium in extracted animal, while along with standard, WAV aqueous extract 500mg kg $^{-1}$ body weight showed less Anti-Estrogenic in nature decrease in the diameter of uterus, height of endometrial epithelium, and thickness of endometrium in extracted animal. It is a suitable plant for developing antifertility drug *Aloe vera* is recommended for working out and should be experimented for antifertility programme. Further studies on mechanism of antifertility action and isolation of the active components responsible for antifertility effect are in progress.

REFERENCE

1. Thakur DS, Kumar P, Kujur A, Kumar P, Kumar R. Contribution of Male Contraception in World Population. J Pharm Sci & Res 2010; 2(7):384-93.
2. Dehghan MH, Martin T, Dehghanan R. Antifertility effect of Iranian neem seed alcoholic extract on epididymal sperm of mice. Iranian Journal of Reproductive Medicine 2005; 3(2):83-89.
3. Gupta RS, Sharma R. A review on medicinal plants exhibiting antifertility activity in males. Natural Product Radiance 2006; 5(5):389-410.
4. Hoesla CE, Saadb F, Pöppela M, Altwein JE. Reversible, NonBarrier Male Contraception: Status and Prospects. Eur Urol 2005 Nov; 48(5):712-22.
5. Montaserti A, Pourheydar M, Khazaei M, Ghorbani R. Antifertility effects of Physalis alkekengi alcoholic extract in female rat. Iranian Journal of Reproductive Medicine 2007; 5(1):13-16.
6. Mishra N, Joshi S, Tondon VL, Munjal A. Evaluation of Antifertility potential of aqueous extract of Bougainvillea spectabilis leaves in Swiss albino mice. Int J Pharm Sci Drug Res 2009; 1(1):19-23.
7. Council of Scientific and Industrial Research. Wealth of India: Raw materials. New Delhi (India); 1956:4.
8. The Wealth of India, Volume-(F-G). In: A Dictionary of Indian Raw Materials and industrial products. New Delhi: Council of Scientific and Industrial Research, 1999. 4:246.
9. Ayodeji F. Ajayl et al, Antifertility activity of cryptolepis sanguniolenta leaf ethanolic in male rats, Journal of Human Reproductive Science 2012;5(1)43-47.
10. Chopra RN, Chopra IC and Varma BS, Supplement to Glossary of Indian Medicinal plants, reprinted edition,CSIR, NewDelhi, 1992;29.
11. Anonymous, the wealth of India, raw material s, council of scientific and in dustrial research, New Delhi, 1952;4:35-36.
12. Anonymous, pharmacopoeia of India, manager of publication, ministry of health, Government of India, Delhi, 1966;2:947-948.
13. Fransworth, N.R. Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences 1966;55:225-276.
14. Agrawal S Shyam et. Al, Antifertility activity of methanolic bark extract of *Aegle marmelos* (l.) in male wistar rats, DARU Journal of Pharmaceutical Sciences 2012;20:94
15. Ramya MC, Shivabasavaiah, Shivanandappa T, Reversible antifertility effect of Opuntia elatior Mill. fruit extract, International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 2015;4(2):392-397.
16. Kokate CK. Practical pharmacognosy. New Delhi: Vallabh Prakashan; 1999:107-108
17. Gupta, S., Sampling and test of significance. In: Gupta, S. (Ed.), Statistical Methods. Sultan Chand and Sons Publishers, New Delhi, 1978: 58–76.
18. Council of Scientific and Industrial Research. Wealth of India: Raw materials. New Delhi (India); 1956:4.
19. The Wealth of India, Volume-(F-G). In: A Dictionary of Indian Raw Materials and industrial products. New Delhi: Council of Scientific and Industrial Research, 1999. 4:246.
20. Chopra RN, Chopra IC and Varma BS, Supplement to Glossary of Indian Medicinal plants, reprinted edition,CSIR, NewDelhi, 1992;29.
21. Jain Sachin, Choudhary Gajendra Pratap, Jain Dinesh Kumar, Pharmacological Evaluation and Antifertility Activity ofJatropha gossypifolia in Rats, BioMed Research International 2013;1-5.
22. Devi Parveena. et, al. Antifertility Activity of Medicinal Plants on Male and Female Reproduction, International Journal of Pharmaceutical Sciences and Research, 2015;6(3): 988-1001
23. Fransworth, N.R. Biological and phytochemical screening of plants. Journal of Pharmaceutical Sciences 1966;55:225-276
24. Harborne, J.B. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, seconded. Chapmann and Hall, London1984:192
25. Warrier PK, Indian medicinal plants, A compendium of 500 species by, Orient long man Ltd, Chennai1996;3:34-35.
26. M. Anithaa et. Al, Evaluation of anti-fertility activity of ethanol extract of cynoglossum zeylanicum (vehl ex hornem) thumb.ex lehm (boraginaceae) whole plant on male albino rats, J. Curr. Chem. Pharm. Sc.: 2013; 3(2):135-145
27. Wassarman PM, Albertini DF, The mammalian ovum. Im Knobil E, Neeill JD, Editors. The Physiology of reproduction, 2dned. Misgan: Raven Press; 1994:79-122.
28. Hodgen AD, Itskovits J. The Physiology of Reproduction. Migan : Raven Press;1988.
29. Vasudeva N, Sharma SK. Post-coital antifertility activity of Achyranthes aspera Linn. Root. J Ethnopharmacol 2006; 107: 179-181.
30. Chauhan A, Agarwal M, Kushwaha S, Mutreja A. Antifertility studies of Aegle marmelos Corr., an Indian medicinal plant on male albino rats. Egyptian Journal of Biology 2008; 10:28-35.