

ANTI-IMPLANTATION EFFECT OF THE AQUEOUS EXTRACT OF *HYPTIS SUVEOLENS* LINN. IN FEMALE ALBINO RATS

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ABSTRACT

According to folklore medicine, the plant *Hyptis suaveolens* Linn exhibits antifertility activity in women. Documented experiments or clinical data are, however, lacking. Therefore, the present study was carried out in female albino rat to explore the anti-implantation activity of aqueous extract of *Hyptis suaveolens* Linn. The effect of the plant extract was observed on early pregnancy in the rat. Aqueous extract when administered in dose of 50,100,150 mg/kg body weight for five days, showed anti-implantation activity (about 56.6%) along with total abortifacient activity (63.6%). There was no noticeable change in the color and size of the implants when compared with those of control group of animals.

KEY WORDS: Abortifacient, Anti-implantation, Female rats, female contraceptive, Folklore medicine, *Hyptis suaveolens* (L.)

INTRODUCTION

The Nature has been a source of medicinal plants for thousands of years and are helpful in treating many diseases. Plants provide safe & effective birth control agents are used for thousands of years for this purpose. Written history documents use of plants as a means of birth control as far back as Ancient Greco-Roman times. Many plants were described for their anti-fertility qualities over 2,000 years ago by the Ancient Greek physician Hippocrates (Riddle, 1997). Today many contraceptives are available in the market but the common side effects include obesity, cholelithiasis, gastric trouble and carcinoma (Umadevi *et al.*, 2013). In countries like India where the masses are extremely impoverished, therefore discovery of some cheapest, safe, sure and effective form of birth control agent of herbal origin is the need of the hour. Implantation is one of critical phase in the ontogeny of eutherian mammals, involving regulated and co-coordinated changes in the uterus, uterine fluid and blastocysts in a sequential order by synergistic action of estrogen and progesterone. It has been noted that any imbalance in estrogen and progesterone inhibit implantation (Prasad and Kalra, 1967). There are many indigenous plants which are reputed to possess the property of preventing births when administered orally (Chaudhary, 1966) or have abortifacient potential (Chondekar *et al.*, 2010). *Hyptis suaveolens* (L.) Poit. (bushmint, pignut) of the family Lamiaceae, a soft suffrutescent herb native to tropical America, is now a pantropical weed, Being introduced and naturalized throughout India. (Murthy *et al.* 2007). Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) studied by Mondal *et al.* (2007). This study was aimed to evaluate anti-implantation effect of *Hyptis suaveolens*.

MATERIALS AND METHODS

a) Study area and Plant Material collection

Amravati district of Maharashtra state lies between 20° 32' and 21° 46' north latitude and 76° 37' and 78° 2' of east longitude. It occupies an area of 12,449.7 sq. km. The major portion of it is identified as Melghat region, forming parts of Satpura Mountains. Korku tribe is the main inhabitant of this Melghat region with rich ethnic knowledge. The present work was undertaken with the aim of documentation of Valuable plants used by korkus traditionally, for medicinal purpose. Fresh plants were collected from study area. They were identified and authenticated by botanist in the Department of Botany, Government Vidarbha Institute of Science and Humanities, Amravati (M.S.) India and the specimen sample of the same was deposited at the Herbarium of the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, (M.S.) India with the voucher accession No.0726 for future reference.

b) Preparation of extracts

The plant materials were dried under shade and grinded to a coarse powder. Powdered

plant material (25g) was subjected to soxhlet extraction with double distilled water (200 ml) and then filtered. Filtrates were concentrated, dried and subjected for activity studies.

c) Experimental Animals

Male and female Albino rats of Wistar strain were obtained and made to feed on pellet diet and water *ad libitum*. The animal room was well ventilated with a temperature range of 25 - 27°C under day and night 12-12 hour photoperiodicity. The protocol was approved by the Institutional Animal Ethics Committee registration no. 1060/ac/07/CPCSEA (IAEC/1/2007-08).

d) Experimental design

Estrus cycle study was done according to the vaginal smear method of Long and Evans (1922) for fixing the date of pregnancy. Female rats of established fertility in proestrous or estrous stage were mated with matured male rats of established fertility (in the ratio female 3: 1 male). The anti-implantation activity was determined according to method of Khanna and Chaudhury (1968) Female rats of established fertility in proestrous or estrous stage were mated with matured male rats of established fertility (in the ratio female 2: 1 male).

The female albino rats of regular estrus cycle were observed for vaginal smear analysis every morning microscopically. Females in an early estrus stage were selected for the study and were left overnight with proven fertile male albino rats in 1:2 ratios. The next morning, vaginal smear of female were observed for the presence of sperms and formation of vaginal plug. The day on which the spermatozoa were found in the smear was considered the first day of pregnancy (day 1). Such pregnant females were grouped into three groups of six each for drug administration as follows.

Group A	- Control group received distilled water orally.
Group B	-Experimental group received successive aqueous extract of the plant extract 50 mg/kg body weight for 1-7 days.
Group C	- Experimental group received successive aqueous extract of the plant extract 100 mg/kg body weight for 1-7 days.
Group D	-Experimental group received successive aqueous extract of the plant extract 150 mg/kg body weight for 1-7 days.

On the 10th day of pregnancy, rats of all groups were laparotomized under anesthesia to know the presence of implantation sites in the uterine horns and corpora lutea to detect the anti-implantation activity.

Animals were observed for:

1. Pre implantation loss = No. of CL on 10th day - No. Implant on 10th day.
2. % Pre Implantation loss = $\frac{\text{No. of CL} - \text{No. of implant}}{\text{No. of CL}} \times 100$

e) Statistical analysis

The results were statistically analyzed using Standard Statistical methods.

The data are presented as mean±SEM. The results were statistically analyzed using one-way ANOVA followed by two tailed Dunnett's multiple comparison test. P values <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

a) Determination of estrus phase

A positive estrus smear is one in which only large, irregular cornified cells are seen indicating maximum growth of the vaginal mucosa. A metestrus smear has many cornified cells, and also some leukocytes and epithelial cells, indicating the postovulatory stage and desquamation of the vaginal mucosa.

A diestrus smear shows few epithelial cells, mucous, and few leucocytes, indicating a quiescent uterus and resting vaginal epithelium. A proestrous smear has many epithelial cells with granular cytoplasm, indicating a rapidly growing vaginal epithelium and the preovulatory stage.

The results are shown in Table 1 and 2; Figure 1.

Table 1: Anti-implantation activity of the aqueous extracts of *Hyptis suaveolens* Linn. before and after treatment.

Dose, mg/kg body Wt. (No. of Animals)	No. of Implantation sites	No. of resorptions	No. of corpus luteum	anti-implantation activity %	early abortifacient activity %	age of total abortifacient activity %
Control (6)	11,9,11,8,11,13	0,0,0,0,0,0	11,9,11,8,11,13	0	0	0
50 (6)	07,09,06,09,08,09	0,1,0,1,0,0	10,12,8,11,10,9	20	3.33	23.33
100 (6)	06,06,08,05,07,08	1,0,1,0,0,1	11,12,10,10,12,13	41.17	4.41	45.58
150 (6)	05,04,06,05,04,05	2,0,1,1,1,0	13,11,12,11,10,09	56.06	7.57	63.63

Fig. 1: Graphical representation of anti-implantation activity of *Hyptis suaveolens*.

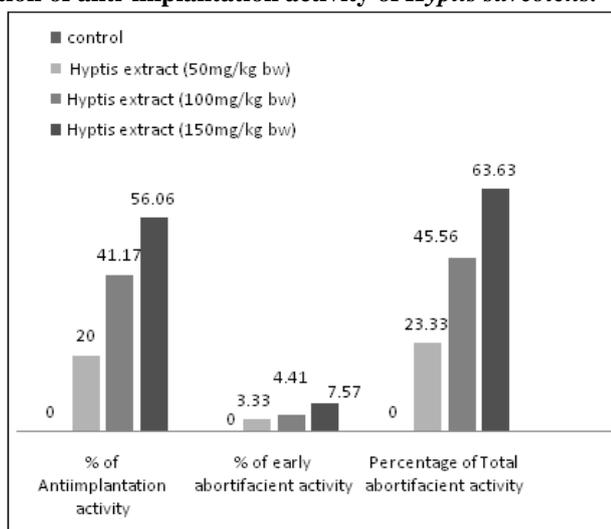


Table 2: Statistical analysis of Anti-implantation activity of the aqueous extracts of *Hyptis suaveolens* Linn before and after treatment.

Drug dose (mg/kg body weight)	Control Mean ± SD	After dose Mean ± SD	**P value
50	10 ± 1.41	8 ± 1.26	0.0066
100	11.33 ± 1.21	6.67 ± 1.21	0.0004
150	11 ± 1.41	4.83 ± 0.75	0.0001

1. F value 16.59. . Test statistic value is much greater than the Critical value $F_{0.05} (2, 15) = 3.68$ so null hypothesis is rejected.
2. P value < 0.001

**Significant at P<0.01

1. F value calculated by using one way ANOVA
2. P value calculated by using two tailed paired t- test

The antiimplantation activity is expressed as the percentage of animals showing absence of implantations in uteri when laparotomised on day 10 of pregnancy (Table -1, Fig. 1). It shows the percentage of anti-implantation and antifertility activities exhibited by each dose of extract in comparison to the corresponding controls. The oral administration of aqueous extract of *Hyptis suaveolens* (L) at 150mg /kg body wt, dose exhibited extremely significant ($p < 0.001$) anti-implantation activity, the highest rate of anti-implantation activity was 56.06% at this dose level. Similarly, at a dose of 100mg/kg body wt, the anti-implantation activity was 41.71% while at 50mg /kg body wt it was observed to be only 20%. It reveals that with the increase in the level of dose of extract from 50 to 150 mg/kg, the anti-implantation activity also increased gradually. In the present study it was observed that in the control group no rat was found without implantation on day 10 of leparotomy and the number of corpus lutea were abundant. However, in each experimental group, after completion of full term, fetal resorption was observed as 3.33%, 4.41% and 7.57% at dose level of 50, 100 and 150 mg/kg body wt. respectively. Table- 2 shows that Test statistic F value is 16.59 which is much greater than the critical value $F_{0.05}(2, 15) = 3.68$. This indicates that all the extracts show significant reduction in number of implants as compared with the respective control.

In the present work plant extract has shown highly significant antiimplantation activity when it was administered from day 1 to 7. *H. suaveolens* is reported to have linoleic acid in its seeds which may be responsible for estrogenic activity (Ayerza and Coates, 1996). Steroidal saponin is one of the active principles of most antifertility agents and reported to exist in various plants (Fransworth et al., 1975; Goldfien, 1995; Debella et al., 1999). Prabhat et al., (2009) studied the *Hyptis suaveolens* shows protective effect on Duodenal ulceration, stimulant, carminative, galactagogue, antispasmodic, antirheumatic, antiinflammatory, antifertility agent and also applied as an antiseptic in burns, wounds. *H. suaveolens* is reported to possess 0.3% saponins which may be responsible for the observed anti-implantation activity (Edeoga et al., 2006).

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. (Psychoyos, 1996) Compounds with hormonal values usually disturb the hormonal milieu in the uterus therefore the anti-implantation activity may be due to estrogenic activity causing the expulsion of ovum from the tube disturbing the luteotrophic activity of blastocyst. Anti-implantation activity of plant extracts were reported by various workers earlier. Bhargav and Prakash (2000) observed anti-implantation effect of herbal preparation from neem bark extract in rats. They noted similar type of observation in rats with the extract of bark of *Azadirachta indica* which showed anti-implantation as well as foetal resorption. Many antifertility plant extracts are known to exhibit estrogenic activity in rats (Vohora et al., 1969; Gebrie et al., 2005). Estrogen causes an increase in protein synthesis and retention of fluid leading to ballooning of Uterus (Rifai et al., 2001). In addition it also induces changes such as increase in diameter of uterus, thickness of endometrium, thus providing non receptive condition for implantation (Dhar, 1995). Different mechanisms might have been involved to produce anti-implantation activity that needs further elucidation. The present study demonstrated the possible therapeutic application of plants as antifertility agents. However, further study on pharmacokinetic profile and active principles of the plants should be carried out.

ACKNOWLEDGEMENT

Authors are thankful to the Dr. Prabha Bhogaonkar, Director, Govt. Vidarbha Institute of Science and Humanities, Amravati, for providing the necessary facilities for carrying out this research work. The authors wish to express their thanks to Prof. Sardesai M.M., Associate Professor, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, and a Taxonomist for his valuable help in identifying and authenticating the plant sample.

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