International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

ANTIFERTILITY ACTIVITY OF PETEROLIUM ETHER EXTRACT OF *HYPTIS SUAVEOLENS* LEAVES IN RATS

Sumalatha.G[1]*,
Revathi.B[1], Babu Rao.Ch[2], Tanuja.M[1]

[1]Department of Pharmacology, Hindu College of Pharmacy,Amaravathi road, Guntur.
[2]Priyadarshini Institute of Pharmaceutical Education and Research, Pulladigunta, Guntur.

ABSTRACT

In the present study the antifertility activity of petroleum ether extract of Hyptis suaveolens leaves in rats was evaluated in an attempt to establish the traditional use. Antifertility activity is assessed by anti-implantation and estrogenic activity. In anti-implantaton activity, oral administration of petroleum ether extract of Hyptis suaveolens leaves at doses 250 and 500 mg/kg b.w from 1st day to 7th day to pregnant rats, has shown significant (p<0.0001) decrease in number of implantation and corpora lutea compared to control in dose dependent manner. Where as in estrogenic activity the oral administration of petroleum ether extract of Hyptis suaveolens showed significant values (p<0.0001) at 250 and 500mg/kg b.w respectively, as indicated by increase in uterine weight, vaginal opening and vaginal cornification in immature female rats when compared to control, but not significantly greater than standard. This is further supported by increase in estrogen dependent biochemical parameters cholesterol, glucose and alkaline phosphatase in uterus also indicates significant (p<0.05) estrogenic activity of petroleum ether extract of Hyptis suaveolens leaves when compared with control group. The phytosterols, triterpenes and flavonoids present in the extract may be responsible for the observed antifertility effect.

Correspondence Author

G.Sumalatha
Associate Professor
Hindu College of Pharmacy,
Amaravathi Road, Guntur-522 001, IN

Email: sumacology@gmail.com

KEYWORDS : *Hyptis suaveolens, petroleum ether extract, antifertility activity, anti-implantation, estrogenic activity, phytosterols, triterpenes and flavonoids etc.*

INTRODUCTION

Rapid population growth is becoming a problem which causes severe pressure on economic, social and cultural resources. Since this problem is getting intense day by day, it is obvious to take appropriate measures to keep it under control by lowering the birth rate[1]. Contraceptive Methods used are Pills, Injectables, Implants, IUD,

Female sterilization, Male sterilization, Condom etc,. The main advantage of these is 99% effective. The side effects include nausea, headache, slight weight gain and in rare cases, stroke or heart attack. Some side effects of these synthetics on normal and natural human body are much more aggressive and unpredictable at prolonged use as long as one which gender expects to use them. Fertility regulation has therefore become the major concern of people of all walks of life. In recent years, plants are perused over steroidal contraceptives because plants are easily available, economic and devoid of harmful side effects. Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care[2].

Ancient Indian literature abounds with information on large numbers of plants reputed to have sterilizing, contraceptive and abortifacient properties. Scholars of Ayurveda have also mentioned several plants in their Ayurvedic treatises. A number of these preparations are still being used by Ayurvedic physicians all over India, who claim their effectiveness but are unable or unwilling to produce data[3]. Control of fertility using traditional antifertility plants has been practiced for many years in India. *Hyptis suaveolens* locally known as *Vilaiti tulsi* is one of many plants used for fertility regulation in India. The genus *Hyptis* is known to be used for traditional medicine for the treatment of various illness and has been found to possess significant pharmacological activity including tumorigenic, mycotoxic and phytotoxic activities. *Hyptis suaveolens* is widely used in traditional medicine as a multiple remedy against many diseases and vectors. Despite the numerous scientifically proven pharmacological activities of *Hyptis suaveolens* there is no data on its potential as antifertility agent. The leaves of *Hyptis suaveolens* found to elicit antifertility activity but results have not been consistent. The present study is done to assess antifertility activity of *Hyptis suaveolens* leaf extract.

MATERIALS AND METHODS

Plant material

The leaves of *Hyptis suaveolens* were collected from Tirunelveli in Tamil Nadu, India and authentified by a Research officer – Botany, in Central Council Research of Ayurveda and Siddha. Specimen of the plant has been deposited at the herbarium of Hindu College of Pharmacy, Guntur, Andhra Pradesh, India.

Preparation of Extracts

The fresh leaves of *Hyptis suaveolens* were collected, shade dried and powdered mechanically. The leaf powder was packed into soxhlet column and extracted successively with petroleum ether (60-80ºc) at a temperature not exceeding the boiling point of the solvent. The extract was filtered and then concentrated in vacuum at 40 ℃ using Rotary evaporator. The dried extract was labelled *Hyptis suaveolens* petroleum ether leaf extract (HSPE) and preserved in refrigerator for further use. Preliminary phytochemical analysis was carried out for the extract. A known volume of each extract was suspended in tween 80(1%) and was orally administered to the animals by gastric intubation using a force feeding needle during the experimental period.

Experimental animals

Female and male of Wistar strain were obtained from Animal house of Hindu College of Pharmacy. They were housed in standard cages at room temperature (25 ± 2^{o}C) and relative humidity (55 ± 5 %) and 12/12 h light/ dark cycle. The animals were provided with standard pellet diet and water *ad libitum*. The study was performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The approved protocol number submitted to CPCSEA is IAEC/P.COLOGY/06/2011-12.

Acute Toxicity Studies

Three female wistar rats were selected for the study. The overnight fasted animals (with water *ad libitum*) were administered with *Hyptis suaveolens* petroleum ether extract at a single dose of 2000 mg/kg body weight by oral route to the animals by gastric intubation using a

force feeding needles. Food is with held for a further 3-4 hours after administration of test extract and was observed for signs for toxicity as per OECD Guideline No. 423 of CPCSEA.

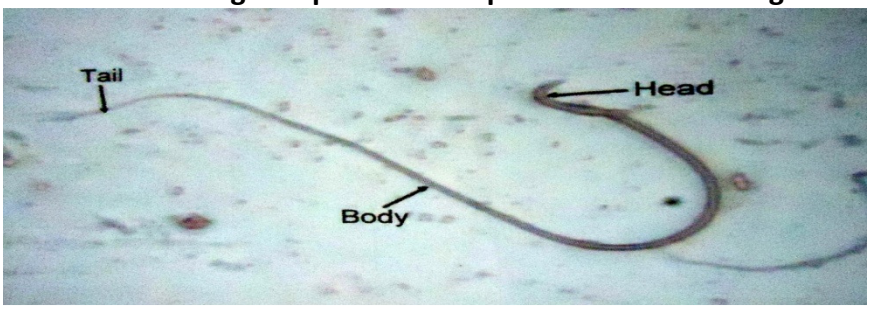
Evalution of Antifertility Activity

Anti-implantation Activity

Female rats of proestrus phase of the estrous cycle were left over night with males of proven fertility (in the ratio of 2 females to 1 male). The female rats were examined for evidence of copulation in the following morning. Female animals which showed the presence of thick clumps of spermatozoa in vaginal smears were separated from the males. The day on which the spermatozoa were found in the vaginal smear was considered the first day of pregnancy (Day 1)[4,5]. These pregnant rats were divided into 3 groups of 6 animals each and the animals. Group I used as a control and treated with saline (2ml per kg b.w) for seven days. Group II and Group III were used as test groups treated with extract at doses of 250 and 500 mg/kg b.w p.o respectively for seven days. On the tenth day[6] animals were sacrificed with cervical dislocation and the uteri horns were examined for number of implants and number of corpora lutea. Percentage of anti-implantation is calculated by using the following formulae,

$$\% \text{ of anti-implantation activity} = 100 - \frac{\text{No. of implantation}}{\text{No. of corpora lutea}} \times 100$$

Figure 1. Slide showing the presence of spermatozoa in the vaginal smear



Estrogenic Activity

Immature female rats of wistar strain 21-23 days old weighing 40-60 gms were divided into 4 groups of 6 animals each. Group I served as a control, treated with saline for seven days. Group II served as a standard, treated with Ethinyl estrdiol (1µ/rat/day). Group III and IV were served as test groups, treated with extract at doses of 250 and 500 mg/kg b.w p.o respectively for seven days. Vaginal opening was observed daily in each animal and vaginal cornification was observed by collecting the sample of vaginal mucous obtained by flushing a few drops of saline solution into the vagina of the rats. The saline and its cellular contents were smeared and analysed using optical microscope to determine the presence or absence of cell types. Positive smears are those containing nucleated or cornified cells and not more than few leukocytes[7].

After 24 hours of last treatment, all the animals were sacrificed by decapitation and uteri horns were dissected out, cleared off the adhesive tissue, blotted on filter paper and weighed quickly on a sensitive balance. The biochemical parameters like cholesterol, glucose, and alkaline phosphatase of uteri were measured[6].

Statistical Analysis

The results were expressed as Mean ± S.E.M. The data of antifertility activity was analyzed by one way analysis of variance (ANOVA) followed by Dunnet's't' test using Graph pad prism 5.02 software.

RESULTS

Anti-implantation activity

This study shows that the mean number of implants and number of corpora lutea in animals were significantly reduced ($p<0.001$, $p<0.0001$) by seven days treatment with HSPE at two dose levels 250 mg/kg and 500 mg/kg, when compared with control group. 500 mg/kg of HSPE-2 group animals has shown very significant ($p<0.0001$) compared

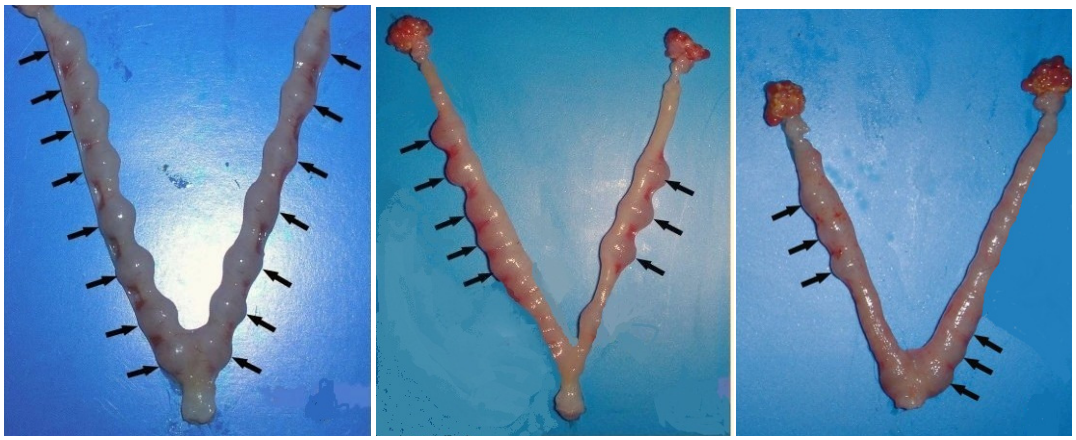
with control group. There was significant difference in the mean values of 250 and 500 mg/kg, shows the dose dependent activity of the extract. The results are shown in the table 1, figure 2 and 3

Table 1: Anti-implantation activity of HSPE

Treatment	Dose (mg/kg)	No. of implantations	No. of corpora lutea	% Anti implantation
Control(saline)	2ml/kg	13.33±0.88	15.00±0.57	10.33±1..85
HSPE-1	250	9.00±0.57**	11.33±0.33*	26.67±1.45
HSPE-2	500	5.34±0.33***	13.66±0.88***	66.00±2.08

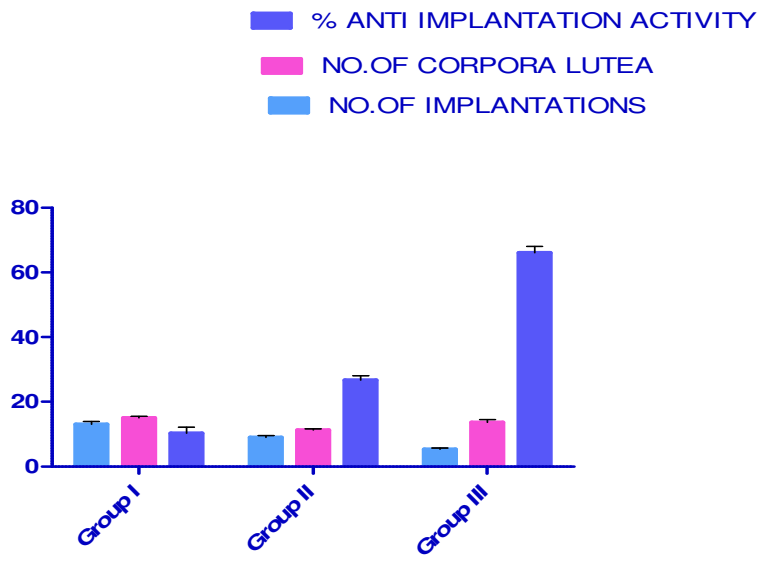
Values were given as Mean ± S.E.M for six rats in each group. Values are statistically significant at ***p < 0.0001, **p < 0.001 and *p < 0.01 as compared with control.

Figure 2: NUMBER OF IMPLANTS SHOWN IN CONTROL AND EXTRACT TREATED ANIMALS



CONTROL HSPE-1(250mg/kg) HSPE-2(500 mg/kg)

Figure 3: Anti-implantation activity of HSPE



Group I Control(saline)
Group II HSPE-1
Group III HSPE-2

Estrogenic activity

In the present study, significant increase in uterine weight ($p<0.01$ and $p<0.001$) after treatment with petroleum ether extract for seven days at dose levels 250 mg/kg and 500 mg/kg, was shown when compared with control group. Vaginal opening induced by the extract and the number of cornified cells were also higher (+++) when compared with control group. However 500 mg/kg of HSPE-2 group animals has shown very significant ($p<0.001$) estrogenic activity as that of standard drug treated group. There was significant difference in the mean values of 250 and 500 mg/kg, shown the dose dependent activity of the extract. The results are shown in the table 2 and figure 4

Table 3: Estrogenic Activity of HSPE

Treatment	Dose mg/kg	Uterine weight(mg)	Vaginal opening	Vaginal cornification
Control(saline)	2ml/kg	110.0±8.660	Not Opened	NIL
Ethinyl estradiol	1µg/rat/day	216.7±12.02***	Opened	+++
HSPE-1	250	161.7±4.410*	Not Opened	+ to ++
HSPE-2	500	186.7±4.410**	Opened	+++

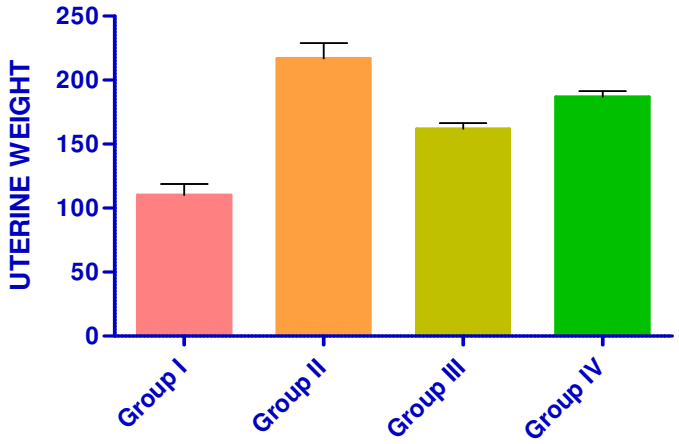
\+ = Nucleated epithelial cells

++ = Nucleated epithelial cells & cornified cells

+++ = Cornified cells

Values were given as mean ± S.E.M for six rats in each group. Values are statistically significant at ***p<0.0001,**P<0.001 and *p<0.01 as compared with control group.

Figure 4: Estrogenic activity of HSPE



Group I Control(saline)

Group II Ethinyl estradiol

Group III HSPE-1

Group IV HSPE-2

Biochemical changes in uterus

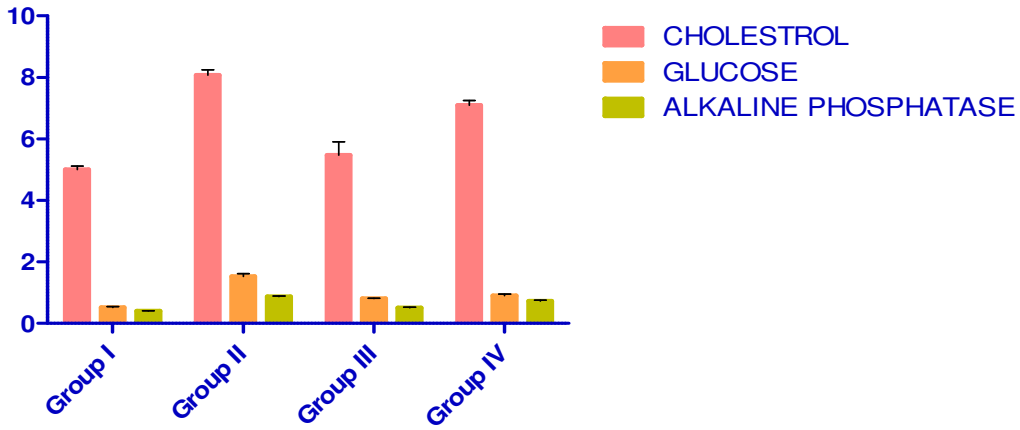
In the present study, significant ($p<0.01$, $p<0.001$) increase in cholesterol, glucose and alkaline phosphatase by seven days treatment with petroleum ether extract at two dose levels 250 mg/kg and 500 mg/kg, was observed when compared with control group. 500 mg/kg of HSPE-2 group animals has shown significant ($p<0.001$) estrogenic activity as that of standard drug treated group. The results are shown in the table 3 and figure 5

Table 3: Biochemical changes in uterus due to administration of HSPE

Treatment	Dose mg/kg	Cholesterol mg/dl	Glucose mg/dl	Alkaline phosphtase IU/dl
Control(saline)	2ml/kg	5.0±0.115	0.523±0.033	0.403±0.008
Ethinyl estradiol	1µg/rat/ day	8.06±0.176***	1.533±0.088***	0.873±0.021***
HSPE-1	250	5.46±0.437	0.816±0.008*	0.513±0.012**
HSPE-2	500	7.10±0.152**	0.906±0.052**	0.730±0.026**

Values were given as mean ± S.E.M for six rats in each group. Values are statistically significant at ***p < 0.0001, **p < 0.001 and *p < 0.01 as compared with control.

Figure 5: BIOCHEMICAL CHANGES IN UTERUS DUE TO ADMINSTRATION OF HSPE



Group I	Control(saline)
Group II	Ethinyl estradiol
Group III	HSPE-1

DISCUSSION

Present eras emphasized on the development of new potent oral antifertility agents from plants despite the fact number of medicinal plants has been reported in the literature to possess antifertility activity; their efficacy has not been confirmed experimentally. For scientific impetus, many of these plants have been screened in laboratory animals, and no single plant is yet available which can be developed further as a potent antifertility agent. The present study deals with the antifertility activity of petroleum ether extract of *Hyptis suaveolens* leaves[8].

In the present study, the petroleum ether extract of *Hyptis suaveolens* leaves were tested for the antifertility activity at the dose 250 mg/kg b.w and 500 mg/kg b.w in female wistar rats. *Hyptis suavolens* ether extract showed significant anti-implantation activity when compared with the control rats. 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[9]. This may indicate that the crude extract of *Hyptis suaveolens* leaves inhibited the process of implantation. Plant extracts can cause endometrial alterations resulting in non-receptive endometrium and resulted in blastocyst implantation failure[10]. In the present study, the very significant ($p<0.0001$) decrease in number of implantations at dose 500 mg/kg is responsible for the anti-implantation activity may be due to disturbance in the level of these hormones and endometrial alteration

resulted in blastocyst implantation failure, result to produce infertility.

Pituitary hormones are essential for first 11 days of pregnancy, progesterone, a pregnancy hormone secreted by corpora lutea is sustained by reduction with FSH through day 1-7, LH becomes the important luteotropic hormone from day 8-12 of pregnancy to maintain the progesterone secretion of corpora lutea and thereafter placenta will take over the function[6]. In the present study the very significant($p<0.0001$) decrease in number of corpora lutea at dose 500 mg/kg is responsible for the anti-implantation activity may be due to decrease in progestrone secretion.

The indication of estrogenic activity is the opening of vagina and cornification of vaginal epithelial cells. Estrogenic compounds are known to cause the keratinization and cornification of vaginal epithelium, causing the superficial cells to shed in to the lumen to form a large squamous cells[11]. Thus the ether extract showed premature vaginal opening and cornification of the epithelium cells. Further, points out the estrogenic nature of the extract.

The administration of extract of *Hyptis suaveolens* to female rats for seven days brought about an increase in the weights of reproductive organs, indicating that the level of estrogen was important for maintaining the weights of reproductive organs. The structural and functional integrity of reproductive tissues depend on the circulating level of estrogen and therefore any small change in estrogen level may result in change in the weights of the reproductive organs. The significant ($p<0.01$,$p<0.001$) difference in the mean weight of uteri of 250 and 500 mg/kg, has shown the dose dependent activity of the extract. The increase in most of the estrogen dependent parameters of female reproductive organs revealed that the internal physiology of female reproductive organ is disturbed because of the change in level of circulating estrogen which is essential for maintenance of their physiology integrity [12,13].

Cholesterol is the precursor of sex hormones and is utilised during steroidogenesis. The cholesterol concentration in uterus increased after extract treatment, indicating increase in estrogen. The treatment of extract caused significant increase in the other estrogen dependent parameters of uterus i.e., glucose and alkaline phosphate. Estrogen is known to stimulate the content of the uterus, there by changing the uterine milieu and creating non receptive conditions in the uterus[14].

Phytoestrogens are any plant compounds structurally and/ or functionally similar to ovarian and placental estrogens and their active metabolites[15]. They include a vast variety of structurally diverse compounds.Plants with estrogenic property can directly influence pituitary action by peripheral modulation of LH and FSH decreasing secretion of this hormones and block ovulation. Hence in the present study the phytosterols, triterpenses and flavonoids present in the extract may be responsible for the observed antifertility effect.

CONCLUSION

Thus, the data obtained from phytochemical analysis, acute toxicity study and pharmacological evaluation of petroleum ether extract of *Hyptis suaveolens* leaves tend to suggests that the extract has shown significant antifertility activity and safe administration of the extract, establishing the traditional use of the plant.

A further study on the exact mechanism of action and isolation of the active constituents is needed and different formulations of extract can be evaluated for antifertility activity. By observing the results of evaluation of antifertility activity of petroleum ether extract of *Hyptis suaveolens* leaves it may be extended as human contraceptive because of its safety and efficacy as reported in the present study.

REFERENCES

1. Anand. How to control the population in India? www.preservearticle.com
2. Phillipson JD. Natural products as drugs. *Trans R Soc Trop Med Hyg*, Suppl 1:S17-9, 1994.

3. Munshi,Shetye and Nair. Antifertility activity of three indigenous plant prepararti ons. *Plant Medica* Vol. 31: 73- 75, 1977.
4. John D, Bancroft, Alan Stevens. Theory and practice of histological techniques. UK: Churchill Livingstone, 3, 465-75, 1990.
5. Khanna V, Chowdhary RR. Antifertility screening of plants, part-I: investigation of Butea monosperma Lam (Kuntze). *Indian J. Med. Res*, 56, 1575-80, 1968
6. Pradeepa M.S, Veerana Gouda, Subodh kumar V Singh, Chetana H, Shambhulingaiah H.M, Ramdas M, Antifertility effect of herb of *Indigofera linnaei ali* in female albino rats. *International Journal of Phytopharmacology*, 3(1):42-49, 2012.
7. Patil, S. R. and Patil, M. B. Evaluation of estrogenic activity of various extracts of *Cuminum cyminum* seeds in female rats. Journal of Cell and Tissue Research Vol. 12(1) 3055-3060, 2012.
8. Dheeraj Ahirwar. Antiferility activity of *Acacia leucophploea.* Der Pharmacia Lettre, 3(3): 411-413, 2011
9. Psychoyos A (1966). Recent Research on Egg Implantation. CIBA Foundation Study Group.
10. Nivsarkar, M., Patel, M., Bapu, C. and Shirvasteva, N. Blastocyst implantation failure in mice due to “non-receptive endometrium”: Endomethrial alterations by *Hibiscus rosa-sinensis* leaf extract. *Contraception*, 71: *227-230,* 2005.
11. Burn, J.H. *Biological Standardization.* Oxford University Press, London(1952).
12. Chatterjee T.K. Herbal options, Publ., Central Book Agency, Kolkata 84. (1995)
13. Guillenim R. and Sakiz E. Quantitative study of responses to LH after hypophysectomy in the ovarian ascorbic acid depletion test: Effect of prolactin. *Endocrinology* 72, 813. 1963
14. Malini, T. and Vanithakumari, G. Estrogenic acticvity of Cuminium cyminium in rats : Indian J. Exp. Bio., 25: 442-444, 1987.
15. Patritia, L.W. and Heater, B. P. Cross- Species and interassay comparison of Phytoestrogen action. *Environmental Health Perspectives,* 109: *51,* 2001.

*****