Eugenol induced changes in reproductive cycle of female albino rats

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ABSTRACT:
Eugenol, one of the potent bioactive components of tulsi (Ocimum sanctum) was used to study its effect on reproductive cycle of female albino rats. Eugenol plus olive oil (0.2ml) was administered intramuscularly (im) in female albino rats up to 30 days. Vaginal cytology was studied by vaginal smear technique. Thin sections of ovary and uterus were taken and stained for histological observation. Body weight and gonadal weight were also recorded. Vaginal cytology revealed that estrous cycles of the experimental rats were disturbed by significant increase in the duration of diestrous phase. The weight of reproductive organs, ovary and uterus were reduced. Histological observations of ovary revealed significant decrease in healthy follicles as compared to control. Uterine endometrium found to be degenerated after 30 days treatment of eugenol. The observed effects of intramuscular administration of eugenol on estrous cycle and gonadal histological suggest antigonadotropic action of eugenol.

Key words: eugenol, estrous cycle, ovary, uterus, female albino rats.

INTRODUCTION:
Eugenol (4-allyl-2 methoxy phenol) is a polyphenolic compound extracted from the leaves and flowering tops of tulsi (Ocimum sanctum, family Labiatae) (Singh et al., 2010). Eugenol is frequently used in the clinical dentistry commonly as zinc-oxide-eugenol (ZOE) cement because of its antiseptic property (Craig; 1980). Eugenol is antibacterial and insecticidal. It is good immune-modulatory agent. Leaf extract of O. sanctum and O. gratissimum have been reported to have antifertility effects in male albino rats (Kantak,1992; Atuboyedia,2010).

It has been reported that, leaves of O. sanctum have antizygotic, anti-implantation and early abortifacient effects in experimental animals (Vora, 1969). Eugenol is one of the potent bioactive components of tulsi, the pharmacological properties documented for tulsi are associated with eugenol (Sen, 1993; Rajeshwari, 1992; Mukherji,1995). Eugenol has structural resemblance to polyphenol which has showed estrogenic properties in albino rats (Farook, 1987). Moreover, ovarian-uterine interrelationship forms an essential prerequisite for normal operation of sexual cycles in mammals (Hafez,1970; Devi,1992 and Guyton, 2009). The present study has been undertaken, prompted by above information to see the possible effects of eugenol on the reproductive cycle of female albino rats.

MATERIALS AND METHODS:
Animals: Healthy adult female albino rats weighing 165±14g with regular estrous cycles were taken from Laboratory rat colony (28±2C, 12:12 hr. L: D) Animals were fed with pellet diet and water ad libitum. Eugenol (98% pure) was obtained from Loba Chem. Animals were divided into 3 groups having 6 rats each. Group I: Control rats: Injected (im) with 0.2 ml olive oil/day/rat up to 30 days as vehicle Group II: Experimental rats : Administered with eugenol + olive oil (im) 0.2ml/day/rat up to 15 days (0.2ml contains 200 mg eugenol). Group III: Experimental rats : Administered with eugenol + olive oil (im) 0.2 ml / day/ rat up to 30 days.

Daily the vaginal cytology of the control and experimental rats was studied by vaginal smear technique as described by Hafez,(1970). Rats exhibiting 4-5 days estrous cycle of proestrus, estrous, metaestrous and diestrous were considered as normal while, any deviation from this pattern in terms of duration and sequence was categorized as abnormal (Gbotolorum et al., 2008).

Ovaries and uterus of control and experimental rats were blotted on filter paper and weighed quickly on a sensitive balance and fixed in Bouins fluid for 24 hrs. The paraffin embedded tissues were cut at 5 µm and stained with hematoxyline-eosin solution for histological studies. A follicle was considered undergoing atresia or regression whenever two or more pycnentic granulose cells could be found in a single section or whenever the oocyte showed various signs of degeneration as fragmentation, loss of nuclear membrane or thinning of the cumulus oophorous (Osman,1985; Baliger and Basappa, 2001).
During the experiment the rats were weighed daily and sacrificed on 16th and 31st day by cervical dislocation. Ovaries and uterus were isolated and weighed.

Students “t” test was used, P< 0.05 was regarded as moderately significant and P< 0.01 as significant (Fischer,1950).

RESULTS AND DISCUSSION:
The olive oil administered control rats exhibited regular estrous cycle and normal duration of each phase of the estrous cycle. Administration of eugenol to female albino rats exhibited an irregular pattern of estrous cycle. These rats showed significant decrease in number of estrous cycles and duration of proestrous, estrous and metestrous phases. Experimental rats showed a prolonged metestrous and diestrous pattern in each cycle as compared to control (Table -1.1)

The percent increase in the body weight of control rat is 3.15 when compared with that of the initial body weight. There is no significant increase in the body weight of the experimental rats after 15 days treatment however, 30 days treatment of eugenol resulted in significant weight gain (3.89%) as compared to control. The weight of ovary and uterus were significantly decreased in the experimental animals after 30 days of treatment with eugenol. The percent loss in the weight of ovary and uterus was found to be 12.56 and 14.54 respectively (Table 1.2). Therefore eugenol is not estrogenic in nature as anethol.

The study of follicular dynamics has revealed a significant decrease in the number of healthy follicles and increase in the number of atretic follicle in eugenol treated rats (Fig.1.2) as compared to control (Fig.1.1). The uterus of control albino rat showed normal epithelium, endometrium and uterine gland. (Fig. 1.3). The 30 days treatment of eugenol resulted into enlargement of endometrium along with enlarged uterine cavity. The epithelial lining of the endometrium was found to be degenerated and showed shrunken blood vessels. Some spaces were observed on the epithelium of endometrium. (Fig.1.4)

The control rats exhibited regular estrous cycle of 4-5 days. Cyclic changes of the vaginal smear observed in the estrous cycle gives a reasonable index of ovarian activity and hormonal synthesis of estrogen and progesterone. The level of estrogen and progesterone are controlled by pituitary gonadotropins and hypothalamus-releasing gonadal hormone (Lerner, 1969). The present study demonstrated that the im administration of eugenol alters the reproductive cycle of female albino rats by prolonging the duration of the diestrous phase, and subsequent lowering the frequency of the estrous. The remarkable extension of diestrous indicates prolongation of life of corpora lutea. (Devi et al., 1992)

Significant decreased weight of ovary after 30 days treatment of eugenol forms the ample evidence towards the inhibited follicular development of ovary. The histological observations revealed the significant decrease in the number of healthy follicles indicating hypogonadotropism. Many antifertility plant extracts are known to exhibit estrogenic activity in rats (Gebrie et al., 2005). Estrogen induces uterophic changes such as increase in diameter of uterus thickness of endometrium, height of endometrial epithelium, providing non receptive condition for implantation (Dhar, 1995). Administration of eugenol up to 30 days resulted into degenerated endometrium and shrunken stratum basale. Hence eugenol has antiestrogenic activity. Muzumdar et al., (1992) in their investigation of anti fertility activity of seeds of Nelumbo nucifera in mice had shown that the seeds can affect the estrous cycle by blocking the biogenesis of ovarian steroids in any intermediary stage. Therefore, in the present study it is concluded that significant decrease in frequency of reproductive cycle is may be due to the hormonal imbalance in any of the stages in hypothalamo - hypophysial ovarian axis or by insensitising the follicular development of surviving follicles into next successive follicular stages and also arrest of estrogen production. This suggests anti-gonadotropic action of eugenol.

REFERENCES:-


Desertation, University of Madras.


Table 1.1: Effect of Eugenol on the Estrous Cycle of female albino rats.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of cycles</th>
<th>Proestrous</th>
<th>Estrous</th>
<th>Metestrous</th>
<th>Diestrous</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.81±0.16</td>
<td>5.01±0.12</td>
<td>7.31±0.29</td>
<td>4.79±0.22</td>
<td>12.06±0.38</td>
</tr>
<tr>
<td>II</td>
<td>2.12±0.20*</td>
<td>1.36±0.22</td>
<td>3.76±0.21</td>
<td>2.67±0.18*</td>
<td>21.62±0.19*</td>
</tr>
<tr>
<td>III</td>
<td>2.00±0.18</td>
<td>1.12±0.11*</td>
<td>3.28±0.18*</td>
<td>2.24±0.20*</td>
<td>23.26±0.34*</td>
</tr>
</tbody>
</table>

*=significant, p<0.05 compared to control
Table 1.2: Effect of Eugenol on body weight and gonadal weight of female albino rats.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Body Weight (g)</th>
<th>Weight of Ovary (mg)</th>
<th>Weight of Uterus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight</td>
<td>168.88±12.07</td>
<td>60.80±3.10</td>
<td>422.07±11.60</td>
</tr>
<tr>
<td>I</td>
<td>174.20 ±11.76 (+3.15)</td>
<td>64.40±1.16 (+5.92)</td>
<td>470.29±21.70 (+11.42)</td>
</tr>
<tr>
<td>II</td>
<td>171.91NS±12.08 (+1.79)</td>
<td>55.91*±1.74 (-8.04)</td>
<td>395.18*±19.12 (-6.37)</td>
</tr>
<tr>
<td>III</td>
<td>175.45* ±12.17 (+3.89)</td>
<td>53.16*±1.37 (-12.56)</td>
<td>360.70±10.40* (-14.54)</td>
</tr>
</tbody>
</table>

Values are mean ±SE of six animals per group and refer to the average combined weight of right and left organs.
*significant, p<0.05 compared to control, NS-Not significant
Figures in parenthesis indicate percent change over control.

Fig.:1.2:T.S. Ovary of female albino rat after 30 days administration of eugenol showing degenerated follicle, disappeared ovum and shrinked corpus luteum (Haematoxylin-eosin, x 100)
(Haematoxylin-eosin, x 100)
Fig.:1.1: T.S. Ovary of control female albino rat showing normal developing follicle, griffin follicle and normal corpus luteum
(Haematoxylin-eosin, x 100)
Fig.:1.4: T.S. of uterus of albino rat after 30 days administration of eugenol showing degenerated epithelium, fluid filled uterine gland and shrinked stratum basale
(Haematoxylin-eosin, x 100)
Fig.:1.3: T.S. of uterus of control albino rat showing normal epithelium, endometrium, myometrium, uterine gland and blood vessels (Haematoxylin-eosin, x 100)