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Research Article

COMPARATIVE ANALYSIS OF EUGENOL COMPOUND AND *OCIMUM SANCTUM* LINN. LEAF EXTRACT ON THE INFERTILITY IN FEMALE ALBINO RATS

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ABSTRACT. The present study is aimed to monitor the effect of Eugenol and OS leaf extract on the antifertility activity through hostological observations and assaying of antioxidant enzymes in female rats. The female rats of 170±20g weight were administered with Eugenol (99% Pure) @ 0.4 ml/day/rat and OS leaf extract @ 500 mg/kg body weight/ orally for 15 days. The present investigation the Catalase levels were significantly increased in liver by both administrations (P<0.001). The AST and ALT levels were significantly elevated in liver by both administrations (P<0.001). The histology of the ovary by the administration of eugenol, showing disorganization of granulose cells and the graafian follicle shows the characteristics of follicular atresia. The significantly elevated ASTactivitys levels in liver tissue clearly demonstrate the liver dysfunctioning and disturbance in the production rates of antioxidant enzymes. The histological observation clearly demonstrates that due to administration of eugenol, Ovary weight was significantly reduced, whereas progressive diminishment in the follicular apparatus and primary follicles.

Keywords: *Eegenol, Ocimum sanctum, Ovary, Uterus, Vagina, Liver.*

INTRODUCTION

Antifertilits agents belong to oral contraceptives and know to control fertility. These drugs affect not only the menstrual cycle and also ovulation in the female sex. In general both estrogen and progesterone put together as birth control pills. In Females, the antifertilits agents prevent fertilization, ovulation, complantation and also destroys suggest leads to absorption. But in the case of the male sex, it affects spermatogenesis, inhibits testosterone and production levels of gonadotropins on reproductive organs [1]. Oestrogen-Progesterone balance plays a vital role in developing an agent, which will prevent pregnancy by interfering with implantation without disturbing the hypothalamic-pituitary-ovarian axis. Medicinal plant forms one of the important sources of drugs to cure several ailments of the world's population. Crude extracts of plants suppose to contain phytochemicals with includes a wide variety of molecules known to effect the biological system [2]. The most commonly available plant Ocimum sanctum, known as holy basil, is very commonly used for treating general ailments like cold, fever, dysentery, hemoschage and dyspepsia, and as well as gastric and hepatic disorders in indigenous systems of medicine.This plant extracts are known to possess several phalmacological properties which includes antifertilits, immunoregulatory, hypoglycemic antibacterial, antifungal, antiimflammatory, antioxidant, anticinogenic and cyclooxigenase inhibitory activites. [4] The leafy extracts suppose to contain a large number of volatile oils including limonene, borneol, copaene, caryophyllene and phenolic compounds like rosmalinic acid, apigenin, cirsimaritin and isothymusin, flavanoids including orientin, vicenin, alomatic compounds such as methyl chavicol and methyl eugenol. Eugenol, a major active constituent known to possess pharmacological activities. [3].

It has been reported that, leaves of Ocimum sanctum have antizygotic, antiimplantation and early abortifacient effects in experimental animals. Eugenol is one of the potent bioactive components of tulsi, the pharmacological properties documented for tulsi are associated with eugenol. Eugenol has a structural resemblance to polyphenol which has showed estrogenic properties in albino rats. Moreover, the normal operation of the sexual cycles in mammals is basically dependent on the ovarian-uterine interrelationship [5].

MATERIALS AND METHODS

Design of study

This study was carried out during November- January 2018. 4 months old (170 ± 20g weight) healthy adult female Wistar albino rats were selected for the present study. Rats were kept in clean polypropylene case under hygienic condition provided sufficient ventilation with 12 : 12 LD cycle at a room temperature of 250C and selective humidity 50% the Rats were ad libitum with commercial laboratory feed obtained from M/S Hindustan Lever Ltd, Mumbai. All the maintenance procedures followed and experiments were conducted strightly in accordance with the CPCSEA guidelines and also following the guidelines for the case and use of laboratory animals [6].

Preparation of Ocimum sanctum leaves extract

The extracts of the leaf were prepared by following the procedures of WHO 1983 [7] Initially soon after collection, the leaves were cut into slices, shed-dried in a shape place, grounded into the powder and extracts were prepared in 95% D/W at 55-60oC. After distillation of the solvent under reduced pressure, the resultant mass was again dried under vacuum and used for further experimental purposes.

Test chemical

Pure compound eugenol (99%) was procured from Sigma Aldrich (St Louis).

Dosage of Animals

The female albino rats were sorted into three groups of 6 each. The initial body weight were recorded.

Experimental design

Group I: Control group administered with 1 m of saline.
Group II: Administered Euenol (99%) a dose of 0.4 ml / day for 15 days through intramuscular injection.
Group III: Administered with OS leaf extract @ dose of 500 mg 1kg BW/day for 15 days. Through oral using gastric gavaging technique [8,5]

Sacrification schedule

Experimental rats were selected, weighed and sacrificed under anesthesia after 24 hrs of the administration of the last dose. Appropriate case was taken to reduce handling stress during blood sample collection, body weight measurements and also sacrificing time.

Body and Organ weights

The animal weights were recorded at the start and end of the 15 day experimental period. The required tissues like, Liver, Ovary, Uterus and Vagina were dissected and weighed accurately, were kept at 4oC further used for biochemical investigations.

Tissue homogenate preparation

Tissues from control and experimental sacrificed rats were collected in ice cold conditions and washed with ice cold physiological saline solution, further homogenized in respective media and for biochemical assays.

Antioxidant Enzyme Assay

The enzymes assays, including catalase [9], LPO [10], SOD [11], ALT [12], AST [12], were assayed by following standard methodologies, with slight modifications.

Histological observation

The histological studies and observations were conducted by adopting the procedure as described earlier by Ramana Ph.D Thesis.

Statistical analysis

The data obtained in the present study were subjected to statistical Analysis performed using SPSS (Version 11.5). Compulsion of data was done using One-Way ANOVA with DUNNETTS Multiple Compulsion Test.

RESULTS AND DISCUSSION

Effect on Antioxidant Enzymes parameters in liver

The results obtained for antioxidant enzymes such as Catalase, LPO, SOD, In liver tissue of both control and experimental group were presented in Table-1. In this present study the results showed there is the Catalase levels were significantly increased in liver by both administrations [P<0.001]. In this present study the results showed there is the Lipid peroxidation levels were significantly decreased in liver by both administrations [P<0.001]. In this present study the results showed there is the Superoxide dismutase activity levels were significantly elevated in liver by both administrations [P<0.001].

Table 1. *Antioxidant activity in female rat liver tissue after experimental treatments*

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Catalase levels (nmoles of H_2O_2 degraded / mg protein/ min)	26.75±1.07 PDC	37.78±1.99 +41.23 [a]	45.70±2.26 + 70.84 [a]
2	Lipid peroxidation levels (µmoles of malondialdehydeformed/g)	43.45±3.26 PDC	35.39±2.68 -18.55 [b]	24.73±1.83 -43.08 [a]
3	Superoxide dismutase activity levels (superoxide anion reduced/ mg protein / min)	38.50±2.17 PDC	62.08±5.24 +61.24 [a]	56.72±4.18 +47.32 [a]

All values are Mean ± SD of six individual observations.
PDC: Percent Deviation over control.
Values are significant at a- p<0.001, b- p<0.01.

The result obtained for AST and ALT in the liver tissue of control and experimental rats were

presented in **Table-2**. Both AST and ALT values were found to be significantly increased (P<0.001) in experimental rats compared to controls.

***Table 2**. Effect of Eugenol and Ocimum sanctum Linn.leaf extract in Liver*

S. No	Name of the parameter	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
1	AST (µmoles of pyruvate formed / mg protein / hour)	32.49±2.64 PDC	54.80±4.76 +68.66 [a]	46.39±3.85 +42.78 [a]
2	ALT (µmoles of pyruvate formed / mg protein / hour)	58.45±4.91 PDC	86.74±7.78 +48.40 [a]	79.69±6.83 +36.33 [a]

All values are Mean ± SD of six individual observations.
PDC: Percent Deviation over control.
Values are significant at a- p<0.001.

Effect on histopathological studies

This study revealed that there was antifertility activity after 15 days of eugenol and *OS* administrations in female rat. The histological changes were observed in female rat tissues like ovary, uterus, vagina and liver in Mag x400. The histology of the ovary by administration of eugenol, showing disorganization of granulose cells and the graafian follicle shows the characteristics of follicular atresia **[Fig. -1].**

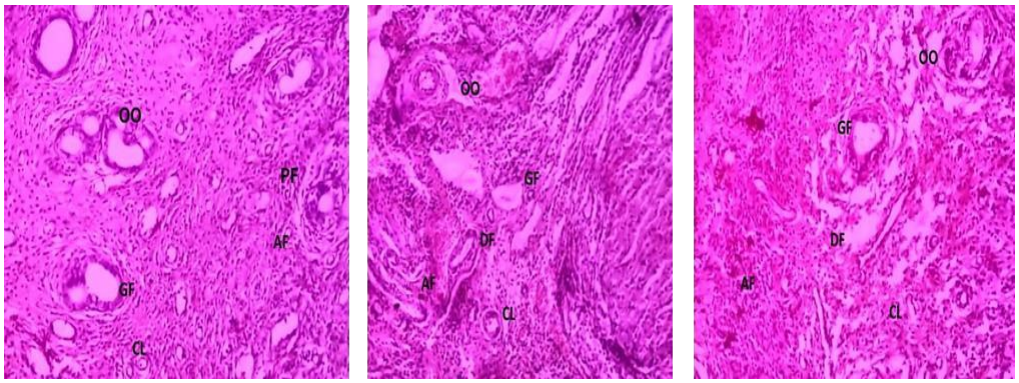
A histological section of the uterus in rat, showed the uterine Lumen of the uterus was significantly enlarged. The epithelial wall height of luminous and myometrial thickness were reduced [Fig. -2]. A histological section of the vagina on rat, showed the administration of eugenol decline in vaginal epithelial thickness, with diminished mucous cells [Fig. -3]. A histological section of the liver on rat, showed The *OS* administered liver showed the normal architecture of the liver. The *Ocimum sanctum* Linn. leaf extract administered in female rat liver. The post menopausal woman show liver damage and liver dysfunction [Fig. -4]. The results obtained in the present study showed that the catalase levels were significantly elevated in the liver by both administrations rat indicate a detoxifying mechanism against the toxicity [14]. The catalase levels were significantly elevated in the liver by both administrations exposures observed in the present study may be an adaptive response to H_2O_2 produced by SOD activity since catalase is responsible for its detoxification to wate [14]. The CAT and SOD activity levels were significantly increased in liver by both administrations, Indicates that its role in the generation of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) is the characteristics feature of liver metabolism [15]. The data of current study showed that LPO levels were significantly reduced in liver by both administrations. Due to enhanced metabolic rates and β-oxidation, the lipid content of liver tissues was considerably decreased, there b y significantly reducing the availability of lipids for peroxidation [16]. The data of current study showed that both administrations of LPO levels were significantly reduced in liver due to a reduction in the excessive production of free radicals rather than the alteration in the antioxidants in rat liver. Scavenging of free radicals is an important mechanism for the inhibitory activity of garlic towards LPO [17]. Liver being a metabolic seat inhabits higher rate of oxygen consumption and also efficient in antioxidant defiance mechanism [15]. Both AST and ALT were considered as marker enzymes for metabolism. ALT primarily located in the cytosol of hepatocytes and

considered as a marker for inflammation in liver tissue, and also provides the quantitative assessment of decrease of damage sustained by liver [18]. A significant increase in AST activity leaves indicates the liver dysfunction and disturbances in the production of enzymes [19]. The histological studies clearly depict the tissue architecture and the relationship between these structures and physiological function. The light microscopy is considered to be an important tool in understanding the structural and functional organization of the tissues, which Intern the organs. The main aim of the present study is to unravel the tissue architecture changes followed by its changes in physiological functions.

In **Fig-1,** the represents the transverse section of the ovary in the administration of eugenol, showing disorganization of granulose cells and the graafian follicle shows the characteristics of follicular atresia. Due to administration of eugenol, the developing follicles, graffian follicles and corpora lutea number were significantly increased.

The significant reduction of corpora lutea in eugenol treated rats indicative of inhibition of conversion of preovulatory follicles in to corpora lueta by arresting ovulation [20]. The developing and graffian follicles reduction in number may be attributed to the decreased/ reduced availability of ovarian estrogens as they are the prime sources of estrogens in the ovary [21]. Pattanayaak [22].

Reported the significant reduction in the number of follicles and corpora lutea in female rats ovary. Several authors also reported that reduction in ovary weight, progressive diminishment of follicular apparatus, complete disintegration of primary and secondary follicles and corpus luteum due to *OS* administration [23,24], but total disintegration was reported by Richa Niranjan [25]. The histological study of ovary clearly reveals that these are an imbalance in the production of these hormones lead to irregularity in the ovarian functions and duration of estrous cycles [26].

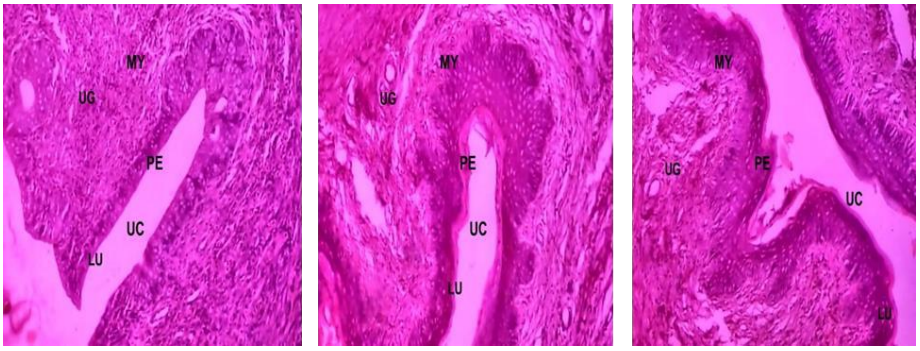


Control EUG administered *OS* administered

Fig. 1. (*T.S of the Ovary), Magnification x400 Stained with Hematoxylin and eosin stain. AF - Atretic follicles, CL - Corpus Lutea, CL - Corpus Lutea, DF – Developing follicles, GF - Graafian follicles, OO – Oocyte, PF - Primary Follicle etc.*

In **Fig-2,** the represents the transverse section of the uterus in the administration of eugenol, showing the uterine lumen was enlarged. The luminal epithelial cell height and myometrial thickness were reduced.Our studies also support the earlier findings showed decreased thickness of myometrium and height of the luminal epithelium in the uterus [27]. Showing increased number and size of uterine glands [28]. In the control administrations with normal saline the histoarchitecture of the uterus appears essentially normal showing the Uterine Gland [UG]. There appeared to be formed of polyps within the uterine cavity [29]. Histometric changes in uterus showed that there was an increase in luminal epithelial height and loosely arranged stroma in endometrium and also increase in total diameter of the uterus. It also showed increases in thickness of endometrium and myometrium significantly. These changes occur in

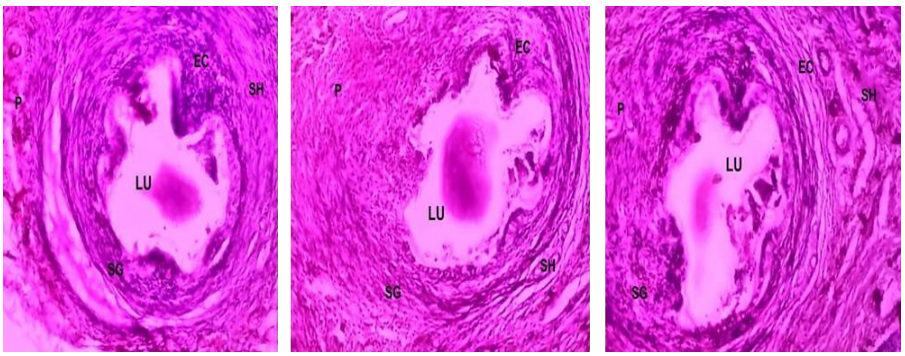
the uterus only due to estrogenic influence. Increase in uterine weight and thickness further supports to the estrogenic activity [25]. In the present study, the uterus, there was poor developed and disorganized endometrial epithelium appeared and contain many degenerating epithelial cells with many cavities [vacuoles] containing degenerating materials [20]. The uterine lumen was enlarged. The luminal epithelial cell height was reduced. The stroma was comparatively less edematous with less active endometrial glands [30].



Control EUG administered *OS* administered

Fig. 2. *(T.S of the uterus), Magnification x400 Stained with Hematoxylin and eosin stain. LU - Lumen, UC - Uterian cavity, MY – Myometrium, UG - Uterine gland, PE – Perimetrium etc.*

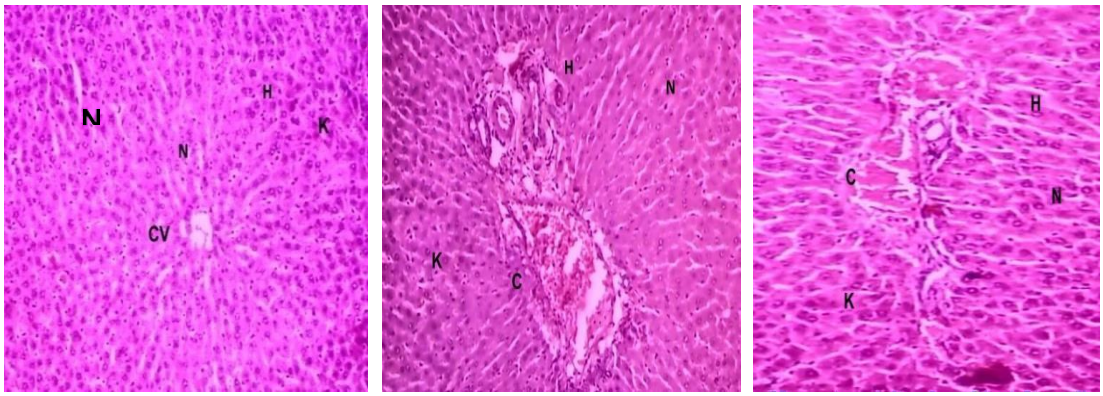
In **Fig-3,** the represents the transverse section of the vagina in the administration of eugenol decline in vaginal epithelial thickness, with diminished mucous cells. The histological details of vagina of female rats, clearly demonstrates the reduction in vaginal epithelial thickness, loss of vaginal epithelial rugae, erythema and also petechia, thinning of epithelium [31]. Due to decrease in premenopausal circulating estrogen levels during perimenopause are after induced menopause, the vagina was shortened and become narrow. Under these circumstances, the vaginal walls show a peculiar condition i.e. Pin point, non – raised, round, purple – red spot, appearance due to intradermal hemorrhage, a condition known as petechiae and later they become very thin, less elastic in nature and finally smoother as rugal fold decreased Susan [32]. Reported the reduction in vaginal blood flow and subabious gland secretions due to *OS* leaf extract administration. Several authors also reported due to *OS* leaf administration atrophy of vaginal epithelium, inactive epithelial luming vaginal mucosa damage, dissolving of vaginal epithelium, increase in superficial layer of vaginal epithelium, carcinoma tissue fibrosis with inflammatory cell infiltration [33,34, 35].



Control EUG administered *OS* administered

Fig.3. *(T.S of the Vagina),Magnification x400 Stained with Hematoxylin and eosin stain. EC- Epethelium cells, SG - Stratum germinativum, LU - Lumen, PC- Pasabasal cells, SH - Submucous haemorrhage*

In **Fig-4,** the represents the transverse section of liver in the administration of eugenol causes, prominence and widening of the liver sinusoids, congestion in central vein, hepatocyte proliferation and kupffer cell activation and coagulative necrosis [36]. The eugenol administered in female rat liver. Hepatocytes radiate like spokes of a wheel, from the central vein, forming anatomizing, fenestrated plates of liver cells [37]. Central vein and portal triad present [38]. In the present study, the *OS* administered liver showed the normal architecture of the liver. The *Ocimum sanctum* Linn. leaf extract administered in female rat liver. The postmenopausal women are more prone to liver damage and exhibit liver dysfunction. Generaly a any form of hepatic cell damage can result in an elevation in the serum glutamate pyruvate transaminase [39].



Control EUG administered *OS* administered

Fig. 4.*(T.S of the Liver), Magnification x400 Stained with Hematoxylin and eosin stain. C –Congestion, K - Kupffer cell, CV - Central Vein, N - Nucleus, H - Hepatocytes etc.*

CONCLUSION

According to the results of the present study, we conclude that the LPO levels were significantly reduced in liver by both administrations. It is possible that decreases the level of lipids in rats by enhancing metabolic rates and β-oxidation, thereby lowering the availability of lipids from peroxidation. Shows histology of *Ocimum sanctum* Linn. leaf extract administered ovary. Ovary like, granulosa cells of mature graafian follicles and the corpus luteum. Imbalance in these hormones leads to irregularity in the ovarian functions and duration of the estrous cycle. The follicle and the corpus luteum in the ovary. This decrease may be due to the no availability of gonadotrophins or steroidal hormones.

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