INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 17, No. 6, p. 373-386, 2020

RESEARCH PAPER OPEN ACCESS

Impact of infertility activity of eugenol and *Ocimum sanctum* Linn. leaf extract on Antioxidant Enzymes study in female albino rats

Venkataramanaiah P[1], Madduru Renuka[2], Yenukolu Aparna[3], Chakali Babu[4], Vasuru Kesavulu[5], Motireddy Srinivasulu Reddy[6*]

[1-6]*Department of Zoology, Sri Venkateswara University, Tirupati-517502, A.P, India*

[6]*Corresponding author: Prof. M Srinivasulu Reddy*

Key words: Eegenol, *Ocimum sanctum*, Infertility, Ovary and Uterus**.**

http://dx.doi.org/10.12692/ijb/17.6.373-386 Article published on December 28, 2020

Abstract

The present study is aimed to evaluate the antifertility activity of the effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Antioxidant Enzymes and Histological study (Reproductive and non-reproductive Organs). Experiments were conducted to investigate the healthy female albino rats were administered with EUG (99% pure) at a dose of 0.4 ml/day/rat and OS Linn. (Tulsi) leaf extract at a dose of 500 mg/kg body weight/day/rat orally for 15 days. One-way ANOVA analysis with Dunnett's multiple comparison test is used for analyzing data. The in vivo results demonstrated that the Catalase (CAT) and Superoxide dismutase (SOD) were significantly increased in all tissues by both administrations ($P<0.001$). The results represented in the GRx, GSH, GST and NO activity levels were significantly increased in all tissues by both administrations ($P<0.001$). The results represented in the LPO, Vitamin-E, Vitamin-C and Vitamin-A Levels were significantly reduced in all tissues by both administrations ($P<0.001$). The results of the present investigation suggest that The ovarian significantly increases in catalase activities suggest a degenerative changes in the ovary which includes a reduction in number of ovarian follicles and atrophic changes in the oocyte and granulosa cells. For instance, during folliculogenesis, GST protects the oocytes from oxidative damage. The suggests a reduction of its defence activity against cellular oxidative damage in the reproductive tissues. Accentuates the potential benefits of the female reproductive system of rats. Vitamin C possesses some functions such as collagen synthesis increment, impact on sexual hormone production, and protection of sexual cells against free radicals associated with infertility.

* **Corresponding Author:** Prof. M Srinivasulu Reddy ✉ pvr9490641036@gmail.com

Introduction

Oxidative stress (OS) is the consequence of an imbalance between the production of reactive oxygen species (ROS) and the total antioxidant capacity (TAC) of the organism. OS contributes to trigger inflammatory pro-cesses and endothelial dysfunction, which is important in the development of cardiovascular diseases (Sies, 2015). ROS are molecules or atoms that contain an unpaired electron in their last external orbital, acquiring a very unstable configuration (Lee, 2005). The defense systems against ROS include; preventive mechanisms, reparatory mechanisms, physical defenses and antioxidant defenses. The antioxidant enzymatic defense mechanisms include superoxide dis-mutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Ascorbic acid (vitamin C), alpha tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, among other molecules, constitutes the non-enzymatic antioxidants (Masella *et al.,* 2005). Oxidative stress is the imbalance of oxidants and antioxidants that can potentially cause cellular injury to the ovary, uterus, and brain. To lessen the oxidative stress in these tissues, antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione-S-transferase (GST) are up regulated in order to protect these tissues from damage (Park, 2018).

Ocimum sanctum L. has antifirtility effect. The leaves of *Ocimum sanctum* L. are said to have abortifacient an effect in women. In Kerala the local women as well as the Ayurvedic physicians have been reported to use the leaves of Tulsi for antifertility activities (Batta, 1971; Khanna *et al.,* 1986). Benzene and petroleum extracts of OS leaves have been reported to produce 60 to 80 % of antifertility in female rats. Ocimum sanctum L. in male rats showed an increased total sperm count, sperm motility and weight of testis (Seth *et al.,* 1981).

Antifertility agents are drugs that control fertility and are also called oral contraceptives. These drugs affect and are involved in the menstrual cycle and ovulation in females. Estrogen and progesterone in combined form are given as birth control pills. The antifertility substance is deemed to be active in females when it prevents fertilization, prevents ovulation, implantation and destroys the zygote or causes abortion. In males, it prevents spermatogenesis, inhibits testosterone or affects the gonadotropin of the organs or the mortality of sperm (Yogesh, 2002). The development of the fertilized ovum and the priming of the uterus for implantation there is a clear understanding of the role of estrogen-progesterone balance serves as the basis of developing an agent that would prevent pregnancy by interfering with implantation but without disturbing the hypothalamus-pituitary-ovarian axis.

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, ovarian-uterine interrelationship forms an essential prerequisite for normal operation of the sexual cycles in mammals. 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 (Kulkarni, 2011).

Materials and methods

Design of study

This study was carried out during November- January 2018. In the present study healthy adult (4 months old, weight 170±20g) female Wistar strain albino rats were used. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. Animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean, air conditioned room, with a photoperiod of 12 hours light and 12 hours dark cycle, at 25 ± 2°C with a relative humidity of 50 ± 5%. The rats were fed with standard laboratory feed (Hindustan lever Ltd, Mumbai) and water *ad libitum*. The experiments were carried out in accordance with the guidelines of

the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (CPCSEA, 2003).

This study was also carried out in accordance with the guidelines for the care and use of laboratory animals (NRC, 1996). The use of animals was approved by the Institutional Animal Ethics Committee (IAEC) (Regd. No. 10(i)a/CPCSEA/IAEC/SVU/ZOOL/CC/ Dt.08-07-2012) at S V University, Tirupati, India.

Preparation of Ocimum sanctum leaves extract

The leaf extract was prepared according to WHO 1983 (WHO, 1983) protocol CG-04. Leaf was sliced, shed-dried, grounded into a fine powder and extracted with 95% D/W (v/v) at 55-60°C for 3h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24° C until use.

Test chemical

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

Dosage of animals

The female albino rats were divided into three groups, each group contains 6 rats. The initial body weight of each animal was recorded.

Experimental design

Group I

First group is controlled rats administered with 1 ml of saline (vehicle).

Group II

Second group is experimental, administered with pure compound Eugenol (99%) at dose 0.4 ml /day for 15 days by intramuscular injection.

Group III

Third group is experimental, administered with *Ocimum sanctum* leaf extract at dose 500 mg /Kg body weight/ day for 15 days administered orally using the gastric gavaging technique (Shankar *et al.,* 2009; Kulkarni, 2011).

Sacrification schedule

Twenty-four hours after their last dose, the rats were weighed and sacrificed under anesthesia. The following steps were taken to minimize the suffering of the rats. First, the rats were handled gently to reduce their discomfort and distress. Second, anesthesia was administered prior to blood sample collection, body weight measurements and before animal sacrifice. Additionally, anesthesia, examinations and animal sacrifice were undertaken in separate rooms to avoid instilling fear in other rats.

Body and Organ weights

The body weight has been recorded on the initial day of the experiment and also on the day of sacrifice (15 day), both the control and experimental groups, by using an automatic balance. The ovary, uterus, vagina and liver were dissected out, freed from adherent tissues, and weighed to the nearest milligram. Organ weights were reported as relative weights (organ weight/body weight ×100).

Collection of Tissues

Both control and experimental animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean air conditioned room. Twenty four hours after the last dose, the animals were autopsied and the reproductive tissues like liver were excised at 4°C and used for biochemical analysis. The blood was collected by puncturing heart.

Tissue homogenate preparation

Rats were sacrificed under ether anaesthesia after 15 days treatment. Liver was quickly removed, trimmed of extraneous tissue, washed with ice-cold physiological saline solution. Liver tissues were homogenized solution for studying the biochemical parameters.

Antioxidant Enzyme Assay

Determination of Catalase activity (CAT)

Catalase activity was measured by a slightly modified version of Aebi, (1984) at room temperature. The tissue was homogenized in ice cold 50 mM phosphate

buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (W/V). The homogenates were centrifuged at 10,000 rpm for 10 min at 40C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 µl of 100% EtOH was added to 100 µl of tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 10 µl of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer and 50 µl of tissue extract was added 250µl of 0.066 M H_2O_2 (in phosphate buffer) and decreases in optical density measured at 240 nm for 60 s in a UV spectrophotometer. The molar extinction coefficient of 43.6 M cm-1 was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded / mg protein / min.

Determination of lipid peroxidation (LPO)

The assay used in MDA levels as described by the method of Okhaw *et al.,* (1979). The tissues were homogenized (5% w/v) in 50mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA, the homogenates were centrifuged at 10,000 rpm for 10 min at 4°C in cold centrifuge.

The separated supernatant part was used for the estimation. 200µl of the tissue extract was added to n50µl of 8.1% sodium dodecyl sulphate (SDS) m vortexed and incubated for 10 min at room temperature. 375µl of 20% acetic acid (pH 3.5) and 375µl of thiobarbutiric acid (0.6%) were added and placed in boiling water bath for 1h. The samples were allowed to cool at room temperature. A mixture of 1.25ml of butanol:pyridine (15:1) was added, vortexed and centrifuged at 1000 rpm for 5 min. The coloured layer (500µl) was measured at 532nm using 1,1,3,3 – tetraethoxy propane as a standard. The values were expressed in µ moles of alondialdehyde formed/g.wet wt. of the tissue. The values were expressed in µ moles of malondialdehyde formed/g. wet wt of tissue.

Determination of superoxide dismutase activity (SOD)

Superoxide dismutase activity was determined according to the method of Misra and Fridovich, (1972) at room temperature. The lung tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (W/V). The homogenates were centrifuged at 10,000 rpm for 10 min at 40C in cold centrifuge.

The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min on a Hitachi U-2000 Spectrophotometer. An activity expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit.

Measurement of GPx activity

The activity of glutathione peroxidase (GPx) was evaluated with Randox GPx detection kit according to the manufac-turer's instructions, as described previously (Kheradmand*et al.*2010; Alirezaei *et al.,* 2011a; Alirezaei *et al.,* 2012b). GPx catalyse the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidised glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP?. The decrease in absorbance was measured spectrophotometri-cally against blank at 340 nm. One unit (U) of GPx was defined as ll mol of oxidized NADPH per min per milligram of tissue protein. The GPx activity was expressed as unit per milligram of tissue protein (U/mg protein).

Reduced glutathione (GSH)

Glutathione (GSH- Reduced) was also determined using 5,5 Dithro-bis 2- Nitrobenzoic acid (DNTB) and Tris-EDTA buffer as described by Tappel (1978). 100ul of the sample was added to 1ml of 0.2 ml Tris-EDTA buffer (pH 8.2) followed by 0.9ml of 20 mM EDTA (pH 4.7) and 20 ul DNTB. The sample was incubated at room temperature for 30 minutes. The mixture was centrifuged and absorbance of the supernan treads at 412nm.

Determination of GST activity

A convenient spectrophotometric method has been developed by Habig *et al.,* (1974) for the analysis of GST activity based on the enzyme-catalysed condensation of glutathione with the model substrate, 1-choloro, 2,4- dinitrobenzene. The product formed (2,4-dinitrophenyl-glutathione) absorbs light at 340 nm with an extinction coefficient of 9.6 mM/cm, which facilitates the analysis of enzyme activity based on product formation.

Vitamin E

The estimation of vitamin E (α-tocopherol) was performed using the method described by Rosenberg (1992). Sample was mixed slowly with 0.1 N sulphuric acid and incubated at room temperature for overnight and then filtered. To the 1.5 ml of tissue extract, 1.5 ml of xylene was added and centrifuged.

Then 1.0 ml of xylene was separated and mixed with 1.0 ml of 2,2-pyridyl and the absorbance was noted at 460 nm. In the beginning, 0.33 ml FeCl3 was added with blank and mixed well. After 15 min, the test and standard was read against the blank at 520 nm.

Vitamin C

Vitamin C estimation was done according to the Chinoy *et al.,* (1976) method. To 2 ml of plant extract, 8 ml of 2,6-dichlorophenol indophenols dye was added. Blank was prepared by using 2 ml of distilled water instead of extract and OD was recorded at 530 nm.

Vitamin A

Vitamin A estimation was performed using the method described by Bayfield and Cole (1980). Homogenate (1.0 ml) was mixed with 1.0 ml of saponification mixture and refluxed for 20 min at 60°C in the dark and cooled with 20 ml of water.

Vitamin A was extracted twice with 10 ml of (40 to 60°C) petroleum ether. TCA reagent (2.0 ml) was added rapidly, mixed and the absorbance was read immediately at 620 nm in a spectrophotometer.

Statistical analysis

The data were expressed as a Mean value with their SD. Reading of the six different groups was compared using one-way ANOVA analysis with a DUNNETTS MULTIPLE COMPRARISION TEST. Statistical analysis was performed using SPSS (Version 11.5; SPSS Inc., Chicago, IL, USA). Using M.S. Office - 2007, Excel Software, the data has been analysed for the significance of the main effects (factors) and treatments along with their interaction (Fischer, 1950). Differences were considered statistically significant a- $p < 0.001$, b- $p < 0.01$, c- $p<0.05$ and d- non significance levels.

Results

Effect on Antioxidant Enzymes parameters in reproductive organs (Ovary and Uterus)

The results of the antioxidant enzyme activities in Ovary and Uterus of control exposed rat are given in Table-1 -10.

Table 1. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extracts on Catalase (CAT) (n moles of H2O2degraded / mg protein/ min) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	17.87±1.24	32.53±2.63	25.64±1.98
			+ 82.03 [a]	+ 43.48 [a]
2	Uterus	19.14±0.97	27.62±1.68	30.36±2.34
			+44.30 [a]	+58.62 [a]

Effect on CAT

The results represented in the Catalase (CAT) were significantly increased in all tissues by both administrations ($P<0.001$).

Effect on LPO

The results represented in the Lipid peroxidase (LPO) Levels were significantly reduced in all tissues by both administrations ($P<0.001$).

Table 2. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Lipid peroxide (LPO) (µmoles of malondialdehyde formed/g) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	39.18±2.77	28.66±1.93	21.44±1.34
			-26.85 [a]	-45.27 [a]
2	Uterus	34.86±2.25	22.54±1.46	18.36±1.08
			-35.34 [a]	-47.33 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

Effect on SOD

The results represented in the Superoxide dismutase (SOD) activity levels were significantly increased in all tissues by both administrations (P<0.001).

Effect on GRx

The results represented in the Glutathione Peroxidase (GRx) activity levels were significantly increased in all tissues by both administrations (P<0.001).

Table 3. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Superoxide Dismutase activity levels (SOD) (superoxide anionreduced/ mg protein / min) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	16.63±1.01	29.47±1.89	28.33±1.76
			+77.20 [a]	+70.35 [a]
2	Uterus	11.42±0.98	20.72±1.37	17.92±1.08
			+81.43 [a]	+56.91 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

Effect on GSH

The results represented in the Reduced Glutathione (GSH) activity levels were significantly increased in all tissues by both administrations (P<0.001).

Effect on GST

The results represented in the Glutathione-s-transferase (GST) activity levels were significantly increased in all tissues by both administrations (P<0.001).

Effect on NO

The results represented in the Nitric Oxide (NO) activity levels were significantly increased in all tissues by both administrations (P<0.001).

Effect on Vitamin-E

The results represented in the Vitamin-E activity levels were significantly reduced in all tissues by both administrations (P<0.001).

Effect on Vitamin-C

The results represented in the Vitamin-C activity levels were significantly increased in the ovary, whereas in uterus these were reduced by both administrations (P<0.001).

Table 4. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Glutathione Peroxidase (GRx) (µmol NADPH oxidized/min/mg protein) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	6.25±0.53	9.78±0.85	9.16±0.79
			+56.48 [a]	+46.56 [a]
2	Uterus	18.18±1.24	13.2±1.01	11.64±0.98
			-26.73 [a]	-35.97 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

Effect on Vitamin A

The results represented in the Vitamin A activity levels were significantly increased in all tissues by both administrations (P<0.001).

Discussion

Effect on Antioxidant Enzymes parameters in reproductive organs (Ovary and Uterus)

CAT is ubiquitous heme enzymes that are found in aerobic organisms, ranging from bacteria to higher plants and animals. CAT is a tetramer of four polypeptide chains, each over 500 amino acids long.

It contains four porphyrin heme group that allows the enzyme to react with the hydrogen peroxide. Functionally, CAT promotes H_2O_2 oxidation by mechanisms that involve ferry intermediates (Sandalio *et al.,* 2001).

Table 5. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Reduced Glutathione (GSH) (µmol/mg protein) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	13.67±1.02	16.95±1.17	17.81±1.38
			+23.99 [a]	+3028 [a]
2	Uterus	36.89±2.68	52.34±4.73	48.53±3.97
			+41.88 [a]	+31.55 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

The ovarian significantly increases in catalase activities suggest a degenerative changes in the ovary which includes a reduction in number of ovarian follicles and atrophic changes in the oocyte and granulosa cells (Beenam, 2016). In uterus were significant increases in the catalase enzyme activities due to the excessive generation of free radicals (Davydov *et al.,* 2004).

Table 6. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Glutathione-s-transferase (GST) (µmol CDNB conjugate formed/min/mg protein) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	0.43±0.03	0.76±0.05	0.64±0.04
			+76.74 [a]	+48.83 [a]
2	Uterus	0.58±0.04	0.93±0.08	0.81±0.06
			+60.34 [a]	+48.27 [a]

Mean± SD of six individual observations. and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

Lipid peroxidation is a key process in many pathological events and it is induced by oxidative stress. Lipid peroxidation is regarded as one of the fundamental mechanisms of cellular damage caused by free radicals having reacted with lipids causing peroxidation that eventually results in the release of products such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and hydroxyl radicals (Kanchana Ganga *et al.,* 2013). The results of the present study indicated that lipid peroxidation in ovary and uterus significant increases in both administrations.

The reduced Lipid peroxidase level observed in the ovary, indicates the normally generated during ovulation due to the presence of inflammatory cells such as in the ovary (Daramola *et al.,* 2016).

Table 7. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Nitric Oxide (NO) levels (µmol nitrite/mg protein) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	1.63±0.12	3.02±0.27	2.74±0.19
			+85.27 [a]	+68.09 [a]
2	Uterus	2.15±0.16	2.98±0.28	2.65±0.20
			+38.60 [a]	+23.25 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

Table 8. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Vitamin-E (mg /g) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	3.45±0.29	2.31±0.17	1.85±0.14
			-33.04 [a]	-46.37 [a]
2	Uterus	2.97±0.21	1.78±0.13	1.26±0.09
			-40.06 [a]	-57.57 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

The uterine was significantly reduced in peroxidase activities; blockage of steroidogenic enzymes and decreased estradiol levels (Shailendra and Preety, 2018). SOD is considered a key enzyme in the regulation of intracellular concentrations of ROS. SOD acts as the first line of defence against ROS, dismutating superoxide to H_2O_2. Thus, increased SOD activity showed that it plays a positive role in controlling the cellular level of these ROS and/or repairing oxidative damage against free radicals by eugenol and OS stress (Muthukumar and Nachiappan, 2010). The increase SOD level observed in ovary, this study suggests that Antioxidants are important in the detoxification of reactive oxygen species generated during the preovulatory gonadotrophin surge in order to make oocyte maturation (Daramola *et al.,* 2016). The enzymic antioxidants SOD significantly increases in the uterine. Elevations in the SOD activity may be a compensatory mechanism for chronic overproduction of free radicals and oxidative stress in the uterine (Ladachart and Martin, 2018).

Table 9. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Vitamin-C (mg /g) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	3.16±0.26	2.43±0.19	2.62±0.21
			-23.10 [a]	-17.08 [a]
2	Uterus	1.83±0.14	1.21±0.09	1.08±0.07
			-33.87 [a]	-40.98 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

Our results showed that GPx in ovary and uterus significant increases in both administrations. Thus, the suggests a reduction of its defence activity against cellular oxidative damage in the reproductive tissues. Accentuates the potential benefits of the female reproductive system of rats (Amos *et al.,* 2020).

Table 10. Effect of Eugenol and *Ocimum sanctum* Linn. Leaf extract on Vitamin-A (µg /g) in Ovary and Uterus.

S. No	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Ovary	21.25±0.16	17.57±0.12	14.32±0.11
			-17.31 [a]	-32.61 [a]
2	Uterus	18.43±0.13	13.74±0.10	11.08±0.07
			-25.44 [a]	-39.88 [a]

Mean± SD of six individual observations. + and – percent increase and decrease respectively, over control. a-p<0.001indicates the level of significance.

Reduced glutathione (GSH), a tripeptide with a reactive sulfhydryl group, is a reductive non-enzymatic antioxidants, which is responsible for the scavenging of free radicals and preservation of intracellular redox status against oxidative insult (Adedara *et al.,* 2014). The oxidative stress in the ovary and uterus significantly increases in GSH level in both administrations. Indeed, GST plays an important role in the cell survival and protection against the oxidative damage (Calabrese *et al.,* 2013).

For instance, during folliculogenesis, GST protects the oocytes from oxidative damage (Angelucci *et al.,* 2006). The results of the present study indicated that Nitric Oxide (NO) in ovary and uterus significant increases in both administrations.

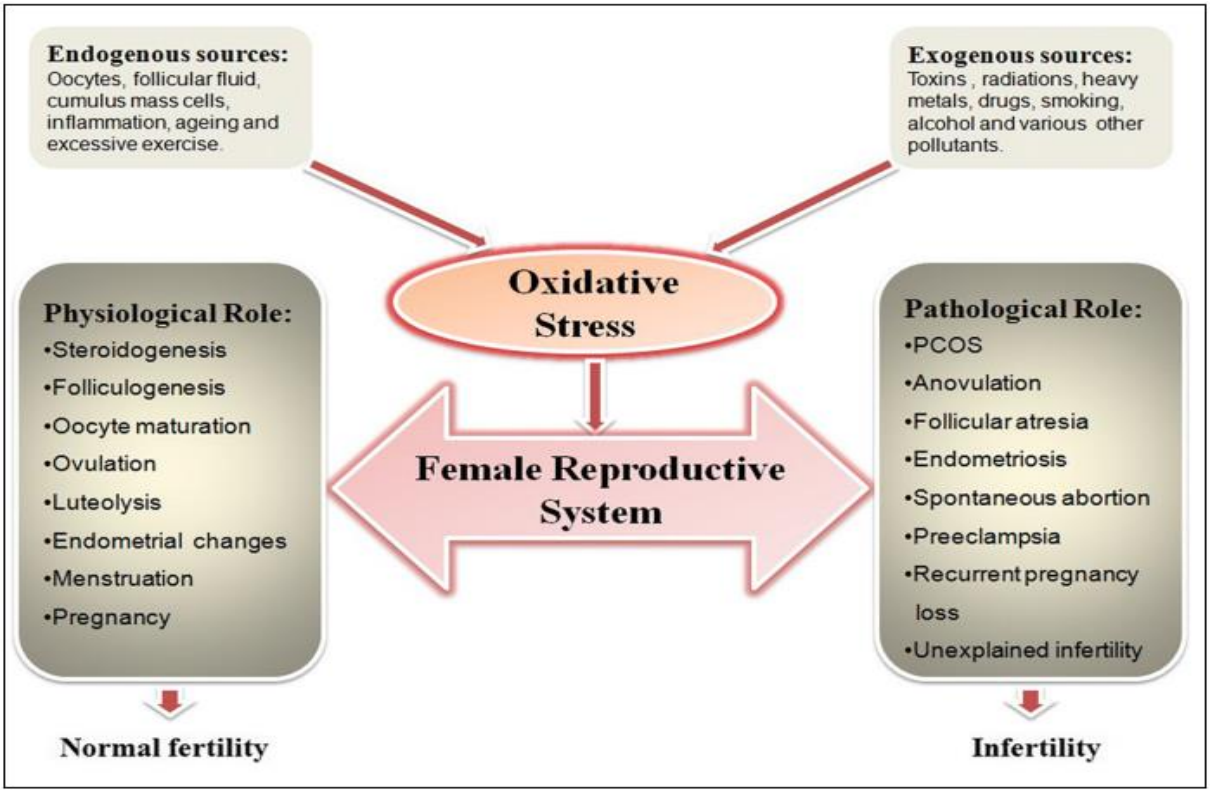

Fig. 1. Relationship between oxidative stress and female reproduction.

This might be due to the ability of the modulates the activity of nitric oxide synthase by acting as an anti-inflammatory agent (Rotelli *et al.,* 2003). The ovary and uterus significantly decreased in Vitamin E level by both administrations. Vitamin-E is a potent antioxidant that is useful in the body to maintain redox homeostasis and has been reported to have a protective effect against endogenous oxidative DNA damage and membrane damage.

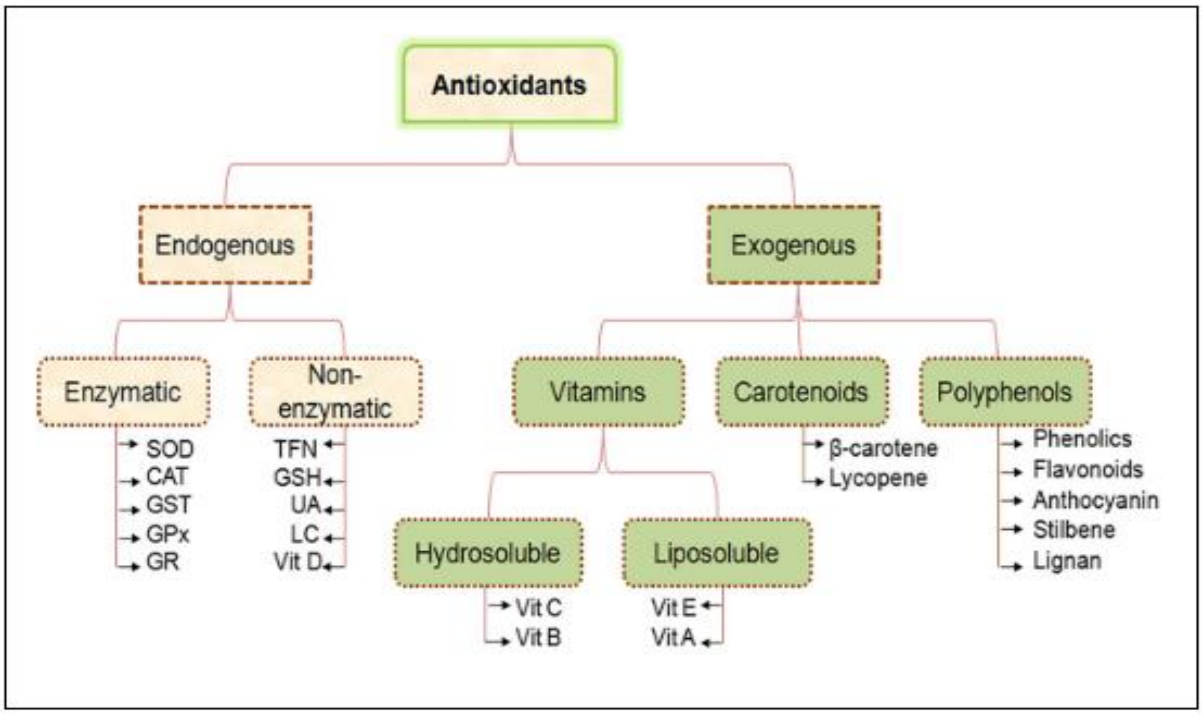

Fig. 2. Schematic representation of general antioxidant classification.

The reduced Vitamin-C level in ovary and uterus, this study suggests that Collagen synthesis is required for follicle growth, for repair of the ovulated follicle, and for corpus luteum development (Luck and Zhao, 1993). Vitamin C and other anti-oxidative substances help to prevent oxidative stress and the production of free radicals that interfere with progesterone production. Vitamin C possesses some functions such as collagen synthesis increment, impact on sexual hormones production, and protection of sexual cells against free radicals associated with infertility (Deane, 1952).

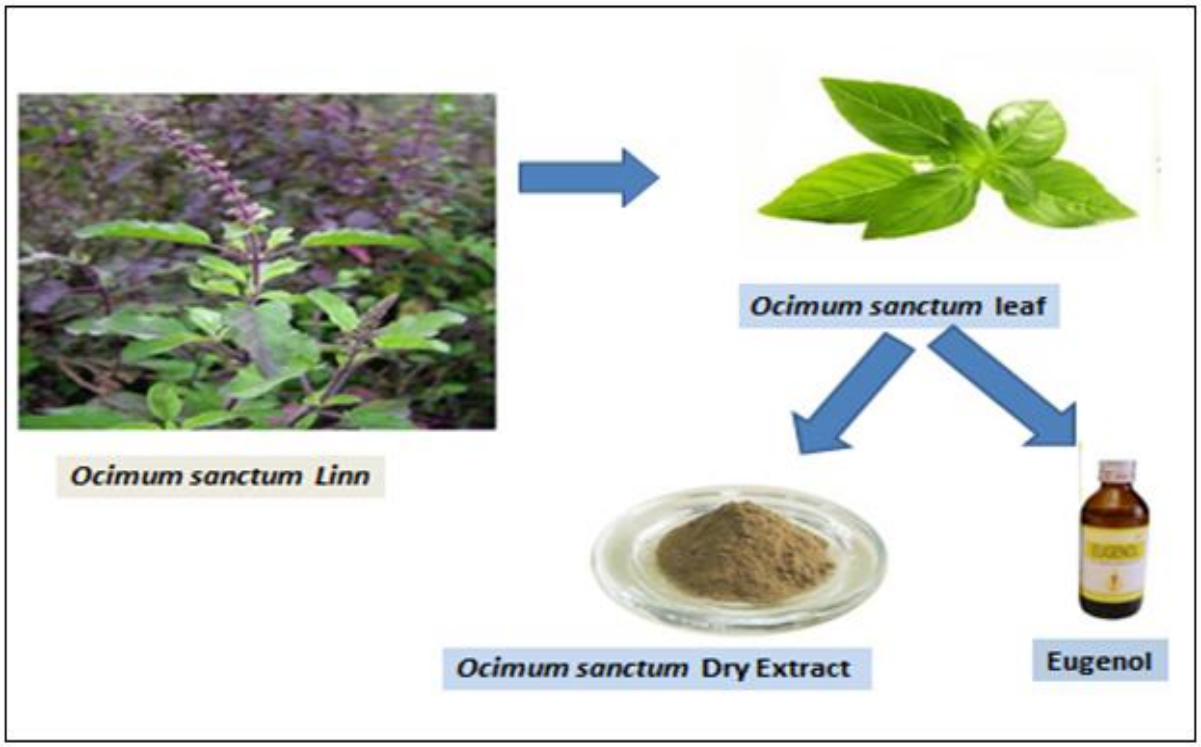

Fig. 3. Graphical abstract.

Our results showed that Vitamin-A in ovary and uterus significantly reduced in both administrations. Vitamin-A is a fat soluble vitamin, which is essential for growth, maintenance of visual function, reproduction and differentiation of epithelial tissue. Its metabolites affect ovarian follicular growth, uterine environment and oocyte maturation. Vitamin A and its metabolites play a crucial role in regulating the differentiation and proliferation of epithelial cells. There is clear in vivo evidence that vitamin A is required for the normal onset of meiotic prophase in ovarian germ cells (Li *et al.,* 2009).

Conclusion

According to the results of the present study, we conclude that the ovarian significantly increases in catalase activities suggest a degenerative changes in the ovary which includes a reduction in number of ovarian follicles and atrophic changes in the oocyte and granulosa cells. Vitamin A is a fat soluble vitamin, which is essential for growth, maintenance of visual function, reproduction and differentiation of epithelial tissue. Its metabolites affect ovarian follicular growth, uterine environment and oocyte maturation.

Acknowledgments

The authors are grateful to the cassava breeding team at Sri Venkateswara University (SVU) for their technical support during the course of this study.

Conflicting interests

The authors of this paper have no financial, personal, or professional conflicts of interest to disclose.

Reference

Abhijith BD, Ramesh M, Poopal RK. 2016. Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of Catla catla during methyl parathion exposure. The Journal of Basic and Applied Zoology **77,** 31–40.

Abkar Raden. 2010. Pegagan (Centella Asiatica) Extract Increases Vaginal Wall Thickness In Menopausal Rats. Folia Medica Indonesiana **46(3),** 222-228.

Adedara IA, Farombi EO. 2014. Influence of kolaviron and vitamin E on ethylene glycol monoethyl ether-induced haematotoxicity and renalapoptosis in rats. Cell Biochemistry & Function **32,** 31–8.

Aebi H. 1984. Catalase in vitro. Methods in Enzymology **105,** 121-126.

Alia Bilal, Nasreen Jahan, Ajij Ahmed, Saima Naaz Bilal, Shahida Habib. 2013. Intifertility Activity of Hydroalcoholic Extract of Ocimum Basilicum Linn. Leaves on Female Wistar Rats. Journal of Reproduction & Contraception **24 (1),** 45-54.

Alirezaei M, Jelodar G, Niknam P, Ghayemi Z, Nazifi S. 2011a. Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. Journal of Physiology and Biochemistry **67,** 605–612.

Alirezaei M, Saeb M, Javidnia K, Nazifi S, Saeb S. 2011b. Hyperhomocysteinemia reduction in ethanol-fed rabbits by oralbetaine. Comparative Clinical Pathology **21,** 1–7.

Amos Abolaji O, Marvis Omozokpia U, Olajide Oluwamuyide J, Temidayo Akintola E, Ebenezer Farombi O. 2020. Rescue role of hesperidin in 4-vinylcyclohexene diepoxide induced toxicity in the brain, ovary and uterus of wistar rats. Journal of Basic and Clinical Physiology and Pharmacology. 20180115.

Angelucci S, Ciavardelli D, Di Giuseppe F, Eleuterio E, Sulpizio M, Tiboni GM. 2006. Proteome analysis of human follicular fluid. Biochimica et Biophysica Acta **1764,** 1775–85.

Baligar PN, Kaliwal BB. 2004. Carbofuran induced block of compensatory ovarian growth in hemicastrated albino mice. Toxicology **204,** 87–95.

Batta SK, Santhakumari G. 1971. The antifertility effect of Ocimum sanctum and Hibiscuc Rosa Sinesis. Indian Journal of Medical Research **59,** 777-81.

Bayfield RF, Cole ER. 1980. Colorimetric determination of vitamin A with trichloroacetic acid. In: McCormick, D.B. and Wright, L.D., Eds. Methods in Enzymology, part F. Vitamins and Coenzymes. New York: Academic Press **67,** 189-195.

Beenam Saxena. 2016. Ovarian toxicity and oxidative stress induced by foodcolors in albino rats. International Journal of Security and Networks **7(4),** 838-842.

Calabrese EJ, Iavicoli I, Calabrese V. 2013. Hormesis: its impact on medicine and health. Human and Experimental Toxicology **32,** 120–52.

Catthareeya thanamool, pittaya papirom, suthida chanlun, sajeera kupittayanant. 2013. Talinum paniculatum (jacq.) gertn a medicinal plant with potential estrogenic activity in ovariectomized rats. International Journal of Pharmacy and Pharmaceutical Sciences **5(2),** 478-485.

Chinnappa Reddy V, Abhay Kumar Kamble, Sharangouda Patil J. 2016. Evaluation of antifertility activity of achyranthus aspera leaves in female mice. International Journal of Pharmaceutical Sciences and Research **7(9),** 3794-3801.

Chinoy JJ, Singh YD, Gurumurthi K. 1976. The role of ascorbic acid in growth, differentiation and metabolism of plants. Journal of Plant Physiology **22,** 122.

Daramola OO, Oyeyemi WA, Odiase LO, Olorunfemi AA. 2016. Effects of Methanol Extract of Telfairia occidentalis Seed on Ovary Antioxidant Enzymes, Serum Hormone Concentration and Histology in Wistar Rats. International Journal of Pharmacognosy and Phytochemical Research **8(8),** 1245-1249.

Davydov VV, Zakharchenko IV, Ovsyannnikov VG. 2004. Free radical processes in the liver of adult and old rats during stress. Bulletin of Experimental Biology and Medicine **137,** 139-42.

Deane HW. 1952. Histochemical observation on the ovary and oviduct of the albino rat during the estrous cycle. American Journal of Anatomy **91,** 363-413.

Djami Tchatchou Arnaud Thierry, Asongalem Emmanuel Acha Nana Paulin, Choumessi Aphrodite, Kamtchouing Pierre, Asonganyi Tazoacha. 2011. Subacute Toxicity Study of the Aqueous Extract from Acanthus montanus. Electronic Journal of Biology **7(1),** 11-15.

Enasa M, Khalil. 2002. Hormonal Profile And Histopathological Study On The Influence Of Silymarin On Both Female And Male Albino Rats. The Egyptian Journal of Hospital Medicine **13,** 112 – 122.

Fatma M, El-Demerdash, Mokhtar I, Yousef Fatma S, Kedwany, Hoda H, Baghdadi. 2004. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats protective role of vitamin E and b-carotene. Food and Chemical Toxicology **42,** 1563–1571.

Fischer RA. 1950. Statistical methods for research workers Oliver Boyd. London.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry **249(22),** 7140–7147.

Heikal T, Mossa AT, Rasoul M, Marei H. 2013. The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. Asian journal of Pharmaceutical and Clinical Research **6(1),** 48-55.

Kanchana Ganga U, Kishori B, Sreenivasula

Reddy P. 2013. Cisplatin and/or etoposide induces oxidative stress intesticular, hepatic and kidney tissues in male albino mice. Journal of Biology and Earth Sciences **3(2),** 249-254.

Khanna S, Gupta SR, Grover JK. 1986. Effect of long term feeding of Tulsi. Indian Journal of Experimental Biology **24,** 302-4.

Kheradmand A, Alirezaei M, Birjandi M. 2010. Ghrelin promotes antioxidant enzyme activity and reduces lipid peroxidation in therat ovary. Regulatory Peptides **162(1–3),** 84–89.

Kulkarni D. 2011. Eugenol induced changes in reproductive cycle of female albino rats. Bioscience Biotechnology Research Communications **4(1),** 98-101.

Ladachart Taepongsorat, Methin Phadungkit. 2018. Effects of Asparagus racemosus Root Extracts on Serum Lipid Profiles, Lipid Peroxidation and Superoxide Dismutase in Ovariectomized Rat. Pharmacognosy Journal **10(5),** 1036-1041.

Lee YM. 2005. Oxidative stress induces vascular heme oxigenase-1 expression in ovariectomized rats. Free. Free Radical Biology and Medicine **39,** 108–117. https://doi.org/10.1016/j.freeradbiomed.2005.02.033.

Li H, Clagett-Dame M. 2009. Vitamin A deficiency blocks the initiation of meiosis of germ cells in the developing rat ovary in vivo. Biology Reproductive **81,** 996–1001.

Luck MR, Zhao Y. 1993. Identification and Measurement of Collagen in the Bovine Corpus Luteium and its Relationship With Ascorbic Acid and Tissue Development. J Reprod Fertil **99,** 647–652.

Masella R, Di Benedetto R, Varì R, Filesi C, Giovannini C. 2005. Novel mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione related enzymes. Journal of Nutritional Biochemistry **16,** 577–586. https://doi.org/10.1016/j.jnutbio.2005.05.013

Misra HP, Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry **247(10),** 3170-3175.

Muthukumar K, Nachiappan V. 2010. Cadmium-induced oxidative stress in Saccharomyces cerevisiae. Indian Journal of Biochemistry and Biophysics **47,** 383-387.

Park SJ, Kim JH, Lee DG, Kim JM, Lee DS. 2018. Peroxiredoxin 2 deficiency accelerates age-related ovarian failure through the reactive oxygenspecies-mediated JNK pathway in mice. Free Radical Biology and Medicine **123,** 96–106.

Rosenberg HR. 1992. Chemistry and physiology of vitamins. Inter Science Publishers Inc. New York 452-453.

Rotelli AE, Guardia T, Juárez AO, De La Rocha NE, Pelzer LE. 2003. Comparative study of flavonoids in experimental models of inflammation. Pharmacology Research **48,** 601–6.

Sandalio LM, Dalurzo HC, Gomez M, Romero PMC, Del-Rio LA. 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. Journal of Experimental Botany **52(364),** 2115-2126.

Seth SD, Johri N, Sundaram KR. 1981. Antispermatogenic effect of Ocimum sanctum. Indian Journal of Experimental Biology **19,** 975-6.

Shailendra Kumar, Preety Sinha. 2018. Toxic effects of induced sodium arsenite on the ovary of swiss albino mice. European journal of pharmaceutical and medical research **5(7),** 372-377.

Shankar Mondal, Bijay R, Mirdha Suhil C, Mahapatra. 2009. The science behind sacredness of tulsi (Ocimum sanctum Linn) Indian journal of physiology and pharmacology **53(4),** 291–306.

Sies H. 2015 Oxidative stress: a concept in redox biology and medicine. Redox Biology **4,** 180–183. https://doi.org/10.1016/j.redox.2015.01.002.

Tappel AL. 1978. Glutathione peroxidase and hydroperoxides. Meth. Enzyme **52,** 506-513.

WHO. 1983. Protocol CG-40. Preparation of Alcoholic Extract for Bioassay and Phytochemical Studies. (APJF/IP, 1001 A) Geneva. World Health Organization.

Yogesh A. 2002. Traditional medicines for jaundice and family planning. Natural Product Radiance **1(6),** 29-35.