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# Eugenol a potent antidyslipidemic compound from *Ocimum sanctum* Linn

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## ABSTRACT

Since atherosclerosis is the major cause of morbidity and mortality world wide. Therefore we have selected antidyslipidemic and antioxidant screening of some medicinal plants. In the present study leaves of the *Ocimum sanctum* Linn. (Tulsi) which is known as holy basil in English, have been taken for this study. The crude ethanol extract of the plant showed promising lipid lowering activity along with increase in high density lipoprotein-cholesterol ratio as compared with the cholesterol fed animals in the Ttriton model. It also showed antioxidant effect. Further the activities were localized in hexane fraction from which the major compound eugenol showed promising lipid lowering and antioxidant activities.



## KEYWORDS

*Ocimum sanctum*;
Leaves;
Lipid lowering,
Antioxidant activities.

## INTRODUCTION

Plants have been used by men from prehistoric times to get rid of suffering & curing ailments. The folk medicine of almost around the world rely on herbal medicine[1]. Plants are the important sources of medicine and a large numbers of drugs in use are derived from plants. The therapeutic uses of plant are safe,economical and effective because of their easy availability[2].

*Ocimum sanctum* Linn. (Tulsi) Commonly known as holy basil in English, belongs to family (labiatae) have been selected for antidyslipidemic and antioxidant screening. Two types of *Ocimum sanctum* are met within cultivation, plants with green leaves and plants with purple leaves. Different parts of the plant such as leaves, flowers, stem, roots and seeds are known to possess therapeutic potentials & have been used by traditional medical practitioners[3-5]. Essential oils and herbal extracts of this plant have attracted scientific research interest greatly. Modern scientific research offers effective evidences of the *Ocimum sanctum.* It reduces the stress, enhances stamina, relives inflammation, lowers cholesterol, eliminates toxins, protects against radiation, gastric ulcer, improves digestion & provides a rich supply of antioxidants & other nutrients[6-8]. *Ocimum sanctum* has been reported effective in supporting the heart, blood vessels, liver &lungs and also regulates blood pressure and blood sugar. The plant is reported to contain eugenol[9].

## MATERIAL AND METHODS

### Collection of the plant material

Fresh leaves of the *Ocimum sanctum* were collected from the local gardens in the month of March. These fresh leaves were shade dried.

### Extraction and isolation procedure

Shade dried leaves (500 g) were extracted with 95% ethanol at room temperature for four times and combined extract was concentrated under reduced pressure below $50^0$C in a rotavapour to a green viscous mass, which was finally dried under high vacuum for 2 hours to remove the last traces of the solvent. The ethanol extract thus obtained (24.5 g) was fractionated in to four fractions by maceration with hexane, chloroform and n-butanol to get hexane, chloroform, n-butanol soluble and n-butanol insoluble fractions. All these extract and fractions were screened for antidyslipidemic activity. The ethanol extract showed potent antidyslipidemic activity, which was localized in hexane fraction only. The major compound eugenol[10] was identified by LCMS in this fraction, which is known in this plant also. This compound was purchased from Sigma Chemical Company St. Luis, MO, USA. and was screened for lipid lowering and antioxidant activities, Promising antidyslipidemic as well as antioxidant activities were shown by this compound.

### Bioassay methodology

Drugs and Standards All chemicals were procured from Sigma Chemical Company, St. Luis, MO, USA.

### Animal model

In vivo experiments were conducted as per the guidelines provided by the animal ethics committee of the Central Drug Research Institute, Lucknow, India. Adult male Charles foster rats (100–120 g) bred in the animal house of the Institute were used. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26ºC, relative humidity 50-70% and 12/12 hr. light /dark cycle.

### Biochemical analysis of plasma/serum

Plasma and lipoproteins were analyzed for their TC, PL and TG by standard procedures reported earlier[11].Plasma LCAT activity was measured using method reported earlier[12].

### Antioxidant activity

Generation of Superoxide anions ($O2^{-}$) (*in vitro*) was measured in an enzymatic system composed of Xanthine (0.122 mg/ml), Xanthine oxidase (12 µl/ ml) and nitrobluetetrazolium (NBT) (0.74 mg/ml) with or without addition of plant products (200 µg / ml)[11]. The amount of formazone formed as a result of reduction of NBT by $O2^{-}$ was measured at 560 nm on spectrophotometer. The in vitro system employed for nonenzymatic generation of $O2^{-}$ comprised of phenazine methosulphate (PMS) (0.28 mg/ml), NADH (1.65 mg/ml) and NBT (1.286 mg/ml) in the absence or presence of plant products (200 µg/ml). After the incubation the amount of formazone formed was measured as above[12]. The in vitro generation of Hydroxyl free radicals and effect of plant products on the formation of hydroxyl free radicals ($OH^{\bullet}$) was measured in an enzymatic system composed of hypoxanthine (0.4 mM), FeSO4·7H2O (Fe+2), sodium salicylate (5 mM) and xanthine oxidase (0.07 units), 3,4-dihydroxybenzoate formed by $OH^{\bullet}$ mediated hydroxylation of salicylate was measured as reported by Richmond et al[13].

## DISCUSSION

The antidyslipidemic activity of the ethanol extract, fractions and the pure compounds of the *Ocimum santum* leaves were evaluated in *in vivo* Triton model[14]. Administration of Triton WR-1339 in rats induced marked hyperlipidemia as evidenced by an increase of the level of TC, PL and TG in the plasma as compared to the control. Treatment of the hyperlipidemic rats with extract, fractions and pure compound eugenol at a dose of 100 mg/kg p.o. reversed the plasma levels of lipid with varying extents (TABLE 1). Ethanol extract and eugenol significantly lowered the TC, PL, and TG, while others showed mild activity. By comparison of data with data of the standard drug gemfibrozil at the same dose decreased the levels of TC, PL, and TG in plasma. With these promising results in hand, a dose-dependent (at 50, 100, 150 mg/kg body weight) studies on ethanol extract and eugenol were performed on a different set of experimental animals, which exhibited dose-dependent effects. Both ethanol extract and eugenol showed similar activity patterns, and at a dose of 150 mg/kg body weight. The ethanol extract and eugenol showed activities comparable to those of the standard drug gemfibrozil (TABLE 2).

It is known that the LCAT enzyme converts free

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cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is subsequently converted into HDL. The ethanol extract and eugenol showed this beneficiary effect as each significantly increased the level of LCAT enzyme in Triton treated hyperlipidemic rats at dose of 100 mg/kg. Furthermore, both ethanol extract and eugenol also increased post heparin lipolytic activities as shown in TABLE 2.

**TABLE 1 : Effect of compound on plasma lipid profile of Triton induced hyperlipidemic rats.**

| Animal groups | TC *(mg/dl)* | PL *(mg/dl)* | TG *(mg/dl)* |
|---|---|---|---|
| Control | 84.37±6.92 | 80.47±6.12 | 86.28±8.00 |
| Triton | 300.62±25.17 $^{c}$(+3.56 F) | 240.61±18.64 (+2.99 F) | 270.17±17.30 (+3.13 F) |
| Triton+EtOH ext. (100mg/kg) | 219.45±1.63 (-27)*** | 180.45±13.00 (-25)*** | 202.62±18.93 (-25)*** |
| Triton+Hexane fr. (100mg/kg) | 266.21±24.00 (-11)* | 211.11±17.00 (-12)* | 250.44±20.70 (-7) $^{NS}$ |
| Triton+$CHCl_3$ fr. (100mg/kg) | 260.52±23.07 (-13)* | 216.66±17.17 (-10)* | 240.14±23.10 (-11)* |
| Triton+Eugenol (100mg/kg) | 234.48±16.77 (-22)*** | 185.26±12.60 (-23)*** | 202.60±17.17 (-25)*** |
| Triton+Qercetin (100mg/kg) | 260.38±22.77 (-13)* | 210.75±13.70 (-12)* | 225.55±23.00 (-17)* |
| Triton+ Gemfibrozil (100mg/kg) | 190.17±16.77 (-36)*** | 155.10±12.14 (-35)*** | 175.18±13.00 (-35)*** |

**Each parameter represents pooled data from 6 rats/group and values are expressed as mean±S.D. $^{c}P < 0.001$, Triton treated group compared with control group and $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ Triton plus compounds groups compared with Triton treated group only; NOTE: NS (non significant) and F (Fold change over control group).**

**TABLE 2 : Dose dependent study of active compound from *Ocimum sanctum* on the plasma lipid profile of Triton induced hyperlipidemic rats.**

| | Lipid profile | | |
|---|---|---|---|
| **Animal groups** | **TC *(mg/dl)*** | **PL *(mg/dl)*** | **TG *(mg/dl)*** |
| Control | 84.37±6.92 | 80.47±6.12 | 86.28±8.00 |
| Triton | 300.60±25.16 $^{c}$ (+3.56 F) | 240.60±18.63 $^{c}$ (+2.99 F) | 270.15±17.31 $^{c}$ (+3.13 F) |
| **Triton+ EtOH ext.** | | | |
| 50mg/kg | 249.49±19.42** (-17) | 199.69±18.88** (-17) | 221.52±18.82** (-18) |
| 100mg/kg | 219.43±21.06*** (-27) | 180.45±11.26*** (-25) | 202.61±12.41*** (-25) |
| 150mg/kg | 216.43±12.54*** (-28) | 178.04±15.92*** (-26) | 202.63±15.92*** (-25) |
| **Triton+Eugenol** | | | |
| 50mg/kg | 258.51±21.04** (-14) | 204.51±19.62* (-15) | 224.22±18.66** (-17) |
| 100 mg/kg | 234.46±17.28*** (-22) | 185.26±13.81*** (-23) | 202.59±10.52*** (-25) |
| 150 mg/kg | 234.43±20.08*** (-22) | 182.26±17.35*** (-24) | 201.88±18.28*** (-25) |
| **Triton+Gemfibrozil** | | | |
| (100mg/kg) | 179.65±15.36*** (-40) | 164.14±14.82*** (-32) | 162.81±14.84*** (-40) |

**Each parameter represents pooled data from 6 rats/group and values are expressed as mean±S.D. $^{c}P < 0.001$, Triton treated group compared with control group and $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ Triton plus compounds groups compared with Triton treated group only; NOTE: NS (non significant) and F (Fold change over control group).**

## RESULTS

### Effect of ethanol extract, fraction and pure compound on lipid profile of Triton induced hyperlipidemic rats

As shown in TABLE 1, the lipid lowering activity of the *Ocimum sanctum* was evaluated in an *in vivo* experiment in Triton model. Administration of Triton WR-1339 in rats induced marked hyperlipidemia as evidenced by an increase of the total cholesterol (TC, 3.56-fold), phospholipids (PL, 2.99-fold), and triglyceride (TG, 3.33-fold) in the plasma level as compared to the control. Treatment of hyperlipidemic rats with the crude extract at a dose of 100 mg/kg b.w per oral reversed the plasma levels of lipid with varying extents (TABLE 1). Ethanol extract and eugenol significantly lowered the TC, PL, and TG by 27%, 25%, and 25%, and 22%, 23%, and 25% respectively, while fractions hexane and chloroform and pure compound quercetin showed mild activity, by comparison of the standard drug gemfibrozil. Gemfibrozil at the same dose level decreased the levels of TC, PL, and TG in plasma by 36%, 35%, and 35%, respectively.

### Effect ethanol extract and eugenol at different doses on lipid profile of the Triton induced hyperlipidemic rats

The analysis of the lipid profile data (TC, PL TG, HDL-C ratio and HDL-C/TC) at different doses (50, 100, 150 mg/kg b.w) suggested that the ethanol extract and eugenol exerted hypolipidemic activity in Triton induced hyperlipidemic rats. The ethanol extract and eugenol showed potent activity at dose of 100 mg/kg b.w. This dose significantly lowered the plasma TC by

27% and 25%, PL by 25% and 22%, TG by 23% and 25%, respectively (TABLE 2).

**TABLE 3 : PHLA and LCAT activity of the *Ocimum sanctum* active compounds at it active dose in Triton induce hyperlipidemic rats.**

| Animal groups | Activity | PHLA *(n mole free fatty acid released/h/L plasma)* | LCAT *(n mole cholesterol released/h/L plasma)* |
|---|---|---|---|
| Control | | 37.52±3.58 | 62.24±5.82 |
| Triton | | 18.64±1.28 $^{C}$ (-51%) | 37.86±2.882.99 $^{C}$ (-67%) |
| Triton+ EtOH extract (100mg/kg) | | 24.60±2.28*** (+33%) | 54.89±4.32*** (+31%) |
| Triton+ Eugenol (50mg/kg) | | 24.23± 1.88*** (+33%) | 53.76±4.84*** (+30%) |
| Triton+ Gemfibrozil (100mg/kg) | | 24.79± 2.42*** (+33%) | 57.54± 5.54*** (+35%) |

**Each parameter represents pooled data from 6 rats/group and values are expressed as mean±S.D. $^{c}P < 0.001$, Triton treated group compared with control group and $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ Triton plus compounds groups compared with Triton treated group only.**

**TABLE 4 : Effect of ethanol extract/fractions / compoundsof the *Ocimum sanctum* on superoxide anions, hydroxyl radicals and lipid-peroxidation.**

| Series of compounds | Dose (µg/ml) | Superoxide anions (%) *(n mol formazone formed/minute)* | Hydroxyl radicals (%) *(n mol MDA formed/h/mg protein)* | Lipid-peroxidation (%) *(n mol MDA formed/h/mg protein)* |
|---|---|---|---|---|
| EtOH ext. | 200 | -25*** | -22*** | -26*** |
| Hexane fraction | 200 | -10* | -8 $^{NS}$ | -10* |
| $CHCl_3$ fraction | 200 | -15* | -12* | -13* |
| Eugenol | 200 | -32*** | -27*** | -36*** |
| Quercetin | 200 | -11* | -9 $^{NS}$ | -9 $^{NS}$ |
| Standards | 200 | -63*** (Allopurinol) | -51*** (Manitol) | -65*** (α-tocopherol) |

**Each value is mean±SD of six values, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ experimental values compared with control values; NOTE: NS (non significant).**

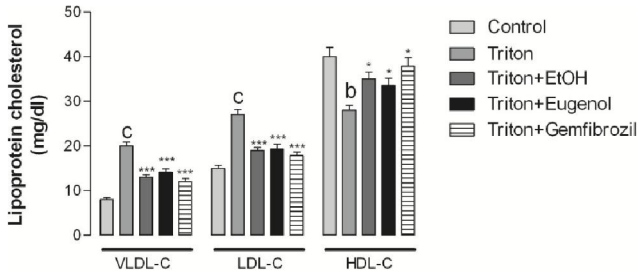


**Figure 1 : EtOH extract and eugenol (100mg/kg) improves the high density lipoprotein-cholesterol ratio in Triton induced hyperlipidemic rats. Each parameter represents pooled data from 6 rats/group and values are expressed as mean±S.D. $^{***}P < 0.001$; $^{**}P < 0.01$ Triton treated, Triton plus EtOH extract, Triton plus Eugenol and Triton plus gemfibrozil groups, compared with control group and $^{b}P < 0.01$; $^{c}P < 0.001$ Triton plus EtOH extract, Triton plus Eugenol and Triton plus gemfibrozil (100mg/kg) groups compared with Triton treated group only.**

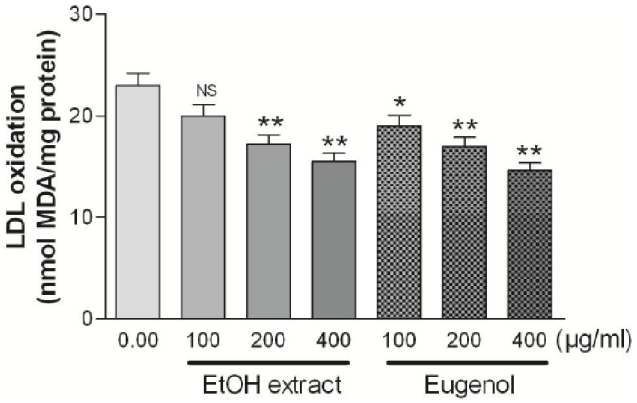


**Figure 2 : EtOH extract and eugenol reduced the LDL-oxidation in dose dependent in normal blood sample. Data expressed as mean ±S.D. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ and NS is non significant as compared to treated values with untreated values and each bar comparison with its corresponding higher dose.**

## The ethanol extract and eugenol reactivates the PHLA and LCAT activity in Triton induced hyperlipidemic rats

Administration of Triton in the experimental rats caused decrease in PHLA and LCAT activities by 51% and 67%, the ethanol extract and eugenol at the dose of 100mg/kg b.w. reactivates the activity of PHLA by 33% and 33%. Similarly LCAT activity increased by 31% and 30% respectively (TABLE 3).

## Effect of ethanol extract, fraction and compound on generation of superoxide anions, hydroxyl radicals and microsomal lipid peroxidation in vitro sys-

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**tem of the antioxidant activity**

The antioxidant activities of Plant extraction, fraction and compound were evaluated by generating free radicals in vitro in the absence and presence of these plant derivatives. The scavenging potential of plant derivatives at 200 µg/mL against formation of $O^{-2}$ and $OH^{\cdot}$ in nonenzymic systems was studied. Further, their effects on lipid peroxidation in microsomes were also studied (TABLE 4). The ethanol extract and eugenol significantly decreased the superoxide anions inhibition by 25% and 32%, hydroxyl radicals inhibition by 22% and 27% and microsomal lipid peroxidation inhibition by 26% and 36%, respectively (each value is mean ± SD of six values $P < 0.001$). The standard drug Allopurinol showed 63% inhibition in superoxide anions at 200 µg/ml. Mannitol and a-tocopherol showed 51% and 65% inhibition of hydroxyl ions and microsomal lipid peroxidation at the dose of 200 µg/ml respectively.

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