Comparative effect of *Ocimum sanctum, Commiphora mukul*, folic acid and ramipril on lipid peroxidation in experimentally-induced hyperlipidemia

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Treatment with *C. mukul* and *O. sanctum*, showed a significant decrease in cholesterol and triglyceride levels respectively. *O. sanctum* also significantly increased serum HDL-cholesterol compared to control. Serum MDA levels were significantly reduced in all the treated groups compared to control suggesting that each of the drugs under study were effective in their free radical scavenging action. Erythrocyte SOD activity was increased in all the treatment groups with *C. mukul* showing the maximum effect followed by *O. sanctum*, folic acid and ramipril. The erythrocyte CAT activity was significantly increased in all the drug treated groups with maximum increase seen in *O. sanctum* and ramipril treated groups, whereas lesser effects were observed with *C. mukul* and folic acid groups. Thus, the indigenous drugs, *C. mukul* and *O. sanctum* had beneficial effect on hypercholesterolemic rabbit model, both in terms of lipid profile as well as antioxidant potential. *Ocimum sanctum* was found to be the most promising of all the drugs. Moreover, it could be hypothesized that these plant products along with folic acid and ramipril can be explored for synergistic effect for treatment for hypercholesterolemic conditions.

**Keywords:** Antioxidant, *Commiphora mukul*, Folic acid, Hypercholesterolemic, *Ocimum sanctum*, Ramipril

Dyslipidemia is an important risk factor in the initiation and progression of atherosclerotic lesions. The beneficial effect of lowering elevated serum cholesterol level in prevention of coronary heart disease is well established. There are evidences that free radical induced oxidation of lipoproteins is an important event in the pathogenesis of atherosclerosis. This association has been explained based on the “oxidative modification” hypothesis of atherosclerosis, which proposes that atherosclerosis is initiated by oxidation of lipids in low density lipoproteins (LDL). As a corollary to this hypothesis, antioxidants that inhibit lipid peroxidation, should limit atherosclerosis and its clinical manifestation. Hence, the choice of an ideal hypolipidemic drug would depend on their antioxidant potential as well. The present study was undertaken to compare effects of *Ocimum sanctum* L., *Commiphora mukul* (Stocks) Hook. along with folic acid and ramipril, on lipid peroxidation in experimentally-induced hyperlipidemia.

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*Ocimum sanctum* (English: Holy basil; Sanskrit: Tulsi; Family: Labiatae) and *Commiphora mukul* (Balsamodendran mukul; Hook-family: Burseraceae) are well known traditional medicinal plants used in the Ayurveda system of medicine. The leaf extract of *O. sanctum* has potent free radical scavenging activity in vitro and antilipid-peroxidation activity in vivo. The aqueous extract of leaves of *O. sanctum* administered intraperitoneally has been shown to increase cellular glutathione, antioxidant enzymes like glutathione transerase, glutathione reductase, glutathione peroxidase as well as superoxide dismutase (SOD).

In various experimental and human studies, *Commiphora mukul* has also shown to decrease atherosclerosis, lower serum cholesterol and triglycerides, and also increase high-density lipoproteins (HDL) cholesterol.

Folic acid, a water-soluble vitamin of the B-complex group when supplemented can improve endothelial function in patients with familial hypercholesterolemia and normal homocysteine levels and also in healthy subjects with hyperhomocysteinemia. Studies have indicated a protective effect of folic acid against oxidative stress
produced in 21-day postpartum rats by chronic maternal ethanol consumption during pregnancy and lactation period\textsuperscript{15}.

Ramilpril is a long-acting, lipophilic angiotensin converting enzyme inhibitor (ACEI), preventing the conversion of angiotensin-I to angiotensin-II. Angiotensin-II enhances the scavenger receptor affinity to ox-LDL and also increases the number of scavenger receptors on macrophages\textsuperscript{16}. In humans too, angiotensin-II binds to LDL in both in vivo and as well as in vitro\textsuperscript{16}. Angiotensin-II can modify native LDL into a modified lipoprotein (Ang-II-LDL) that is taken up at enhanced rate by macrophages. These effects appear to be the first stages of atherogenesis. Thus, ACEIs have been shown to increase enzymatic and non-enzymatic antioxidant defenses in vitro and in vivo in human and animal studies\textsuperscript{17-20}.

Since lipid peroxidation in addition to high level of serum lipids in blood is known to play a role in the development of atherosclerosis, so there is a continuous search to find lipid lowering drugs with antioxidant potential. Therefore, the present study was undertaken to compare and analyze the antioxidant effects of these four drugs and also their effects on the lipid profile in experimentally-induced hyperlipidemia in rabbits.

**Materials and Methods**

*Animals*—Male albino rabbits (weighing 1.5-2 kg) were obtained from the Central Animal House, University College of Medical Sciences, Delhi. Animals were housed under standard laboratory condition of 12 h light/dark cycle at 22\textdegree±2\textdegree C, given standard rabbit feed and water ad libitum. Care of animals was taken as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and in accordance to the ethics prepared by INSA, Animal Welfare Division of the Ministry of Environment & Forest, Council of International Environment & Forest, Council of International Population Studies and Training, Government of India and were approved by the Ethics Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

*Plant material*—The dried leaves of *Ocimum tenuiflorum* L. (syn *O. sanctum*) were collected from New Delhi, India and authenticated in the Department of Genetics, Indian Council of Agricultural Research, Pusa, New Delhi, India. The gum resin of *Commiphora wightii* (syn. *C. mukul*) (Am.) Bhandari was obtained from Cipla, Mumbai.

**Drugs and dose schedule**—Aqueous extract of *O. sanctum* was prepared by the procedure as described earlier\textsuperscript{7}. The extract was lyophilized to form a powder, which was reconstituted in distilled water to feed the rabbits at a dose of 100 mg/kg/day for 6 weeks, by intragastric feeding. The selection of dose was based on previous studies\textsuperscript{21,22}. Alcoholic extract of *C. mukul* was prepared\textsuperscript{23} using ethanol (95%), lyophilized in lyophiliser and concentrated in vacuum to dryness. A yellow powder was obtained and was reconstituted in distilled water and administered at a dose of 100 mg/kg/day for 6 weeks. Ramipril [obtained from Aventis, India] was suspended in distilled water and administered (0.6 mg/kg/day) orally for 6 weeks. Folic acid [obtained from Cipla, India] was suspended in distilled water and administered (0.5 mg/kg/day) orally for 6 weeks.

*Induction of hypercholesterolemia*—Rabbits were subjected to hypercholesterolemia\textsuperscript{24} by administering cholesterol (obtained from Loba Chemie, India) at a dose of 500 mg/animal/day prepared in 10 ml of 1% starch mucilage for 8 weeks.

*Blood collection and preparation*—After respective treatment, blood samples were drawn from the marginal ear vein of overnight fasted rabbits. Blood was allowed to clot and the serum was centrifuged at 3000 rpm for 5 min and used for estimation of serum lipids\textsuperscript{25-27} and malondialdehyde (MDA)\textsuperscript{28}.

Another sample of heparinised blood (250 units of heparin in 5 ml blood) was also collected and centrifuged at 500 rpm for 10 min. Then, RBC (0.5 ml) were taken and washed with normal saline (3 ml) three times and added cold distilled water (1.5 ml) in the test tube. Clear red haemolysate was obtained, and utilized for the estimation of SOD\textsuperscript{29} and catalase (CAT)\textsuperscript{30} activities.

**Biochemical estimation of serum lipid profile**—Biochemical estimation kits (Merck, USA) were used for the photometric estimation of total cholesterol, HDL-cholesterol and triglycerides. Cholesterol was estimated using CHOD-PAP method\textsuperscript{25} whereas triglycerides were measured by GPO-PAP method\textsuperscript{26,27}.

*Estimation of hemoglobin (Hb) in erythrocyte hemolysate*—The amount of hemoglobin in erythrocyte hemolysate was determined by cyanmethemoglobin method\textsuperscript{31}.

*Estimation of parameters of oxidative stress*—Serum MDA levels as lipid peroxides were estimated as described earlier\textsuperscript{28}, erythrocyte SOD activity\textsuperscript{29}, and erythrocyte CAT activity\textsuperscript{30} were estimated as described earlier.
Results

Effect of drugs on lipid profile—Administration of cholesterol for 8 weeks raised the total cholesterol and triglycerides, and decreased HDL levels significantly in all the groups compared to initial levels at 0 week. Thereafter, drug administration for 6 weeks reduced the cholesterol levels significantly in C. mukul, O. sanctum and folic acid treated groups, reduced triglyceride levels in C. mukul and O. sanctum treated groups and raised HDL levels in O. sanctum treated group compared to their levels at 8 weeks. In addition, at 14 weeks, C. mukul and O. sanctum caused reduction in total cholesterol. Ramipril, C. mukul and O. sanctum caused reduction in triglycerides and C. mukul increased the HDL levels significantly compared to control (Tables 1-3). Maximum reduction in total cholesterol (about 70%) and triglycerides (about 40%) as compared to controls was observed in animals treated with O. sanctum at 14 weeks.

Effect of drugs on serum MDA levels, SOD and CAT activity—Administration of cholesterol for 8 weeks reduced SOD and CAT activities and raised MDA levels significantly in all the groups as compared to their initial levels at 0 week. Thereafter, drug administration for 6 weeks raised the SOD and CAT activities significantly in all the drug treated groups compared to their levels at 8 weeks. In addition, at 14 weeks, all the drug treated groups caused significant increase in SOD and CAT activities and reduction in MDA levels as compared to control (Tables 4-6). Maximum increase in SOD activity as compared to controls was observed in animals treated with C. mukul and MDA was reduced by 30%.

Discussion

Hypercholesterolemia is known to induce oxidative stress manifested by increase in MDA levels. We found an increase in serum MDA levels in the hypercholesterolemic rabbits (Table 5) similar to the observations of Cavalea and co-workers.

Serum MDA levels were significantly reduced in all the treatment groups compared to control. This suggested that each of the drugs under study were effective in reducing oxidative stress.
effective in their free radical scavenging action. Erythrocyte SOD activity was increased in all the treatment groups indicating a positive influence on the enzymatic antioxidant defense in hypercholesterolemic animal. *C. mukul* showed the maximum effect followed by *O. sanctum*, folic acid and ramipril. The erythrocyte CAT activity was significantly increased in all the drug treated groups with maximum increase seen in *O. sanctum* and ramipril treated groups, whereas lesser effects were observed with *C. mukul* and folic acid groups.

Recent studies have also suggested acute antioxidant effect of folic acid independent of its effect on homocysteine concentrations\(^{34}\). Increase in SOD and CAT activity as shown by folic acid has also been shown by Cano et al.\(^{35}\). Folic acid was shown to reduce endothelial NO synthase (eNOS) and xanthine oxidase (XO)-induced superoxide generation in vitro in patients with hypercholesterolemia, thus suggesting a free radical scavenging action and a role in reversing the derangement in NO metabolism that occurs in hypercholesterolemia\(^{34,35}\). Thus, folic acid may have important clinical implications in hypercholesterolemia.

Ramipril, a non-sulphydryl containing ACEI also inhibited lipid peroxidation as evidenced by reduction in MDA levels and elevation in SOD and CAT activities after chronic ramipril administration. Earlier, fosinopril, another non-sulphydryl containing ACEI also showed significant increase in HDL-cholesterol and triglyceride levels and *O. sanctum* showed a significant decrease in cholesterol and triglyceride levels and *O. sanctum* also showed significant increase in HDL-cholesterol compared to control.

Recently, different fractions of gum guggul (*C. mukul*) have been isolated. Of these, fraction A contains ketonic steroid compounds, guggulsterone-Z and guggulsterone-E (Fig. 1A) responsible for its hypolipidemic property\(^{37,39}\). Guggulsterones in the body are easily reduced into guggulsterols which behave as powerful antioxidants\(^{40}\). Our study showed significant increase in SOD and CAT activity on *C. mukul* administration which further strengthened the earlier results. These sterones have a steroid-like structure and behave as powerful antioxidants\(^{41}\).

Table 4—Effect of *Ocimum sanctum*, *Commiphora mukul*, folic acid and ramipril on erythrocyte superoxide dismutase (SOD) activity

<table>
<thead>
<tr>
<th>Drugs (mg/kg/day)</th>
<th>SOD activity (U/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 week</td>
</tr>
<tr>
<td>Control</td>
<td>1876.46±17.26</td>
</tr>
<tr>
<td>Ramipril (0.6)</td>
<td>1497.73±20.24</td>
</tr>
<tr>
<td>Folic acid (0.5)</td>
<td>2046.38±27.04</td>
</tr>
<tr>
<td><em>C. mukul</em> (100)</td>
<td>2384.37±43.10</td>
</tr>
<tr>
<td><em>O. sanctum</em> (100)</td>
<td>2395.52±29.11</td>
</tr>
</tbody>
</table>

P values: a<0.001 as compared to control at 0 week; b<0.001 as compared to ramipril at 0 week; c<0.001 as compared to folic acid at 0 week; d<0.001 as compared to *C. mukul* at 0 week; e<0.001 as compared to *O. sanctum* at 0 week; f<0.001 as compared to ramipril at 8 week; g<0.001 as compared to folic acid at 8 week; h<0.001 as compared to *C. mukul* at 8 week; i<0.001 as compared to *O. sanctum* at 8 week; j<0.001 as compared to control at 14 week.

Table 5—Effect of *Ocimum sanctum*, *Commiphora mukul*, folic acid and ramipril on serum malondialdehyde (MDA) levels

<table>
<thead>
<tr>
<th>Drugs (mg/kg/day)</th>
<th>0 week</th>
<th>8 weeks</th>
<th>14 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.41±0.04</td>
<td>2.67±0.04</td>
<td>2.52±0.03</td>
</tr>
<tr>
<td>Ramipril (0.6)</td>
<td>1.12±0.04</td>
<td>2.69±0.23</td>
<td>1.66±0.03</td>
</tr>
<tr>
<td>Folic acid (0.5)</td>
<td>1.45±0.03</td>
<td>2.64±0.04</td>
<td>1.75±0.02</td>
</tr>
<tr>
<td><em>C. mukul</em> (100)</td>
<td>1.44±0.04</td>
<td>2.70±0.05</td>
<td>1.76±0.03</td>
</tr>
<tr>
<td><em>O. sanctum</em> (100)</td>
<td>1.39±0.03</td>
<td>2.67±0.03</td>
<td>1.58±0.02</td>
</tr>
</tbody>
</table>

P values: a<0.001 as compared to control at 0 week; b<0.001 as compared to ramipril at 0 week; c<0.001 as compared to folic acid at 0 week; d<0.001 as compared to *C. mukul* at 0 week; e<0.001 as compared to *O. sanctum* at 0 week; f<0.001 as compared to ramipril at 8 week; g<0.001 as compared to folic acid at 8 week; h<0.001 as compared to *C. mukul* at 8 week; i<0.001 as compared to *O. sanctum* at 8 week; j<0.001 as compared to control at 14 week.

Table 6—Effect of *Ocimum sanctum*, *Commiphora mukul*, folic acid and ramipril on serum catalase (CAT) activity

<table>
<thead>
<tr>
<th>Drugs (mg/kg/day)</th>
<th>0 week</th>
<th>8 weeks</th>
<th>14 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.79±0.08</td>
<td>2.84±0.01</td>
<td>2.85±0.01</td>
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<tr>
<td>Ramipril (0.6)</td>
<td>3.51±0.05</td>
<td>2.83±0.03</td>
<td>3.43±0.01</td>
</tr>
<tr>
<td>Folic acid (0.5)</td>
<td>3.26±0.04</td>
<td>2.83±0.00</td>
<td>3.04±0.01</td>
</tr>
<tr>
<td><em>C. mukul</em> (100)</td>
<td>3.34±0.01</td>
<td>2.84±0.01</td>
<td>3.23±0.01</td>
</tr>
<tr>
<td><em>O. sanctum</em> (100)</td>
<td>3.78±0.02</td>
<td>2.87±0.00</td>
<td>3.52±0.01</td>
</tr>
</tbody>
</table>

P values: a<0.001 as compared to control at 0 week; b<0.001 as compared to ramipril at 0 week; c<0.001 as compared to folic acid at 0 week; d<0.001 as compared to *C. mukul* at 0 week; e<0.001 as compared to *O. sanctum* at 0 week; f<0.001 as compared to ramipril at 8 week; g<0.001 as compared to folic acid at 8 week; h<0.001 as compared to *C. mukul* at 8 week; i<0.001 as compared to *O. sanctum* at 8 week; j<0.001 as compared to control at 14 week.
Recent chromatographic studies have shown (GSH) levels with leaf extract of *O. sanctum* on the basis of evidence of increased glutathione oxidized myocardial infarction in experimentally has been inhibited lipid peroxidation in alcoholic extract of *O. sanctum* extract.* Earlier reports have suggested that aqueous leaf structure with H, CH₃ and O bonds indicating that the drug may quench free radicals due to its antioxidant effect. This study gives valuable evidence suggesting the role of guggulsterol as a hypolipidemic due to its antioxidant action.

The aqueous extract of *Ocimum sanctum*, was seen to reduce total cholesterol, and triglycerides, and increase serum HDL-cholesterol levels as compared to control. Sarkar *et al.* have also reported similar results. A reduction of serum MDA on treatment with *O. sanctum* extract was observed in our study, thereby suggesting free radical scavenging action. A methanol extract and aqueous suspension of *O. sanctum* leaves have been found to have anti-inflammatory, analgesic and immunostimulatory properties. *O. sanctum* seed oil has also been shown to exhibit antidiabetic, antihypercholesterolemic and antioxidant effects.

Earlier reports have suggested that aqueous leaf extract of *O. sanctum* (50 mg/kg) protected against radiation induced lipid peroxidation in mice. The alcoholic extract of *O. sanctum* has been found to inhibit lipid peroxidation in experimentally has been induced myocardial infarction. We found that *O. sanctum* showed significant increase in erythrocyte SOD and CAT activities. SOD enzyme is the first line of defense against superoxide free radicals involved in myocardial cell damage. Thus, increased SOD activity may indicate protection of myocardial cell function. Increased CAT activity can be explained on the basis of evidence of increased glutathione (GSH) levels with leaf extract of *O. sanctum* and previous knowledge that both CAT and GSH subserves similar function of scavenging hydrogen peroxide.

Recent chromatographic studies have shown *Ocimum sanctum* to contain various active constituents viz. eugenol, luteolin, ursoic acid, and oleanolic acid, among which eugenol content ranged from 0.175 to 0.362% (w/w). Eugenol (1-hydroxy-2-methoxy-4-allylbenzene; Fig. 1B), the active constituent present in *Ocimum sanctum* L., has been found to be largely responsible for the therapeutic potentials of Tulsi. Since the active component of *O. sanctum* is eugenol, it can be presumed that the above results could be due to the antioxidant potential of eugenol.

Thus, hypercholesterolemia and increased oxidative stress are the two major contributors to cardiovascular morbidity. The treatment of coronary artery disease by drugs with hypolipidemic and antioxidant potential may be the most effective solution to this growing pandemic. In our study, we found that the indigenous drugs, *C. mukul* and *O. sanctum* had beneficial effect on hypercholesterolemic rabbit model, both in terms of lipid profile as well as antioxidant potential. Folic acid improved the antioxidant status without affecting the lipid profile, whereas ramipril lowered the triglyceride levels in addition to its antioxidant effect. *Ocimum sanctum* has emerged as the most promising of all the drugs evaluated in our study, having a favorable effect on both the antioxidant status and lipid profile by augmenting HDL cholesterol as well. The possible synergistic effect of these drugs as cardioprotective agents is the subject of further study.

**References**


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