INTRODUCTION

Lipoproteins become particularly susceptible to oxidative modification, giving rise to hydroperoxidase, lysophospholipids, oxysterols and aldehyde breakdown products of fatty acids and phospholipids. High density lipoproteins (HDL) particles modified by HOCI-mediated chlorination function poorly as cholesterol acceptors, a finding that links oxidative stress with impaired reverse cholesterol transport, which is one likely mechanism of the antiatherosclerotic action of HDL [1]. Increased intracellular generation of reactive oxygen species (ROS) plays an important role in chronic inflammatory responses to atherosclerosis [2]. ROS are generated in aerobic organisms during physiological or physiopathological oxidative metabolism of mitochondria. ROS may react with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue, thereby interfering with cell function. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases [3]. The production of free oxidative radicals may induce endothelial dysfunction which predisposes long term atherosclerotic lesions and proposed as an important diagnostic and prognostic factor for coronary syndromes [4]. Main sources of oxidative substances and ROS in atherosclerotic vessels are macrophages and smooth muscle cells. Reactive oxygen species are increasingly recognized as the major factor responsible for compromising endothelial cell function [5]. Hypercholesterolemia stimulates the production of superoxide anion radicals (O2-) from the smooth muscle cells of vessels that lead to increased oxidation of LDL. The increased production of ROS reduces the production of NO, leading to vasoconstriction, platelet aggregation and adhesion of neutrophils to endothelium [6]. Oxidative stress by hydrogen peroxide (H2O2) increases phosphorylation of tyrosin kinases leading to stronger binding of neutrophil cells on endothelium and alteration of vessel permeability [7]. LDL can be oxidatively modified by all major cells of the arterial wall[8]. Role of lipid oxidation in the development of atherosclerotic lesions is its ability to modify LDL sufficiently to promote its uptake by macrophages. Oxidized LDL is taken up by the scavenger receptor pathway in macrophages leading to appreciable cholesterol ester accumulation and foam cell formation [9].

MATERIALS AND METHODS

This study was undertaken at the Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India. Animals were housed in polypropylene cages [28 cm x 22 cm x 14 cm] bedded with husk in groups of six under controlled environmental conditions [Temp-23±2 °C, Humidity 65-70% and 12 hrs light/dark cycles] at Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. Animal Ethical Committee of Rajah Muthaiah Medical College and Hospital [Registration No.160/1999/CPSEA] approved the study. All studies were conducted in accordance with the National Institute of Health "Guide for the care and use of Laboratory Animals" (NIH, 1985). The study was approved by the Animal Ethical Committee of Rajah Muthaiah Medical College and Hospital [Registration No.160/1999/CPSEA]. Male Wistar rats of Wistar strain weighing 150-200 gms were used in the present study. They were purchased from the Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India. Animals were housed in polypropylene cages [28 cm x 22 cm x 14 cm] bedded with husk in groups of six under controlled environmental conditions [Temp-23±2 °C, Humidity 65-70% and 12 hrs light/dark cycles] at Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet [VRK Nutritional Solutions, Baramati Agro Limited, Sangli, Maharashtra, India] and water ad libitum. Hypercholesterolemia was induced in all groups except control group by feeding cholesterol diet (1% cholesterol, 0.5% sodium cholate, 1% coconut oil) in rat chow [10].

Study design

The rats were divided into 6 groups of six each (n=6).

GROUP I (n=6) = Control.

GROUP II (n=6) = High cholesterol diet.

GROUP III (n=6) = Atorvastatin 5 mg/kg with the high cholesterol diet.

GROUP IV (n=6) = Lutein 25 mg/kg with the high cholesterol diet.

GROUP V (n=6) = Lutein 50 mg/kg with cholesterol diet.

GROUP VI (n=6) = Lutein 100 mg/kg with cholesterol diet.
GROUP V (n=6) = Lutein 50 mg/kg with the high cholesterol diet.

GROUP VI (n=6) = Lutein 100 mg/kg with high cholesterol diet [11]

The drug treatment was carried out every day morning using intragastric tube for 16 weeks.

Tissue sampling

The animals were sacrificed by cervical dislocation and the Liver from all the groups of rats were dissected out. They were processed for tissue antioxidant study.

Table 1: Effect of lutein on TBARS and antioxidants in plasma of Hypercholesterolemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tbars (nmol/ml)</th>
<th>SOD (NBT/min/mg Hb)</th>
<th>Catalase (Mmol/min/mg Hb)</th>
<th>Gpx (Mmol)</th>
<th>GSH (Mmol)</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>2.07±0.03 a</td>
<td>6.93±0.04 a</td>
<td>192.26±0.91 a</td>
<td>17.11±0.04 a</td>
<td>32.58±0.09 a</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>6.52±0.02 b</td>
<td>5.13±0.02 b</td>
<td>156.04±0.40 b</td>
<td>10.91±0.04 b</td>
<td>25.94±0.20 b</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>5.07±0.04 c</td>
<td>6.05±0.02 c</td>
<td>163.14±0.65 c</td>
<td>13.11±0.03 c</td>
<td>29.90±0.05 c</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>5.74±0.01 d</td>
<td>6.45±0.02 d</td>
<td>161.84±0.22 d</td>
<td>14.19±0.04 d</td>
<td>30.23±0.05 d</td>
<td></td>
</tr>
<tr>
<td>Group V</td>
<td>4.03±0.03 e</td>
<td>6.60±0.03 e</td>
<td>174.00±0.18 e</td>
<td>15.50±0.06 e</td>
<td>31.20±0.03 e</td>
<td></td>
</tr>
<tr>
<td>Group VI</td>
<td>3.09±0.02 f</td>
<td>6.83±0.02 f</td>
<td>183.04±0.15 f</td>
<td>16.40±0.02 f</td>
<td>32.08±0.04 f</td>
<td></td>
</tr>
</tbody>
</table>

Legend: 1Values are expressed as Means±SD for six in each group. Values not sharing a common superscript differ significantly at p<0.05.

Table 2: Effect of Lutein on Malondialdehyde and Antioxidants in Liver of Hypercholesterolemic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD(U/mg)</th>
<th>CAT (mmol/min/mg)</th>
<th>GPx (mmol/mg)</th>
<th>GSH (mmol/mg)</th>
<th>MDA (mmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>14.51±0.31 a</td>
<td>36.2±2.1 a</td>
<td>5.93±0.39 a</td>
<td>2.36±1.73 a</td>
<td>24.48±0.21 a</td>
</tr>
<tr>
<td>Group II</td>
<td>9.02±0.18 b</td>
<td>22.5±0.9 b</td>
<td>2.93±0.17 b</td>
<td>15.87±1.26 b</td>
<td>6.76±0.32 b</td>
</tr>
<tr>
<td>Group III</td>
<td>14.62±0.25 c</td>
<td>36.9±2.4 c</td>
<td>6.01±0.43 c</td>
<td>24.31±1.35 a</td>
<td>2.51±0.18 a</td>
</tr>
<tr>
<td>Group IV</td>
<td>11.23±0.17 d</td>
<td>27.9±1.0 d</td>
<td>4.27±0.38 c</td>
<td>19.52±0.9 c</td>
<td>3.68±0.23 a</td>
</tr>
<tr>
<td>Group V</td>
<td>12.01±0.29 e</td>
<td>31.2±1.5 e</td>
<td>4.86±0.25 c</td>
<td>21.19±1.10 d</td>
<td>3.17±0.32 a</td>
</tr>
<tr>
<td>Group VI</td>
<td>13.86±0.43 f</td>
<td>34.5±1.8 f</td>
<td>5.47±0.44 d</td>
<td>22.29±2.07 d</td>
<td></td>
</tr>
</tbody>
</table>

Legend: 2 Values are expressed as means±SD for six in each group. Values not sharing a common superscript differ significantly at p<0.05.

RESULTS

Effect of lutein on plasma antioxidants

From this study it was observed that plasma Thio barbituric acid reactive substances (TBARS) level was significantly higher in high cholesterol fed rats. The plasma superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (Gpx) levels were significantly (p<0.05) higher in high cholesterol fed rats treated with Lutein in a dose dependent manner compared to untreated hypercholesterolemic rats. No statistically significant difference was seen between Atorvastatin 5 mg/kg body wt and Lutein 25 mg/kg body wt.

Effect of lutein on liver antioxidants

The liver tissue Malondialdehyde (MDA) levels of Lutein treated groups, Lutein 25 mg/kg, Lutein 50 mg/kg, Lutein 100 mg/kg body wt and Atorvastatin 5 mg/kg body wt and Atorvastatin 5 mg/kg body wt showed significantly low in comparison with the high cholesterol diet group. There was no statistically significant difference between Lutein 50 mg/kg and Lutein 100 mg/kg lowering of MDA levels. The liver tissue superoxide dismutase, catalase, glutathione, glutathione peroxidase in all Lutein treated groups and Atorvastatin 5 mg/kg body wt were statistically significantly compared to high cholesterol diet group.

DISCUSSION

Increased intracellular generation of reactive oxygen species (ROS) plays an important role in atheroma formation. Continuous formation of free radicals in living cells & uncontrolled generation of ROS leads to disease states like hyperlipidemia, hyperglycemia, atherosclerosis, cancer & arthritis [12]. Free radicals react with lipids causing pro-oxidative changes & this results in enhanced lipid peroxidation [13]. The antioxidant enzyme neutralises free radicals & reactive oxidant species (ROS). These enzymes help to protect the cell against free radical induced cell damage by meta bolising oxidative toxic intermediates. Thio barbituric acid reactive substances (TBARS) are formed as a byproduct of lipid peroxidation. ROS have very short half-lives and hence are difficult to measure directly. Hence TBARS, the degradation products of fat are measured.

Blood sampling

At the end 16 weeks, blood samples were taken by retro-orbital puncture under I. M. Ketamine from all the groups of rats for biochemical analysis.

Statistical analysis

Values of Biochemical analysis were expressed as means±S.D for six rats in each group. The data was analyzed by Duncan’s Multiple Range Test (DMRT), by SPSS software. Values not sharing a common superscript differ significantly at p<0.05.
was more effective in elevating liver catalase level compared to lutein, whereas lutein at 100 mg/kg (group VI) increased the plasma SOD level to near normal level, comparable to the control group (group I). Glutathione peroxidase functions in scavenging hydrogen and lipid peroxide. It is found abundantly in fruits & vegetables. GpX is the most important antioxidant that inhibits CD95 induced apoptosis in cultured breast cancer cells. Gpx 3 is a tumor suppression in hepatocellular carcinoma and may have therapeutic and prognostic value in HCC [18]. Rats fed with diet rich in cholesterol & saturated fatty acid resulted in inhibition of reduced glutathione and glutathione peroxidase of both liver & plasma (group II). At all three doses lutein elevated the plasma values of both GSH &Gpx. The effect of lutein at 25, 50 & 100 mg/kg superior to the effect of atorvastatin. Lutein at 100 mg/kg dose (group VI) resulted in near normal values of reduced glutathione. In liver although lutein increased the levels of both GSH &Gpx in all dose ranges (Group IV, V, VI), the effect on atorvastatin (Group III) in elevating the levels of GSH &Gpx was superior & comparable to control values (Group I). Malondialdehyde results from lipid peroxidation of polyunsaturated fatty acids and the degree of lipid peroxidation can be estimated by the amount of malondialdehyde in tissues [19]. Reactive oxygen species degrade polyunsaturated lipids forming malondialdehyde [20]. Being a reactive aldehyde, it causes toxic stress in cells forming covalent protein adducts called advanced lipidation end products. The production of malondialdehyde is used as a biomarker to measure the level on oxidative stress in an organism [21]. Malondialdehyde reacts with deoxyadenosine monophosphate in DNA forming DNA adducts and hence is also mutagenic [22]. It is oxidized by aldehyde dehydrogenase. It has been found in heated edible oils like sunflower and palm oils. Malondialdehyde is found tissue sections of patients suffering from osteoarthritis keratoconus & bullous keratopathy of cornea [23, 24]. Malondialdehyde is a highly reactive compound and not available in pure form. It is generated by hydrolysis of 1, 1, 3, 3-tetramethoxy propane [25]. Malondialdehyde assay (MDA) gives an indication about the malondialdehyde level. Atorvastatin group (group III) has reduced the MDA levels in liver considerably in our study. Lutein also has reduced the liver MDA levels in a dose dependent manner. The effect of lutein 100 mg/kg (group VI) is compared to atorvastatin group. The effect of lutein on lipid peroxidation & antioxidant enzymes of plasma & liver and its effect on liver malondialdehyde reveals the antioxidant nature of lutein and its therapeutic benefit in atherosclerosis.

CONCLUSION
Lutein reduced plasma TBARS & liver malondialdehyde levels. Lutein was found to increase the antioxidant levels. This study proved that Lutein had significant antioxidant effect against the hypercholesterolemia induced oxidative stress. Hence Lutein may be added as an adjuvant therapy for preventing or slowing the progression of atherosclerosis. Futher studies in human are yet to be done to substantiate its clinical significance.

ACKNOWLEDGEMENT
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CONFLICT OF INTERESTS
Declared None

REFERENCES