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Original Article

EFFECT OF LUTEIN IN HYPERCHOLESTEROLEMIA INDUCED OXIDATIVE STRESS IN MALE WISTAR RATS

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ABSTRACT

Objective: Oxidative stress induced by reactive oxygen species (ROS) plays an important role in the etiology of several diseases, including atherosclerosis and coronary heart disease. Hypercholesterolemia is reported to be associated with the oxidative stress that results from the increased production of ROS or impairment of the antioxidant system. Hence the objective of this study was to evaluate the antioxidant effect of lutein in hypercholesterolemiainduced oxidative stress in male wistar rats.

Methods: Male Wistar rats were divided into 6 groups of 6 each. Group I served as control. Group II III, IV, V & VI rats were received high cholesterol diet. Group III was treated with Atorvastatin 5 mg/kg. Group IV, V & VI rats was treated with 25 mg/kg, 50 mg/kg & 100 mg/kg of Lutein. After 16 weeks, blood samples &liver tissue samples were collected from all the groups of animals to evaluate antioxidant levels in blood & tissue samples.

Results: Catalase, superoxide dismutase, glutathione peroxidase, glutathione levels significantly increased in both plasma & liver in lutein treated groups. TBARs level is increased in plasma in hypercholesterolemicrats and Malondialdehyde level in the liver tissue is also significantly increased in hypercholesterolemic rats.

Conclusion: The results of this study indicate Lutein is an effective nutritional supplement to prevent oxidative stress in hypercholesterolemia.

Keywords: Hypercholesterolemia, Oxidative stress, Liver, Lutein.

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 HOCl-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 anti atherogenic 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, there by 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 (O_2^-) 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 (H_2O_2) 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 atherosclerosis 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. 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/(CPCSEA)] Annamalai University, Annamalai Nagar, Tamilnadu, India(Proposal No.1064, dated 29.11.2013).

Reparation of drug

Lutein 50 mg/ml. Atorvastatin powder was dissolved in dimethyl sulfoxide (DMSO) to make a solution of 10 mg/ml. Healthy adult male 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 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 intra-gastric 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.

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 $p<0.05$.

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

Groups	Tbars (nmol/ml)	SOD (NBT/min/mg Hb)	Catalase (Mmol/min/mg Hb)	Gp_x (Mmol)	GSH
Group I	2.07±0.03[a]	6.93±0.04[a]	192.26±0.91[a]	17.11±0.04[a]	32.58±0.09[a]
Group II	6.52±0.02[b]	5.13±0.02[b]	156.04±0.40[b]	10.91±0.04[b]	25.54±0.20[b]
Group III	5.07±0.04[c]	6.05±0.02 [c]	163.14±0.65[c]	13.11±0.03[c]	29.90±0.05[c]
Group IV	5.34±0.01[c]	6.45±0.02[d]	161.84±0.22[c]	14.19±0.04[d]	30.23±0.05[c, d]
Group V	4.03±0.03[d]	6.66±0.03[e]	174.00±0.18[d]	15.50±0.06[e]	31.20±0.03[d]
Group VI	3.09±0.02[e]	6.83±0.02[f]	183.04±0.15[e]	16.40±0.02[f]	32.08±0.04[a]

Legend: 1Values are expressed as Means±SD for six in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT).

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

Groups	SOD(U/mg)	CAT (µmol/min/mg)	GPx (nmol/mg)	GSH (nmol/mg)	MDA (nmol/mg)
Group I	14.51±0.31[a]	36.2±2.1[a]	5.93±0.39 [a]	23.69±1.73[a]	24.48±0.21 [a]
Group II	9.08±0.18[b]	22.5±0.9 [b]	2.93±0.17 [b]	15.87±1.26[b]	6.76±0.32[b]
Group III	14.62±0.25[a]	36.9±2.4[a]	6.01±0.43 [a]	24.31±1.35[a]	2.51±0.18 [c]
Group IV	11.23±0.17[c]	27.9±1.0 [c]	4.27±0. 38[c]	19.52±0.9 [c]	3.96±0.23 [d]
Group V	12.01±0.29[d]	31.2±1.5 [d]	4.86±0.25 [c]	21.19±1.10[d]	3.17±0.32[e]
Group VI	13.86±0.43[a]	34.8±1.8 [e]	5.47±0.41 [d]	22.29±2.07[d]	2.82±0.25 [e]

Legend: 2 Values are expressed as means±SD for six in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$. (DMRT).

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 Atorvastatin5 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 showed significantly low in comparison with the high cholesterol diet group. There was no statistically significant difference betweenLutein 50 mg/kg and Lutein 100 mg/kgin lowering of MDA levels. The liver tissue superoxide dismutase, catalase, glutathione, glutathione peroxidase in all Lutein treated groups andAtorvastatin 5 mg/kg body wt were statistically significantcompared 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 to assess the damage produced by oxidative stress reported that lipid peroxidation resulted in higher incidence of carotid atherosclerosis. [14]. Elevated levels of TBARS in plasma of high cholesterol diet rats group (Group II) indicates excessive formation of free radicals resulting in activation of lipid peroxidation. The flavonoid Lutein has significantly reduced the TBARS level at all three doses in plasma in a dose dependant manner. Lutein at 100 mg/kg (group VI) was more effective than the standard drug. Atorvastatin (group III) in reducing plasma TBARS and this indicates that it is a potent antioxidant. Superoxide dismutase (SOD) catalyses the breakdown of superoxide anion into singlet oxygen (O_2) Copper & Zinc SOD are present in cytosol but Manganese SOD present in mitochondria is biologically important. SOD acts both as antioxidant and as anti-inflammatory agent. Such is its importance that mice lacking SOD dies due to oxidative stress. Broccoli, brussels, sprouts & cabbage are rich sources of SOD. Recent reports suggest that manganese SOD is decreased in early breast cancer and increased in late stages [15]. The clinical significance of the manganese SOD and its elevated levels in hepatitis C virus (HCV) related hepatocellular carcinoma (HCC) [16]. Lutein elevated the SOD levels at all three dose levels. Lutein 100 mg/kg was more effective than Atorvastatin in elevating plasma SOD level. Lutein also increased the liver SOD levels in a dose dependant manner. When compared to cholesterol rich diet group (group II) the liver SOD levels of lutein treated groups (group IV, V, VI) were increased significantly but those effects were found to be not so superior to the effect of Atorvastatin, which had greater effect in elevating the liver SOD levels. The antioxidant catalase catalyses the conversion of hydrogen peroxide to H_2O & O_2. Levels of catalase is decreased in neuromuscular, cardiovascular & metabolic diseases [17]. At all three doses lutein increased both plasma & liver catalase enzyme levels. Of the three doses used Lutein 100 mg/kg doses (group VI) resulted in the maximum response in both liver & plasma. However atorvastatin

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 & lipid peroxidase. It is found abundantly in fruits & vegetables. Gp_X5 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 dose levels, 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 malondialdehydeintissues [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 lipoxidation 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&deoxyguanosine in DNA forming DNA adducts and hence is also mutagenic [22]. It is oxidised 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 osteoarthritiskeratoconus & 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 dependant 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. Furthur studies in human are yet to be done to substantiate its clinical significance.

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CONFLICT OF INTERESTS

Declared None

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