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The Effect of Alpha Crystallin on Diet Induced Hypercholstrolemic Rats

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Abstract: To explore the effect of bovine α-crystallin on high cholesterol diet-induced rats. Rats were divided into three groups (n=6 in each) control, hypercholesterolemic and α crystallin treated. The lipid profiles, antioxidant and membrane bound ATPase status of serum and various tissues were investigated in hypercholesterolemic rats and the effect of α-crystallin on defense systems. The results showed that there was a decrease in cholesterol, triglyceride level on α-crystallin treated hypercholesterolemic rats. Cholesterol enriched diet caused a significant increase in the lipid peroxide and free radical scavenging enzyme concentrations of serum, liver and heart. In addition, a significant decrease in glutathione (GSH) content, were found in serum, liver and heart. This study indicated that administration of α-crystallin may play an important role in suppressing oxidative stress, and thus, may be useful for the prevention of hypercholesterolemia.

Key Words: α-crystallin, Hypercholesterolemia, oxidative stress, lipid profile, Antioxidant, ATPase

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I. INTRODUCTION

Hypercholesterolemia is a well known risk factor in the development of atherosclerosis and subsequent coronary heart disease [1]. A diet of high lipids continuously cause hyperlipidemia[2], a condition is marked by an increase in total cholesterol, triglycerides, Low Density Lipoprotein-Cholesterol (LDL-C), and a decrease in High DensityLipoprotein-Cholesterol (HDL-C).[3] Hyperlipidemia increase the occurrence of atherosclerosis[4], one of factors that triggering cardiovascular disease, as hypertention; coronary heart[5] and stroke[6]. Oxidative stress has been prescribed as the main mechanism responsible for cardiovascular diseases while

hypercholesterolemia under oxidative stress could trigger the progression of atherosclerosis and abnormal lipid metabolism[7]. It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease anti oxidative enzyme activity[8]. It also causes oxidative stress resulting in increased lipid peroxidation in multiple organs[9]. Antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation[10].

α-crystallin is a major lens protein in vertibrates. It is constituted by two subunits, the αA (acidic) and αB (basic). Both independent polypeptides have sequence similarity with the small heat shock protein family (sHsp) and are expressed in low quantities in extracellular tissues[11, 12]. In myocardium, elevated levels of heat shock proteins (HSPs) have been associated with cardiac protection against injury caused by ischemia/reperfusion or other stressful treatments such as hypoxia, ATP depletion, glucose deprivation, and hypotoxicity [13]. The use of sHSPs as targets for the development of novel drug therapies has been demonstrated using α-crystallin, which acts as molecular chaperone to protect against protein aggregation invitro [14]. There is some information that indicates that α-crystallin can behave as an antioxidant and free radical scavenger. α-crystallin was able to decrease thiol groups oxidation in conditions of oxidative stress [15]. α-crystallin can also protect lipids from oxidative modification. The interaction of α-crystallin with lipids has been studied by the group of Borchman et al[16, 17].

The objective of the present study was to test the efficacy of α-crystallin, as therapeutic agent to treat hypercholesterolemia in rats. The present work is carried out to study the potent pharmacological effects of α-crystallin on the lipid composition, antioxidant enzyme level and thiobarbituric acid reactive substance activities in serum, liver and heart of hypercholesterolemic rats. We want to determine α- crystallin administration has any influence on lipid profile, lipid peroxidation, antioxidant and membrane bound enzymes in hypercholesterolemic rats.

II. MATERIALS AND METHODS

Male wistar strain albino rats weighing 150-200 gms were obtained from Tamilnadu Veterinary and Animal Sciences University, Madhavaram, Chennai, Tamilnadu ,India. The animals were maintained in the poly acrylic cages under hygienic conditions at normal room temperature (28-30 ^{0}C) on a 12- h light/dark cycle. The animals were fed with commercial pellet diet (Hindustan lever Ltd., Bangalore, India) and had free access to water. The surgical procedures were performed as per NIH animal ethical guidelines, approved by the animal care committee of Central Leather Research Institute , Chennai, Tamilnadu.

2.1 Isolation and purification of bovine α-crystallin

Bovine eyes obtained from local slaughter house were decapsulated and eye lens capsules were removed. These lenses were mixed with a three fold quantity of 0.1 M Tris buffer (containing 100mM NaCl, 1mM EDTA, and 0.1% sodium azide pH 7.4 at 4°c and gently homogenized[18]. The mixture was gently stirred overnight and the soluble fraction was decanted from the remaining insoluble part of the lens. After centrifugation at 6000×g for 30 min, the supernatant was collected and its absorbance measured at 280 nm. One unit of absorbance was taken as equivalent to 1mg of protein perml in the lens extract. The extract was distributed in aliquots and conserved at -20°C. Purification was done using 0.8x50 sephacryl S-200 column equilibrated at 4°C with eluting buffer (contains Tris 0.1mM ,NaCl 0.2M, pH 7.2). The eluted proteins was collected and filtered through a Sartorious membrane (cutoff 100,000) and the concentrated fraction was dialysed against distilled water, distributed in aliquots, and conserved at-20 °C. The purity of α--crystallin was confirmed by 12% SDS-PAGE[19].

2.2 Preparation of hypercholesterolemic diet

Diet was prepared according to Vinitha *et al.,* with modification[20]. The commercial feed 50g was pulverized and mixed with 0.2g sodium cholate, 20g sucrose, 2g lactose, 0.4g choline chloride and 0.15g 2-thiouracil. 20g hydrogenated oil was melted separately with 15g cholesterol dissolved in hot fat. The feed was made into dough and 10g per day dose given to rats for 35 days. The animals had free access to water.

2.3 Experimental design

The animals were divided into three groups, each group consists of six animals . Group I (control) were fed with commercial feed, group II &III were on hypercholelesterolemic diet (atherogenic diet) for 35days. Lipid profiles (total cholesterol, HDL cholesterol and triglycerides) were measured to confirm the hypercholesterolemic condition. A 0.1 ml saline was injected intraperitoneally to group I & II rats whereas group III receives α-crystallin (50 μg/100g body weight) for seven days. The effective dosage was fixed on the basis of our previous report[21].

2.**4 Biochemical Analysis**

Animals were fasted overnight and sacrificed by cervical dislocation. Blood was collected from all subjected animals at the end of seventh day and allowed to stand for 10 minutes and centrifuged at 1500g for 10 minutes at 4°C. The heart and liver tissues were weighed and homogenized in 0.1 M Tris buffer pH7.4. The homogenates was then centrifuged at 5000×g for 10 minutes at 4°C, and the supernatant was used for all the estimations. The serum obtained was diluted 10 times before estimation of protein by Lowry's method. Other parameters like antioxidant enzymes, lipid peroxidation, ,ATPase, , GSH and lipid profile were studied.

2.5 Protein content:

Protein content of the serum and homogenates were determined by the method of Lowry et al[22] using bovine serum albumin as standard.

2.6 Thiobarbituric acid reactive substances (TBARS)

The Lipid peroxidation products were determined by the TBA reaction described by Ohkawa et al[23].

2.7 Antioxidant enzyme assays: -

2.7.1 Superoxide dismutase (SOD) activity

SOD activity was assayed according to the method of Misra and Fridovich[24]. The assay is based on the inhibition of epinephrine-adrenochrome transition by the enzyme.

2.7.2 Catalase activity

The activity of catalase was determined by the method of Beers and seizer[25]. The breakdown of hydrogen peroxide on addition of enzyme is followed by observing the decrease in light absorption of peroxide solution in the ultraviolet (UV) region.

2.7.3 Total Reduced Glutathione (GSH)

The method of Moron et al[26] was followed to determine the total reduced glutathione. The method is based on the reaction of glutathione with DTNB to give absorption at 412 nm.

This method is based on the reaction of glutathione with DTNB to give absorption at 412 nm. Briefly 1 ml of the tissue homogenate was precipitated with 5% TCA and centrifuged. 0.5 ml of supernatant was added with 0.5 ml of 0.2 M phosphate buffer, pH 8.0, followed by 2.0 ml of DTNB reagent (0.6 mmol DTNB IN 0.2 M phosphate buffer, pH 8.0). The color developed was read at 412 nm in a Perkin Elmer spectrophotometer against a blank containing 5% TCA instead of sample. A series of standards were treated in a similar manner. The amount of glutathione was expressed as μg/mg protein.

2.8 Ascorbic acid

Ascorbic acid was estimated by the method of 0maye *et al*, 1979[27]. The ascorbic acid is oxidized by copper to form dehydro ascorbic acid and diketoglutaric acid. These products are reacted with 2, 4 dinitrophenyl hydrazine (DNPH) to form a compound, which in the presence of concentrated H_2SO_4, undergoes rearrangement to form a product with absorbance maximam at 520nm. The reaction is seen in the presence of thiourea to provide a reducing medium with buffer to prevent interference from non-ascorbic acid chromogen.

2.9 α-Tocopherol estimation

α-tocopherol was estimated by the method of Desai 1984[28] using bathophenanthroline, which forms complex with ferrous ions. Briefly 3.0 ml of aliquot of hexane extract was pipetted into suitable reaction tubes and evaporated to dryness under nitrogen or vacuum. The residue was carefully dissolved in 1 ml of absolute ethanol. Tubes containing α-tocopherol standards were treated exactly the same way as the test samples and to each tube was added 0.2 ml of 0.2% batho-phenonthroline reagent and the contents of the tubes were thoroughly mixed. The assay was proceeded very rapidly from this point. Care was taken to reduce unnecessary exposure to direct light. Added 0.2 ml of ferric chloride reagent and vortex method. After 1 min, 0.2 ml of orthophosphoric reagent was added and mix thoroughly. The absorbance was read at 536 nm against the reagent blank containing ethanol. The values are expressed as mg/dl in plasma

2.10 Determination of membrane bound ATPases

Activities of sodium potassium ATPase(Na^+ K^+ ATPase), calcium ATPase (Ca^+ ATPase) and magnesium ATPase (Mg2+ ATPase) were determined by the following method of Bonting[29], Hjerten and Pan[30], and Ohinishi etal[31], respectively. The tissue homogenates of heart and liver were assayed for the membrane bound ATPase activities. The activities were indirectly quantified by estimating phosphorous liberated after the incubation of tissue homogenate in a reaction mixture containing the substrate ATP with the co-substrate elements at 37°C for 15 minutes. The reactions were stopped by adding 1.0 ml of 10% TCA. The phosphorous content from TCA supernatants then determined by the method of Fiske and Subbarao[32].

Statistical Analysis

Results were statistically valuated using on-way analysis of variance (ANOVA) followed by the Turkey's test was applied to determine the significant differences amongst the groups. P values less than 0.05 were considered significant.

III. Results

3.1 Lipid Profile

Table 1 shows the effect of α-crystallin on serum lipid concentrations of HCD fed rats, wherein there is a significant ($P<0.05$) increase in total cholesterol (4.2 fold), triglyceride (1.7fold), LDL (3.5 fold) and VLDL (2.0 fold), whereas decrease in HDL (34.1%) concentrations was observed in HCD fed rats. All these abnormalities were considerably reduced upon treatment with α-crystallin.

Table 1 Effect of α-crystallin on serum lipid status of HCD fed groups compared with the control animals

Parameters Mg/dl	Group I Control	Group II HCD	Group III α-crystallin treated
Total cholesterol	63.19± 4.12	236.24 ± 21.8 ^{a*}	144.2 ± 19.13 ^{b**}
Triglyceride	88.97± 6.48	145.4 ± 12.12 ^{a*}	100.37 ± 9.66 ^{b*}
HDL	35.38 ± 3.96	2 3.31 ± 1.98 ^{a*}	30.41 ± 3.28 ^{b*}
LDL	22.28 ± 1.16	52.39± 5.068 ^{a*}	35.08 ± 2.56 ^{b**}
VLDL	15.86± 1.42	27.14 ± 2.21 ^{a*}	20.48 ± 1.66 ^{b**}

Units: Total cholesterol, Triglyceride, HDL,LDL, and VLDL; mg/dl. Comparisions are made between: [a]Group I and II, [b]Groups II and III. [*]P<0.05, [**]P<0.01 [***]P<0.001 Values are expressed as mean ± S.D for 6 animals

3.2 Lipid peroxidation

Elevated concentrations of LPO are recorded in plasma, heart and liver (P<0.05) of hypercholesterolemic group when compared to control and α-crystallin treated groups (Table 2). Hypercholesterolemic group showed 110%, 123% and 72% increase in the concentration of plasma, heart and liver respectively. On treatment with α-crystallin, a significant reduction of TBARS concentration in the plasma, heart and liver (15% 20% and 19%) was observed when compared to hypercholesterolemic rats.

Table 2 Determination of TBARS in the plasma, heart and liver of Control and treated animals

Parameters	**Group I**	**Group II**	**Group III**
Plasma TBARS (nmols of MDA released/mg protein	1.17±0.13	2.46±0.07[a*]	1.34±0.11[b**]
%Variation of TBARS production compared to control	-	110.34	14.52
Heart TBARS (nmols of MDA released/mg protein	0.84±0.08	1.88±0.04[a*]	1.09±0.04[b***]
%Variation of TBARS production compared to control	-	123.80	20.23
Liver TBARS (nmols of MDA released/mg protein	2.87±0.12	4.95±0.54[a*]	3.25±0.30[b***]
%Variation of TBARS production compared to control	-	72.47	18.88

Values represent the group mean ±SD for six rats. Different superscripts (a) indicate Comparision between [a]Group I and Group II, [b]Group II and Group III. significant difference between Groups.
P<0.05, [**]P<0.01, [***]P<0.001, superscript NS-indicates not significant.

3.3 Antioxidant status

The anti oxidant status of plasma, heart and liver tissue with reference to non-enzymatic (GSH, ascorbic acid, and α-tocopherol) and enzymatic antioxidants (GPx, SOD,CAT,GR,GST and G6PDH) was determined.
The levels of anti oxidants, GSH, ascorbic acid and α-tocopherol in plasma, liver and heart tissue are presented in tables 3, 4, 5 respectively.

Table 3 Non-enzymatic antioxidants GHS, Ascorbic acid and α - tocopherol in plasma of control and experimental group of rats

(ug/mg protein)	Group I	Group II	Group III
Reduced glutathione	10.95±0.81	7.62±62 ^{a***}	9.31± 1.30 ^{b*}
Ascorbic acid	0.90+0.02	1.45+0.01 ^{a***}	1.37±0.05 ^{b**}
α - tocopherol	1.36±0.01	0.63±0.002 ^{a***}	0.95±0.01 ^{b***}

*P<0.05 **P<0.01 ***P<0.001
Values are mean ± S.D of 6 rats in each Group, ^{a}Group I was compared with ^{b}Group II and Group II compared Group III. GSH- glutathione expressed as μg/mg protein.

Table 4 Nonenzymatic antioxidants GSH, Ascorbic acid and α- tocopherol in liver tissue of control and experimental group of rats.

(ug/mg protein)	Group I	Group II	Group III
Reduced glutathione	7.21±0.5	4.32±0.1 ^{a***}	7.31± 0.2 ^{b***}
Ascorbic acid	2.4±0.2	1.89±0.09 ^{a***}	2.23±0.1 ^{b**}
α - tocopherol	5.33±0.90	3.2±0.89 ^{a***}	4.17±0.93 ^{b***}

**P<0.01 **P<0.001 NS-Not significant Values are mean ± S.D of 6 rats in each Group, Group II was compared with Group I and Group III was compared with Group II.

Table 5 Non - enzymatic antioxidants GSH, Ascorbic acid and α - Tocopherol in Heart tissue of control and experimental groups of rats

(ug/mg protein)	Group I	Group II	Group III
Reduced glutathione	5.27±0.33	3.26 ± 0.29 ^{a***}	4.21 +0.15 ^{b***}
Ascorbic acid	1.96 ± 0.09	1.66 ± 0.09 ^{a***}	1.71± 0.07 ^{bNS}
α - tocopherol	5.82 ± 0.91	4.91 ± 0.22 ^{a*}	5.43 ±0.37 ^{b**}

*P<0.05 ***P<0.001 NS - Not significant Values are mean ± S.D of 6 rats in each Group, Group II was compared with Group I and Group III was compared with Group II.
GSH was marked depression in Group II rats in plasma, liver and heart tissues (p<0.001).
α-crystallin treated rats (Group III) as compared to Group II displayed in increase in GSH in plasma (p<0.05) liver and heart tissues (p<0.001). Ascorbic acid in plasma in contrast to hepatic and cardiac tissues, was markedly increased in Group II rats (p<0.001). Tissue concentration of ascorbic acid was however markedly depressed in this Group (p<0.001).
α-crystallin treated decreasing ascorbic acid concentration in plasma (p<0.01), increased the same in liver (p<0.001). Heart tissue displayed a slight increase in ascorbic acid which was however not significant.
Hypercholesterolemic diet fed Group II rats had significantly depressed α-tocopherol concentrations in plasma (p<0.001), liver (p<0.01), and heart (p<0.05) tissues. α-crystallin treated rats served to increase α-tocopherol concentrations in plasma (p<0.001) but apparently had no positive effect on liver concentrations where as cardiac tissue concentration of α-tocopherol in Group III rats were raised (p<0.05).

Table 6 Effect of α-crystallin treatment on enzymatic antioxidants of plasma

(ug/mg protein)	Group I	Group II	Group III
GPX ^{a}	102.54 ± 4.95	85.67 ± 6.22 ^{a***}	95.39 ± 4.67 ^{b*}
SOD ^{b}	17.9 ±2.30	8.8 ± 3.27 ^{a***}	11.8 ± 1.37 ^{bNS}
CAT ^{c}	218.81 ± 15.3	285.33 ± 11.1 ^{a***}	210.01 ± 11.0 ^{b***}
GR ^{d}	17.9 ± 1.63	15.7 ± 0.92 ^{a*}	16.9 ± 0.52 ^{b*}
GST ^{e}	256.92 ± 7.3	245.71 ± 4.60 ^{a***}	259.55 ± 8.21 ^{b**}
G6PDH ^{b}	1.91 ± 0.09	0.35 ± 0.03 ^{a***}	0.61 ± 0.03 ^{b***}

*P<0.05 ***P<0.001, **P<0.01 NS - Not Significant Values are mean ± SD of 6 rats in each Group, Group II was compared with Group I and Group III was compared with Group II.

a - μg of GSH consumed / min/ mg protein
b - units /mg protein
c - μ moles of H_2O_2 consumed / min / mg protein
d - n moles of NADPH oxidized min/mg protein
e - n moles of CDNB conjugated /min/protein

Table 7 α-crystallin Treated Enzymatic Antioxidants of Liver Tissues

Parameters	Group I	Group II	Group III
GP x[a]	97.57±7.37	42.33±4.22[a***]	62.31± 9.27[bNS]
SOD [b]	11.55±0.25	8.36±0.91[a***]	10.11±0.90[b**]
CAT[c]	62.71±3.93	69.66±3.91[a*]	59.39±d.26[b**]
GR[d]	25.98±3.2	15.32±2.9[a***]	20.12±2.2[b**]
GST[e]	1150.27±51.3	889.73±60.33[a***]	1098.69±99.6[b**]
G6 PDH[b]	1.02±0.1	0.29±0.1[a***]	0.52±0.09[b***]

*P<0.05 ***P<0.001, **P<0.01 NS - Not Significant Values are mean ± SD of 6 rats in each Group. Comparisons are between a Group I and Group II , b Group II and Group III.

a - μg of GSH consumed / min/ mg protein
b - units/mg protein
c - μmoles of H_2O_2 consumed / min / mg protein
d - n moles of NADPH oxidized min/mg protein
e - n moles of CDNB conjugated /min/protein

Table 8 α-crystallin Treated Enzymatic Antioxidants of Heart Tissues

Parameters	Group I	Group II	Group III
GPx[a]	58.26±3.36	12.29±7.32[a***]	20.63±2.90[b*]
SOD[b]	15.31±1.11	9.33±0.71[a***]	13.91±1.96[b***]
CAT[c]	101.51±4.77	156.95±5.83[a***]	1.31.85±6.23[b***]
GR[d]	20.88±.1.2	12.67±2.8[a***]	19.81±3.17[b**]
GST[e]	215.76±7.3	101.25±8.37[a***]	188.20±7.23[b***]
G6 PDH[b]	1.23±0.1	0.89±0.05[a***]	0.99±0.09[b*]

*P<0.05 ***P<0.001, **P<0.01 NS - Not Significant Values are mean ± SD of 6 rats in each Group, Comparisions are between a Group I and Group II , b Group II and Group III.

a - μg of GSH consumed / min/ mg protein
b - units /mg protein
c - μ moles of H_2O_2 consumed / min / mg protein
d - n moles of NADPH oxidized min/mg protein
e - n moles of CDNB conjugated /min/protein

Cholesterol-loading in the diet caused a marked depression in GPx (P<0.001), SOD (P<0.001) and G6 PDH (P<0.001) in plasma, heart and liver. GR an enzyme of the redox cycle was slightly decreased in plasma of Group II rats (P<0.05) whereas liver and heart tissue displayed a significant decrease in this enzyme (P<0.001) and (P<0.001), GST, another enzyme of the redox cycle, was decreased by the atherogenic diet in plasma (P<0.001) liver (P<0.001) and heart (P<0.001). There was a marked difference in catalase activity in Group II

rats. Plasma levels of catalase increased significantly (P<0.001) as did levels in liver tissue (P<0.05) and heart tissue (P<0.001).

It was observed that α-crystallin treated rats apparently had a positive effect on restoring the imbalance in enzymatic antioxidants of plasma, liver and heart.

α-crystallin treated rats served to elevate plasma concentration of GP x (P<0.05) as well as in liver (P<0.001) and heart (P<0.05). SOD levels were apparently not restored to normal values in plasma as opposed to tissue levels. Liver tissue displayed a marked increase in SOD activity (P<0.01) and did heart tissue (P<0.001) in-Group III. The enzymes of the redox cycle were all markedly increased in Group III rats. GR activity increased in plasma (P<0.05) liver and heart (P<0.001). G6 PDH activity was elevated in Group III rats in plasma (P<0.001) liver (P<0.01) and heart (P<0.05). Catalase activity which displayed a marked elevation in Group II rats was decreased in α-crystallin treated rats in plasma (P<0.001) liver (P<0.01) and heart (P<0.001).

3.4 Membrane bound ATPase

The membrane transport enzymes such as Na^+/K^+, Mg^{2+}, Ca^{2+}, ATPase activities in erythrocyte membrane, heart and liver of the control, hypercholesterolemic and α-crystallin treated animals have been presented in Tables respectively. Results indicate that the Na^+, K^+ ATPase activity was significantly reduced in the cholesterol fed groups as compared with control (p<0.001). α-crystallin treatment normalize the activity in the hypercholesterolemic rats .

Table 9 The Effect of α-crystallin on ATPase activity in the erythrocyte membrane of hypercholesterolemic rats

Membrane ATPases Units/ min/per mg protein	Group I	Group II	Group III
Na^+K^+ ATPase	2.44 ± 0.22	1.47 ± 0.12 ^{a*}	2.17 ± 0.18 ^{b*}
Ca^{2+} ATPase	1.35 ± 0.12	0.90 ± 0.10 ^{a*}	1.19 ±0.12 ^{b***}
Mg^{2+} ATPase	1.63 ± 0.11	1.19 ± 0.10 ^{a*}	1.46 ± 0.14 $_{b***}$

Values are expressed as mean ± SD for Six animals in each group. Units N^{a+} K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase, umol of pi liberated / mg protein / hr. Comparisions are made between ^{a} Group I and Group II, ^{b} Group II and III. *P<0.05, ** P<0.01 *** P<0.001

The levels of Mg2+ ATPase in erythrocyte membrane, heart and liver of the control, hypercholesterolemic and treated animals have been shown in Table 9. Hypercholesterolemia causes a significant change in Mg2+ ATPase activities in the cholesterol fed groups and there was significant difference between the α-crystallin treated groups. The effects of α-crystallin on cholesterol fed groups showed significant increase in Mg2+ ATPase activity (p<0.05).

Table 10 The Effect of α-crystallin on ATPase activity in the heart tissue of hypercholesterolemic rats

Membrane ATPases Units/ min/per mg protein	Group I	Group II	Group III
Na^+K^+ ATPase	0.57 ±0.07	0.37 ± 0.04 ^{a*}	0.49 ± 0.04 ^{b**}
Ca^{2+} ATPase	0.28 ± 0.026	0.13 ± 0.02 ^{a*}	0.22 ± 0.03 ^{b***}
Mg^{2+} ATPase	0.42 ± 0.04	0.27 ± 0.02 ^{a*}	0.37 ± 0.05 ^{b**}

Values are expressed as mean ± SD for Six animals in each group. Units N^{a+} K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase, umol of pi liberated / mg protein / hr. Comparisions are made between ^{a} Group I and Group II, ^{b} Group II and III. *P<0.05, ** P<0.001 ***P<0.001

The levels of Ca^{2+} ATPase in erythrocyte membrane, heart and liver of the control, hypercholesterolemic and treated animals have been shown in Table. Results indicate that the Ca^{2+}ATPase activity was significantly less in the cholesterol fed groups as compared with control (p<0.01). Ca^{2+} ATPase levels were increased in α-crystallin treated rats when compared with group II (p<0.05).

Table 11The effect of α-crystallin on ATPase activity in the Liver tissue of hypercholesterolemic rats

Membrane ATPases Units/ min/per mg protein	Group I	Group II	Group III
Na^+K^+ ATPase	0.84± 0.02	0.45 ± 0.03 ^{a*}	0.71 ± 0.04 ^{b*}
Ca^{2+} ATPase	0.94 ± 0.01	0.46 ± 0.07 ^{a*}	0.90 ± 0.13 ^{b***}
Mg^{2+} ATPase	0.88 ± 0.29	0.56 ± 0.28 ^{a*}	0.65 ± 0.21 $_{b***}$

Values are expressed as mean ± SD for Six animals in each group. Units N^{a+} K^+ ATPase, Ca^{2+} ATPase, Mg^{2+} ATPase, umol of pi liberated / mg protein / hr. Comparisions are made between ^{a} Group I and Group II, ^{b} Group II and III.
$^*P<0.05$, $^{**}P<0.01$ $^{***}P<0.001$

IV. DISCUSSION

Dietary cholesterol exerts a profound influence on the physiology and pathology of the cardiovascular system. Various studies indicate that high serum levels of cholesterol are strongly related to coronary atherosclerosis and increased risk of coronary heart disease. Clinical studies in animals reveals that lowering levels of serum cholesterol with diet or drugs decreases the incidence coronary heart disease[33]. An increase in serum cholesterol in rat fed with high cholesterol diet suggests cholesterol rich diet enhances the level of cholesterol and triglyceride[34]. Our results indicated that cholesterol rich diet caused significant increases in TC and TG concentrations of serum. The serum cholesterol levels especially the total cholesterol, and triglycerides were reduced significantly in α-crystallin treated rats. Superoxide production is increased in a number of disease states including hypercholesterolemia[35]. The precise mechanisms leading to this increase remain uncertain. The group II shows increase in antioxidant enzyme (SOD) and catalase by increasing lipid peroxidation clearly shows that oxidative stress in group II. Antioxidant molecules capable of scavenge free radicals in extracellular compartment[36]. α-crystallin has been ascribed antioxidant and free radical scavenging properties[37]. The substantial decrease in antioxidant enzyme in-group III reveals that crystallin acts as an antioxidant and scavenged free radicals. Decreased oxidative stress leads to the low expression of antioxidant enzyme. Above results correlates with our data (group III).

The MDA level was found to be increased in hypercholesterolemic rat suggests more lipid peroxidation. Increased MDA suggests an increase in the level of oxygen free radicals, which may be due to decreased antioxidant reserve. Group II rats were found to agree with this observation. The increase in catalase and SOD level in-group II can be related to increased MDA level. An increase in lipid peroxidation causes a decrease in antioxidant enzyme level, and that is not obeyed on α crystallin treatment suggesting that the decreases in lipid peroxidation may be due to its chaperoning activity. The α-crystallin protects the antioxidant enzyme by binding to them and thereby functions more actively. This study demonstrates that α-crystallin may be a useful therapy for hypercholesterolemia through reducing oxidative stress and cholesterol level .The serum thiobarbituric acid reactive substance level decreased after treatment of α-crystallin indicating that it could prevent hypercholesterolemic disease through reducing lipid peroxidation.

An increase in cholesterol content of cell membrane results in a decrease in membrane fluidity and decreases membrane permeability, thus altering the function of membrane-bound enzymes, ion channels and receptors[38]. A significant reduction of Ca^{2+}, mg^{2+} ATPases in-group II animals were observed and level of Ca^{2+}, mg^{2+} ATPases increased in α-crystallin treatment. Administration of high cholesterol diet has been shown to inhibit Na^+ - K^+ - ATPase activity in hypercholesterolemic rats[39]. Our findings show that hypercholesterolemia reduces Na^+, K^+ ATPase content. Rats fed a high cholesterol diet show a significant hypercholesterolemia and a decreased activity of Na^+ - K^+ ATPase as compared with the normal and α-crystallin treated groups. These results indicate that cholesterol exerts an inhibitory role on myocardial Na^+, K^+ - ATPase activity. Cholesterol lowering treatment with α-crystallin influences Na^+, K^+ transport systems.

A decrease in intracellular glutathione confirmed oxidative stress upon incubation with hydrogen peroxide[40]. High fat induces decreases in normal activities of glutathione reductase enzymes and glutathione contents in the tissues. The decreased activity of glutathione reductase should normally result in a decreased concentration of reduced glutathione. The treatment with α-crystallin has elevated the levels of these parameters in tissues of experimental rats fed the high fat diet.

All of these results showed that cholesterol feeding caused important changes in the relations between tissue oxidant-antioxidant balance and lipid parameters, and α-crystallin treatment partly decrease these relations. The present study demonstrated that α-crystallin treatment to the hypercholesterolemic rats led to a recovery in antioxidant enzymes and membrane bound ATPase activities.

V. CONCLUSION

We have demonstrated that high dietary cholesterol may result in hypercholesterolemia and lead to decrease in the enzymatic antioxidant defense potential of tissues and lead to oxidant stress. As a result of these metabolic events, some important changes occur in serum, tissues and lipid parameters. Administration of α-crystallin may activate antioxidant enzymes and scavenge toxic free radicals. Therefore, it can be concluded from the present study that α-crystallin administration may serve as a potent therapeutic agent in hypercholesterolemic diet induced rats.

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REFERENCES

[1]. Khoo K, H.Tan, Y. Liew, J.Deslypere and E.Jansus, Lipids and coronary heart disease in Asia.Atherosclerosis,2003; 169; 1-10 .

[2]. Matos, S., H. Paula, M. Pedrosa, R. Santos, E. Oliveira, D. Junior and M. Silva. Dietary models for inducing hypercholesterolemia in rats.Brazilian Archives of Biology and Technology and Internationa Journal, 2005, Vol.48 No. 2 pp: 203-209.

[3]. .

[4]. Vafa, M., E. Haghighatjoo and A. Ziaee. Effect of apple consumption on lipid profile of hyperlipidemic and overweight men. International Journal of Preventive Medicine. 2011.Vol. 2 No.2 pp:84-100.

[5]. .Ling, W., Q. Cheng, J. Ma and T. Wang. Red and Black Rice Decrease Atherosclerotic Plaque Formation and Increase Antioxidant Status in Rabbits. The Journal of Nutrition.2001. Vol. 131 No. 5 pp:1421-1426.

[6]. . Kumar, K., R. Reddy, N. Reddy and J. Anbu. Lipid Lowering Activity of Lercanidipine inHyperlipidemic Rats. Iranian Journal Of Pharmacology and Therapeutics. 2010.Vol. 9 pp:73-75.

[7]. . Kreisberg R.A. and J.E.B. Reusch. Hyperlipidemia (High Blood Fat). The Journal of Clinical Endocrinology and Metabolism, 2005. Vol. 90 No. 30 .

[8]. Zulkhairi HA, Khairunnuur AF, Hafipah MRN, Azrina A,Rasadah MA, Kamilah KAK, et al. An aqueous extract of Citrus mitis posessess antioxidative properties and improves plasma lipid profiles in rat induced with high cholesterol diet. J Med Plant Res2010; 4: 49-57.

[9]. Slim, R.M., Toborek, M., Watkins, B.A., Boissonneault, G.A.,Hennig, B.,Susceptibility to hepatic oxidative stress in rabbits fed different animal and plant fats. J. Am. Coll. Nutr1996, 15(3), 289–294.

[10]. Cai J, Yang L, He HJ, Xu T, Liu HB, Wu Q et al. Antioxidant capacity responsible for a hypocholesterolemiais independent of dietary cholesterol in adult rats fed rice protein. Gene. 2014;533:57-66

[11]. Sheweita, S.A., Abd El-Gabar, M. and Bastawy, M:Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: Role of antioxidants. Toxicology.2001, 165(2–3):217–24.

[12]. Bloemendal H, De Jong W.W, Progress in nucleic acid research and molecular biology. Cohen WE, Moldave K, eds. San Diego

a. Academic Press.1991; 41:259-81.

[13]. Joseph Horwitz. Alpha crystallin. Exp Eye Res. 2003; 76:145-153.

[14]. Mestril R, Dillmann WH. Heat shock proteins and protection against myocardial ischemia. J mol cell cardiol 1995; 27(1): 45-52.

[15]. Golenhoyen N, Htun P , Ness W, Koob R, Schaper W, Drenckhahn D. Binding of the stress protein αB-crystallin to cardiac myofibrils correlates with the degree of myocrdial damage during ischemia/reperfusion invivo. J Mol Cell Cardiol 1999; 31: 569-580.

[16]. Wang K and Spector A. Alpha crystallin can act as a chaperone under conditions of oxidative stress. Invest. Opthol Vis Sci 1995; 369 (2):311-21.
[17]. Borchman D and Tang D. Binding capacity of alpha crystallin to bovine lens lipids. Exp Eye Res. 1996; 63:407-410.
[18]. Tang D and Borchman D. Temperature induced structural changes of beta-crystallin and spingomyelin binding. Exp Eye Res. 1998; 67:113-118.
[19]. Luthra M , Balasubramanian D. Nonenzymatic Glycation alters protein structure and stability . J Biol Chem 1993; 268 (24): 18119-27.
[20]. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227(259): 680-5.
[21]. Vinitha R , Thangaraju M , Sachdanandam P. Effect of tamoxifen on lipid metabolizing marker enzyme in experimental atherosclerosis in wister rats. Mol.cell Biochem 1997; 168 : 13-9.
[22]. J.G.Masilamoni, E.P.Jesudason, S.N.Bharathi, R.Jayakumar. The protective effect of α-crystallin against acute inflammation in mice, Biochim.Biophys.Acta 1740 (2005) 411-420.
[23]. Lowry O.H, Rosenburg N.J, Farr A.I, Randall R.J. Protein measurment with the Folin phenol reagent. J Biol Chm 1951; 193:265-275
[24]. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction..Anal Biochem 1979; 95:351-358.
[25]. Misra H..P, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol chem 1972; 247: 3170-3175
[26]. Beers R.F, Seizer I.W. A spectroscopic method for measuring breakdoen of hydrogenperoxide by catalase. J Biol chem 1952; 115: 133-140.
[27]. Moron M.S, Depierre J.W, Mannervik B. Levels of glutathione reductase and glutath ione-S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979; 582: 67-70.
[28]. Omaye ST, Turnball J, Sauberllich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol 1979, 62: 1-11.
[29]. Desai ID. Vitamin E analysis methods for animal tissues. Methods Enzymol 1984; 105:138-47.
[30]. Bonting S.L. in E.E.Bittar (eds) Membranes and ion transport vol1, Wiley in terscience, London, 1970; 257-263.
[31]. Hjerten S, Pan H. Purification and characterization of two forms of a low-affinity Ca 2+ ATPase from erythrocyte membranes. Biochim Biophys Acta. 1983; 728: 281-288.
[32]. Ohnishi T, Suzuki T, Suzuki Y, Ozawa K. A comparative study of plasma membraneMg 2+ ATPase activities in normal, regenerating and malignant cells. Biochim Biophys Acta 1982; 684:67-74.
[33]. Fiske C.H, Subbarao Y. The colorimetric determination of phosphorous. J Biol Chem 1925; 66: 375-400.
[34]. Mary Astuti.,Yustinus Marsono and Nyoman Sukana, The role of Tempe on lipid profile and lipid peroxides in hyperlipidemic rats. Second International symposium on the role of soy in preventing and treating chronic disease 1996; Sep15-18, Brussells, Belgium
[35]. Marcia Barbosa Aguila., Carla Cota Loureiro., Alessandra da Rocha Pinheiro., Carlos Alberto Mandarim-de-Lacerda. Lipid metabolism in rats fed diets containing different types of lipids. Arg Bras Cardiol 2002; 78 (1) : 32-8
[36]. Nourooz-Zadeh J, Smith CC, Betteridge DJ. Measures of oxidative stress in heterozygous familial hypercholesterolemia. Atherosclerosis 2001; 156 (2): 435-41.
[37]. Aruoma OI. Nutrition and health aspects of free radicals and antioxidants. Food chem Toxicol 1994; 32 (7): 671-83.
[38]. Manzanares D, Bauby C , de la pena D, Garcia JC , Sanchez R , Martinez S , Romay CH , Lopez-Reconde JL, Pino E, Lissi EA. Antioxidant properties of α-crystallin. J Protein chem 2001;3: 181-9.
[39]. Bolotina M, Najibi S, Palacino JJ, Pagano PJ, Cohen RA. Nitric oxide directly activates calcium dependent potassium channels in vascular smooth muscle. Nature 1994; 368: 850-853.
[40]. Ademoglu E , Gokkusu C , Palanduz S . Vitamin E and ATPases: protection of ATPase activities by vitamin E supplementation in various tissues of hypercholesterolemic rats. Int J Vitam Nutr Res 2000; 70 : 3-7.
[41]. F.Shang and A.Taylor. Oxidative stress and recovery from oxidative stress are associated with altered ubiquitin conjugating and proteolytic activities in bovine lens epithelial cells. Biochem.J 1995; 1: 307 : 297-303.

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