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Original Research Article

Effect of Coenzyme $Q_{10,}$ Riboflavin, Niacin, Selenium (CoRNS) and *Emblica officinalis* on Lysosomal Enzymes Studied on Experimental Atherosclerosis

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ABSTRACT

Objective: Our study was aimed to show the effect of nutraceuticals such as Coenzyme $Q_{10,}$ Riboflavin, Niacin and Selenium (CoRNS) and *Emblica officinalis* on high cholesterol diet (HCD) induced atherosclerotic rat model and to show the effect of this formulated drug on the activity of lysosomal enzymes and protein bound carbohydrate (PBC).
Method: The atherosclerotic induced rats were treated with (CoRNS) and *Emblica officinalis* and the activities of the lysosomal enzymes and protein bound carbohydrates were studied.
Result: Treatment with (CoRNS) and *Emblica officinalis* has decreased the activity of the lysosomal enzymes and PBC.
Conclusion: The atherosclerotic rats were brought to normal conditions on treatment with (CoRNS) and *Emblica officinalis*.

Key words: cardiovascular disease, cathepsin, hexosamine and hexuronic acid, hexose.

INTRODUCTION

Atherosclerosis is one of the major cardiovascular disease (CVD), has emerged as a major health burden worldwide. Atherosclerosis has become dreadful affecting the Indian population at a younger age than the other groups. Prevalence of coronary artery disease is seen both in the urban as well as in rural India. [(1)] Change in food pattern and lack of exercise may lead to increase in the prevalence of obesity and consequently CVD. [(2)] Intake of high fat diet may cause accumulation of fatty substance in the walls of the arteries and block the blood flow, leading to atherosclerosis. Oxidative stress may be due to increase in diet-induced hypercholesterolemia. Many researches have proved that the major risk factors of CVD are cholesterol, blood pressure, smoking and lack of physical activity. [(3)] The use of herbal plants has been practiced by many researches for the cure of many diseases including heart diseases. *Emblica officinalis* (EO) commonly called Amla by Indians and gooseberry in English has been used for the cure for CVD. [(4)] The powdered Amla fruit along with Coenzyme $Q_{10,}$ Riboflavin, Niacin and Selenium (CoRNS) was given to the atherogenic rats in our study. The activity of the lysosomal enzymes and protein bound carbohydrates were studied. The high fat diet given to the rats caused changes in the activity of the lysosomal enzymes and protein bound carbohydrates. The induction of the

nutraceuticals viz (CoRNS) and EO has reverted the condition to normal.

MATERIALS AND METHODS

Chemicals Coenzyme Q_{10} used for our study was purchased from Kaneka Corporation, Japan. Selenium in the form of Sodium Selenite, Riboflavin and Niacin were purchased from Madras Pharmaceutical, India. Powered form of *Emblica officinalis* was purchased from IMCOPS, Chennai. The other chemicals, solvents of analytical grade, cholesterol and cholic acid were obtained from Sisco Research Laboratories, Mumbai, India.

Animal model

Male albino rats of Wistar strain (150±10g) were housed in spacious cages and were given food and water *ad libitum* under standard conditions of controlled temperature (25°C±2°C) with 12h/12h day-night cycle. They were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai. Animals were used after obtaining prior permission and handled according to the University and Institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on animals (IAEC No.02/027/09), Ministry of Social Justice and Empowerment, Government of India.

Experimental setup

The animals were divided into five groups of six rats each. Group I served as control. Group II, Group IV and V were fed with high cholesterol diet (HCD) comprising of the normal chow supplemented with 4% cholesterol, 1% cholic acid for 30 days. After 30 days, Group IV animals were treated with the reference drug Simvastatin (10mg/kg bwt/day). Group V animals were treated with CoRNS and *Emblica officinalis* (CoQ_{10} (20mg/kg bwt/day), Riboflavin (40mg/kg bwt/day), Niacin (100mg/kg bwt/day), Selenium (0.17mg/kg bwt/day) and *Emblica officinalis* (EO) (100mg/kg bwt/day). Group III animals served as drug control (CoRNS and EO alone). CoQ_{10} was dissolved in corn oil. All the other drugs were dissolved in water and were given by oral gavage for 30 days. At the end of the experimental period, all the animals were sacrificed by cervical decapitation. Heart was immediately excised and rinsed in ice cold physiological saline. The tissues were homogenized in ice cold 0.01M Tris-HCl buffer, pH 7.4 and centrifuged to give 10% homogenate and aliquots of this were used for the assay. Blood was collected with EDTA as anticoagulant for the preparation of plasma. Plasma was separated by centrifugation for 20 minutes.

Assay of lysosomal Enzymes

D-Glucuronidase was assayed by the method of Kawai and Anno. [(5)] DGalactosidase activity was assayed by the method of Kawai and Anno. [(5)] Lysosomal proteases- Cathepsin D aspartyl protease Cathepsin D activity was assayed by the method of Sapolsky. [(6)] Estimation of Acid phosphatase**.** This enzyme was assayed by the method of King [(7)] using disodiumphenyl phosphate as the substrate.

Estimation of protein bound carbohydrates in plasma

Hexose was estimated by the method of Niebes. [(8)] Hexosamine was estimated by the method of Wagner. [(9)] Hexuronic acid was estimated by the method of Bitter and Muir. [(10)] Sialic acid was determined by the method of Warren. [(11)]

Statistical analysis

The values are expressed as mean ± standard deviation (S.D) for six animals in each group. Difference between groups was assessed by one way analysis of variance (AVONA) using SPSS 20 software package for windows. Post hoc testing was formed for inter-group comparison using the least significance difference (LSD) test, significance at *p*-values <0.001, <0.01, <0.05 have been given respective symbols.

RESULTS AND DISCUSSION

Table 1and 2 depict the activities of lysosomal enzymes namely acid phosphatase (ACP), glucuronidase (-Glu),

galactosidases (-gal), N-acetyl- D glucosaminidase (NAG) and Cathepsin-D(Cat-D) in liver, plasma and heart of control and experimental animals. The activity of these enzymes were significantly increased in the HCD animals (Group II) when compared to the control rats in the plasma and the tissues such as liver and heart. The Group IV and V animals treated with the standard drug and CoRNS and EO have shown significant decrease in the activities of these enzymes when compared to Group II animals. There was no significant change observed in the drug control animals.

Lysosomes are the group of organelles present in numerous animal tissues. The vesicles that are present in the cytoplasm have hydrolytic enzymes. They convert cellular components into simple low molecular weight compounds by intracellular digestion that can be reused by the cell. Disturbances in the Lysosomal membrane can result in the release of lysosomal enzymes. [(12)] This reduces the membrane integrity. [(13)] Lysosomal enzymes have been studied extensively in atherosclerosis. In most of the animals, lysosomal damage is well established as a biomarker of stress. Damage in the Lysosomal membrane causes the release of hydrolytic enzymes which in turn damage the cells and tissues. The atherosclerotic condition may elevate the activity of these enzymes. Lysosomal enzymes play an important role in the inflammatory process. The modification of tissue constituents caused by these enzymes which are of mitochondrial and lysosomal origin may play an important role in myocardial ischemia. The inflammatory mediators like oxygen radicals, prostaglandins are stimulated by the Lysosomal enzymes which are mediators of acute myocardial infarction. [(14)] Decreased lysosomal membrane stability is caused by elevated lysosomal enzymes in the extracellular fluid. Glycosaminoglycans, glycoprotein, collagen in different connective tissue would be affected. Membranous instability would cause cardiac damage. The oxygen free radicals generated during ischemia, and the release of lysosomal enzymes may cause cardiac damage. [(15)] The membrane deterioration of lysosomes by HCD induced LPO could have resulted in the leakage of enzymes from the enclosed sacs. The increased activities of glycohydrolases and cathepsin-D observed in this study indicate the possible infiltration of inflammatory cells at the site of infarction.

The free radicals' presence may cause the release of-Glucuronidase, a sensitive marker of lysosomal integrity. [(16)] Cathepsin-D(Cat-D) is a ubiquitous aspartyl endoprotease sorted to the lysosomes and found intracellularly. During some physiological and pathological conditions, Cat-D is secreted by various cell types. Human bovine, and rat milk has procathepsin D. [(17)] The elevated activity of these enzymes may be due to the leakage of these enzymes due to cellular damage by the free radicals generated due to HCD supplemented to the Group II animals. The animals treated with CoRNS and EO has decreased activity of these enzymes due to their antioxidant property which quench the free radicals. The reduced oxidants maintain the cell integrity and thus, maintain the normal levels of these enzymes.

Table 1: Effect of CoRNS and EO on the activity of lysosomal enzymes on liver of control and experimental animals

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
ACP	1.9±0.17	5.21±0.39^{a*}	1.9±0.17	2.50±0.25^{b*}	2.47±0.20^{c*}
β-Glu	22.97±3.00	39.12±4.17$^{a@}$	31.82±3.64	25.92±2.95$^{b@}$	22.25±2.41$^{c@}$
β-gal	13.64±1.25	22.94±1.58^{a*}	13.50±1.16	18.25±1.7^{b*}	17.34±1.61^{c*}
NAG	32.49±4.34	40.18±3.98^{a*}	35.24±2.81	33.18±2.18^{b*}	32.27±4.01^{c*}
Cat-D	0.15±0.01	0.31±0.03^{a*}	0.14±0.01	0.19±0.02^{b*}	0.18±0.02^{c*}

Values are expressed as mean ± S.D of six animals. Unit: ACP-µmoles of phenol formed/h/mg protein: β-Glu, β-gal and NAG-µmoles of p-nitrophenol formed/h/mg protein:

Cat-D- µmoles of tyrosine liberated/ h/mg protein. For statistical significance a-Group I Vs Group II; b-Group II Vs Group IV; c-Group II Vs Group V. $^{@}$ p<0.05, # p<0.01, *p<0. 001.

Table 2: **Effect of CoRNS and EO on the activity of lysosomal enzymes on plasma and heart of control and experimental animals**

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V
Plasma					
ACP	1.50±0.08	3.42±0.27^{a*}	1.60±0.09	2.07±0.16^{b*}	2.09±0.16^{c*}
β-Glu	2.25±0.16	4.80±0.38 ^{a*}	2.31±0.16	2.47±0.24 ^{b*}	2.44±0.21 ^{c*}
β-gal	1.50±0.11	2.40±0.19 ^{a*}	1.52±0.12	1.74±0.14 ^{b*}	1.72±0.14 ^{c*}
NAG	2.40±0.16	3.6±0.35 ^{a*}	2.38±0.17	2.93±0.19 ^{b*}	2.90±0.18 ^{c*}
Cat-D	0.30±0.02	0.07±0.06 ^{a*}	0.31±0.02	0.39±0.04 ^{b*}	0.37±0.04 ^{c*}
Heart					
ACP	0.52±0.04	0.94±0.07 ^{a*}	0.50±0.04	0.57±0.04 ^{b*}	0.55±0.04 ^{c*}
β-Glu	18.69±1.97	28.74±3.19$^{a@}$	18.51±1.92	23.98±2.49$^{b@}$	20.16±2.17$^{c@}$
β-gal	3.25±0.25	5.34±0.38 ^{a*}	3.23±0.24	3.70±0.31 ^{b*}	3.64±0.30 ^{c*}
NAG	31.32±2.66	74.35±6.31 ^{a*}	32.15±2.51	40.12±3.11 ^{b*}	39.60±3.29 ^{c*}
Cat-D	0.13±0.01	0.48±0.04 ^{a*}	0.11±0.01	0.25±0.02 ^{b*}	0.24±0.02 ^{c*}

Values are expressed as mean ± S.D of six animals. Unit: ACP-µmoles of phenol formed/h/mg protein: β-Glu, β-gal and NAG-µmoles of p-nitrophenol formed/h/mg protein: Cat-D- µmoles of tyrosine liberated/ h/mg protein. For statistical significance a-Group I Vs Group II; b-Group II Vs Group IV; c-Group II Vs Group V. $^{@}$ p<0.05, # p<0.01, *p<0. 001

Table 3 and 4 show the levels of glycoprotein components such as hexose, hexosamine, hexuronic acid and sialic acid in plasma, heart, liver and kidney of control and experimental animals**.** Group II animals fed with HCD have shown significant increased levels of hexose, hexosamine, hexuronic acid and sialic acid when compared to the control animals. The Group IV and Group V animals treated with Simvastatin and CoRNS and EO showed significant reduction in the glycoprotein component levels. Drug control animals did not show any significant changes. Glycoproteins play an important role in mediating cell-cell recognition, cellular adhesion, antigenicity, intracellular processing of proteins and cell activation. It has been reported that changes in collagen, elastin, and glycosaminoglycans of an arterial wall of humans and experimental atherosclerosis. [(18)] In the aorta of diet induced atherosclerotic monkeys, the glycosaminoglycans of an arterial wall are in the protein bound form such as proteoglycosaminoglycans. These are reported to be involved in altered permeability, lipid metabolismand homeostasis. [(19)] Sialic acids are a family of *N*- and *O*-substituted derivatives of neuraminic acid, an amino sugar, contributes to the pathogenesis of atherosclerosis and as a predictor of cardiovascular events. These are generally found as terminal sugar residues on oligosaccharides of both glycoprotein and glycolipid. Their functions include conformational stabilizations, cellular recognition, protein targeting, developmental regulation etc., Increased sialic acid in blood has been reported as one of the markers for atherosclerosis, cancer, etc. Feeding the rats with HCD may cause secretion (or) shedding of glycoproteins from cell membrane into the circulation due to peroxidative damage by free radical formation. [(20)] Increased inflammations with concomitant elevation in plasma and tissue hexose, hexosamine, hexuronic acid and sialic acid have been reported in animals fed with cholesterol. On treatment with Coenzyme Q_{10}, Riboflavin Niacin, Selenium and EO, glycoprotein components were reverted back to near normal levels. This could be due to the cyto-stabilizing property of this drug. This drug might have altered cell membrane glycoprotein synthesis and structure. The drug also has anti-inflammatory property. They preserve an endothelial function during myocardial infarction. The cyto-stabilizing property and anti-inflammatory property has attributed to

the reduced levels of protein bound carbohydrates in Group V animals.

Table 3: Effect of CoRNS and EO on the protein bound carbohydrates in plasma and heart of control and experimental animals

PBC	Group-I	Group-II	Group-III	Group-IV	Group-V
Plasma					
Hexose	121.62±14.59	200.62±17.05[a]	123.71±14.91	157.42±20.46[b]	156.81±20.38[c]
Hexosamine	27.14±2.44	41.80±5.42 [a]	27.29±3.24	35.17±5.00 [b]	34.82±4.87[c]
Hexuronic acid	43.54±4.57	67.01±10.05[a]	43.11±4.04	55.72±6.15[b]	54.90±4.03[c]
Sialic acid	38.23±4.20	61.82±4.94[a]	38.45±4.75	52.10±6.90[b]	50.81±5.10[c]
Heart					
Hexose	2.62±0.25	3.82±0.46[a]	2.52±0.20	3.30±0.38[b]	3.24±0.24[c]
Hexosamine	1.91±0.17	3.10±0.39[a]	1.93±0.16	2.54±0.30[b]	2.52±0.30 [c]
Hexuronic acid	2.17±0.21	3.24±0.43 [a]	2.21±0.26	2.70±0.33[b]	2.65±0.32 [c]
Sialic acid	170.14±14.63	268.29±30.8 [a]	164.68±19.77	229.95±2.66[b]	222.57±21.75 [c]

Values are expressed as mean ± S.D of six animals. Units: Plasma-Hexose, Hexosamine, Hexuronic acid and Sialic acid-mg/dl. Heart- Hexose, Hexosamine, Hexuronic-mg/g tissue. Sialic acid-µg/g tissue.For statistical significance a-Group I Vs Group II; b-Group II Vs Group IV; c-Group II Vs Group V. The symbols [a b c] represent significance at [@] $p<0.05$.

Table 4: Effect of CoRNS and EO on the protein bound carbohydrates in liver and kidney of control and experimental animals

PBC	Group-I	Group-II	Group-III	Group-IV	Group-V
Liver					
Hexose	2.40±0.24	3.63±0.43[a]	2.41±0.19	2.54±0.28[b]	2.50±0.27[c]
Hexosamine	2.05±0.25	2.98±0.23 [a]	2.05±0.25	2.49±0.24 [b]	2.36±0.33 [c]
Hexuronic acid	3.40±0.35	6.08±0.72 [a]	3.40±0.27	4.73±0.52 [b]	3.69±0.41 [c]
Sialic acid	210.22±22.07	342.61±47.31 [a]	204.90±19.18	235.21±24.69 [b]	225.25±23.40 [c]
Kidney					
Hexose	2.95±0.28	4.64±0.62 [a]	2.95±0.36	3.81±0.48 [b]	3.03±0.40 [c]
Hexosamine	2.05±0.25	2.99±0.45 [a]	1.98±0.18	2.38±0.6 [b]	2.08±0.22 [c]
Hexuronic acid	3.24±0.37	5.01±0.43 [a]	3.32±0.41	4.11±0.55 [b]	3.36±0.31 [c]
Sialic acid	198.26±21.89	293.26±3.60 [a]	195.28±25.67	222.12±25.54 [b]	208.17±23.35 [c]

Values are expressed as mean ± S.D of six animals. Unit: Hexose, Hexosamine, Hexuronic-mg/g tissue. Sialic acid-µg/g tissue. For statistical significance a-Group I Vs Group II; b-Group II Vs Group IV; c-Group II Vs Group V. The symbols [a b c] represent significance at [@] $p<0.05$.

CONCLUSION

The combined effects of the nutraceuticals have the membrane protective action and thus have reverted back to normal condition and hence have cardio protective action.

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