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**R E S E A R C H A R T I C L E**

# Combined effect of Coenzyme $Q_{10}$, Riboflavin, Niacin, Selenium (CoRNS) and *Emblica Officinalis* studied on experimental atherosclerosis in rats

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**A B S T R A C T**

Cardiovascular diseases are becoming more prevalent in the world. Atherosclerosis is one of the most widespread and leading diseases which causes death in most developed countries. The present study is aimed at bringing about the anti-atherogenic effect of the herbal drug, *Emblica Officinalis* in combination with natural respiratory antioxidants viz., Coenzyme $Q_{10}$, Riboflavin, Niacin and Selenium (CoRNS) in a high cholesterol (HCD) induced atherosclerotic rat model. The HCD brought changes in the activity of the lipid metabolizing enzymes and membrane bound ATPases in the rats. Upon treatment with CoRNS and *Emblica Officinalis*, the activity of the enzymes was brought to normal.

**Key words:** Lipid, lipid metabolizing enzymes, ATPases, cardiovascular disease.

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**C O N T E N T S**



## 1. Introduction

Atherosclerosis is one of the major cardiovascular diseases [CVD] causes 80% deaths in the world [1]. The change in life style and food habits are the causes for the deaths in the developed countries. It is caused due to the deposition of carbohydrates, fatty substances and other cellular debris in the arterial walls which becomes hardened and blocks the arteries. [2]. All these substances forms plaque, which block the flow of blood leading to cardiac arrest. The most well

known risk factor is cholesterol which is the main cause for the formation of atherosclerotic plaque[3]. A dietary food rich in saturated fat and cholesterol leads to elevated levels of circulating cholesterol levels particularly, LDL-C. The free and esterified cholesterol may undergo oxidation when exposed to oxidative stress. It has been proved that hypercholesterolemia increases the incidence of ischemic disease by restricting blood flow in lesion-prone arteries. Diet mixed with cholesterol and cholate may promote atherosclerosis [4]. Advancement in treatment and therapies has been evolved for the treatment of atherosclerosis. Nutraceuticals have been used by many researches to carry out research for the prevention of cardiovascular disease and cancer [5]. These nutrients are rich in vitamins and minerals that provide health or disease prevention. Nutraceuticals may act as an essential amino acid drug, phytosterols, etc. Medicinal plants and herbs have shown significant inhibition of cell inflammation. Studies show that Coenzyme $Q_{10}$, selenium, copper, manganese, and zinc act as anti-oxidant, preventing cardiovascular disease in cardiac cells [6]. Niacin is found to protect heart cells [7]. The tannoids, phyllemblic acid and two hydrolysable tannins emblicanin A and B, present in aqueous extract of the fruits of *Emblica Officinalis* [EO], commonly called as Amla, were found to scavenge free radicals and superoxides and also possess antioxidant property[8][9]. FAD dependant enzyme, glutathione reductase works in conjugation with Riboflavin, a vitamin acting as antioxidant that participates in the redox cycle of glutathione. Riboflavin is involved in regenerating glutathione, the main cellular protector against free radical damage. Riboflavin has anti-atherosclerotic property secondary to antioxidant action. It protects against lipid peroxidation and oxidation of LDL. Individually all these nutrients have antioxidant property. When combined and given as drug, they show potent antioxidant activity and hence have anti-atherogenic property.

## 2. 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 purchasedfrom 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$^{o}$C±2$^{o}$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* (Amla),($CoQ_{10}$),(20mg/kg bwt/day), Riboflavin (40mg/kg bwt/day), Niacin (100mg/kg bwt/day), Selenium (0.17mg/kg bwt/day) and *Emblica Officinalis* (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 activity of lipid metabolizing enzymes in plasma, heart and liver:** Total lipase was assayed by the method of Bier [10]. The lipoprotein lipase [LPL] was assayed by the method of Schmidt [11]. Cholesterol ester synthetase [CES] and Cholesterol ester hydrolase [CEH] was assayed by the method of Kothari et al., [12] with slight modification by Kritchevsky and Kothari [13]. Lecithin-cholesterol acyl transferase [LCAT] was assayed by the method of Legraudet al.,[14] with modifications of Hitzet al.,[15].

**Assay of activity of erythrocyte membrane bound ATPases:** Preparation of hemolysate and isolation of erythrocyte membrane was carried out by the method of Dodge et al., [16]. Erythrocyte lipid peroxide content was determined by the method of Cynamonet al. [17] Estimation of adenosine triphosphatases in erythrocyte membrane. Estimation of phosphorus content by the method of Fiske and Subbarow [18]. $Na^{+}$, $K^{+}$ ATPase was assayed according to the method of Bonting [19]. $Ca^{2+}$ ATPase was estimated as described by the method of Hjerten and Pan[20]. $Mg^{2+}$ATPase was assayed by the method of Ohnishi et al., [21].

**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 19.0 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.

## 3. Results and Discussion

**Results:** Table 1 and 2 show the activity of lipid metabolizing enzymes in plasma, liver and heart of control and experimental animals. The enzymes such as lipoprotein lipase [LPL], cholesterol ester hydrolase [CEH], cholesterol

ester synthetase [CES] and lecithin cholesterol acyl transferase [LCAT] are the enzymes that metabolize the lipids in the plasma as well as liver and heart tissues. The activities of all these enzymes were significantly altered in the atherogenic animals [Group II]. In atherogenic animals, decreased activity of LPL and CEH and increased activity of CES were observed in plasma and also in heart and liver tissues.

The activity of LCAT was also observed to be decreased in plasma and tissues. No significant alterations in the activity of these enzymes were observed in the drug control animals. The Group IV and V animals treated with Simvastatin and CoRNS and EO respectively showed increased activity of LPL and CEH and decreased activity of CES in plasma as well as in tissues. The activity of LCAT in plasma was increased to near normal levels. The Group V animals treated with CoRNS and EO showed normalization in the levels of all the enzymes.

Fig 1 indicates the levels of lipid peroxides in erythrocyte membrane of control and experimental animals. An elevated level of LPO was observed in the Group II animals when compared to the control rats. The Group IV and V animals showed significant reduction in the LPO levels when compared to Group II animals. No significant alteration in drug control animals [Group III] when compared to Group I animals was observed. Figure 2 depicts the activity of ATPases enzymes in the erythrocyte membrane of control and experimental animals. The activity of $Na^+/K^+$, $Mg^{2+}$ and $Ca^{2+}$ATPases were significantly decreased in Group II atherogenic animals when compared to control animals. A significant increase in the activities of the ATPases was observed in Group IV and V animals treated with Simvastatin and CoRNS and EO respectively when compared with Group II animals. No significant alteration was found in drug control animals.

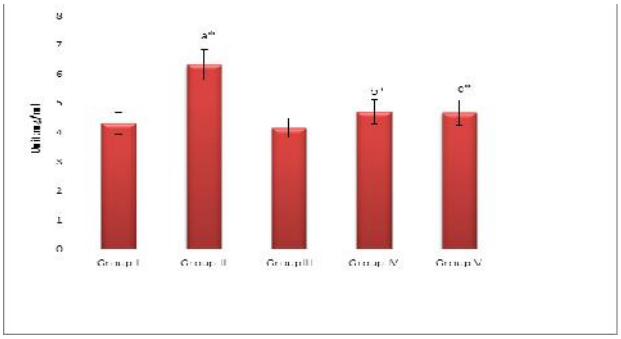

**Fig 1 :**Effect of CoRNS and EO on Erythrocyte LPO in control and experimental animals

Values are expressed as mean ± S.D of six animals. Unit: µmoles of MDA released /mg protein. 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 also represents statistical significance* p<0.001.

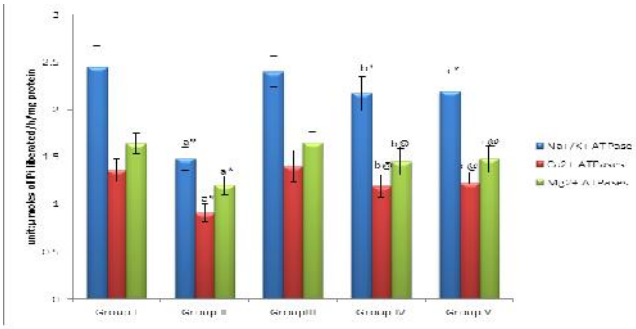

**Fig 2:**Effect of CoRNS and EO on membrane bound ATPases of control and experimental animals

Values are expressed as mean ± S.D of six animals. 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 1:**The effect of CoRNS and EO on the activities of plasma lipid metabolizing enzymes in the control and experimental animals

| Enzymes | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---|---|---|---|---|---|
| LPL | 5.20±0.63 | 3.46±0.28$^{a@}$ | 5.30±0.51 | 4.50±0.59$^{b@}$ | 4.85±0.45$^{c@}$ |
| LCAT | 7.52±0.77 | 4.95±0.74$^{a@}$ | 7.64±0.72 | 6.12±0.45$^{b@}$ | 6.95±0.67$^{c@}$ |
| CES | 3.90±0.34 | 5.81±0.76$^{a@}$ | 3.84±0.46 | 4.25±0.53$^{b@}$ | 3.98±0.50$^{c@}$ |
| CEH | 5.31±0.60 | 3.45±0.31$^{a@}$ | 5.47±0.68 | 4.31±0.43$^{b@}$ | 4.86±0.56$^{c@}$ |

**Table 2:**The effect of CoRNS and EO on the activities of lipid metabolizing enzymes in hepatic and cardiac tissue of control and experimental animals

| Enzymes | Group-I | Group-II | Group-III | Group-IV | Group-V |
|---|---|---|---|---|---|
| **Heart** | | | | | |
| LPL | 10.23±0.95 | 6.90±0.64$^{a@}$ | 10.31±0.85 | 8.73±0.88$^{b@}$ | 9.43±1.05$^{c@}$ |
| CES | 8.42±0.78 | 13.01±1.68$^{a@}$ | 8.53±0.71 | 10.80±1.08$^{b@}$ | 9.17±1.39$^{c@}$ |
| CEH | 3.11±0.30 | 2.11±0.29$^{a@}$ | 3.21±0.38 | 2.96±0.34$^{b@}$ | 3.10±0.32$^{c@}$ |
| **Liver** | | | | | |
| LPL | 19.16±1.65 | 12.60±1.63$^{a@}$ | 19.53±2.37 | 16.52±1.75$^{b@}$ | 17.36±2.16$^{c@}$ |
| CES | 16.32±1.85 | 27.75±1.95$^{a@}$ | 16.07±1.98 | 18.70±1.97$^{b@}$ | 17.78±1.75$^{c@}$ |
| CEH | 19.21±1.82 | 13.78±1.70$^{a@}$ | 19.35±2.37 | 17.13±2.05$^{b@}$ | 18.92±2.08$^{c@}$ |

Values are expressed as mean ±S.D for six animals. Units; LPL-µmoles of Free fatty acids liberated/mg protein/h; LCAT-µmoles of cholesterol esterified /mg protein/h; nmoles of cholesterol esterified /mg protein/h; CEH- nmoles of cholesterol esterified /mg protein/h.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 also represents statistical significance @$p<0.05$.

**Discussion**

Lipids are involved in maintaining the integrity and the fluidity of the cellular membrane components and their modification changes the quality in the membrane which may affect electrolyte balance, enzyme activity and hence the overall cell functions. Increased intake of fats through diet may enhance the development of atherosclerosis. It has been reported that increase in the synthesis of fatty acid and triglyceride in the liver may be due to cholesterol fed to rats [22].The reduced rate of esterification of cholesterol may have decreased the activity of LCAT and other lipid metabolizing enzymes such as LPL and CEH and increased activity of CES in atherosclerosis. This causes increase in the levels of total and free cholesterol in atherosclerotic animals[23].In the aorta, the cholesterol is deposited as cholesterol esters when high cholesterol diet is taken [24]. LPL is an enzyme bound to the walls of the blood capillaries hydrolyzing triglycerides to form glycerol and fatty acid, renders a good proportion of lipids to tissue and thus, protects the system from deleterious effects of lipid abnormalities [25]. LCAT and other metabolizing enzymes showed elevated levels and decreased activity of CES in animals treated with EO along with CoRNS.

The increased level of LCAT causes the transport of cholesterol in the blood and to the liver for further metabolism [26]. So combination of all these nutrients given as drug is likely to reduce the lipid profiles to near normal levels in Group V animals. The drug may increase the activity of LPL, LCAT and other metabolizing enzymes anddecreases activity of CES and hence, keeps the lipid profile in a controlled manner. All the drugs possess hypolipidemic property individually. When given in combination, the additive effect of all these components may be responsible for the highly normalized level of lipid components in the animals treated with the drug. The results indicate that the nutraceuticals possess hypolipidemic effect and hence, can act as an anti-atherogenic agent in combination.

The enhanced level of lipid peroxides observed in the Group II animals may be due to HCD feeding, which induce free radical production in rats. Enhanced malondialdehyde [MDA] production may be due to Lipid peroxidation [27]. Peroxides cause damage to membranes. The animals treated with CoRNS and EO [Group V] showed marked reduction in LPO. This may be due to the free radical quenching property of these nutraceuticals. ATPases are transmembrane enzymes transports solutes across the membrane, typically against their concentration gradient. Under high cholesterol diet, Sodium-potassium exchanger [$Na^+/K^+$ ATPase], activity has been found to be altered in various cell types. They help in the transport of sodium and potassium ions across the cell membrane with the help of the energy released by the hydrolysis of ATP. Reduction in the activity and $Na^+/K^+$ ATPase in erythrocyte membranes is due to lipid peroxidation and increased free radical production in the erythrocytes due to changes in antioxidant status. [28][29]. In many heart problems, $Na^+/K^+$ ATPase may be considered as an index for the complications [30]. The plasma membrane have many transporters. One such is the $Ca^{2+}$ ATPase which is the major active calcium transport protein which maintains the normal intracellular calcium levels in almost all the cell types. In cardiac dysfunction and other conditions, abnormal $Ca^{2+}$ATPase activity and intracellular calcium levels were reported as important one [30]. Inhibited activity of $Ca^{2+}$ ATPase in the Group II animals may be due to activated oxygen and depletion of thiols status. Reactive oxygen species formed may attack the membranes of intracellular organelles and lead to a decrease in cardiac $Ca^{2+}$ ATPase activity [31].Increased oxidative stress which induces decreased membrane fluidity has been linked to the abnormalities in calcium metabolism [32]. $Mg^{2+}$ ATPase present in the plasma membrane, actively transport of magnesium across cell membrane.

The reduced activity of $Mg^{2+}$ ATPase may be due to the LPO caused due to high fat diet, which might have reduced the antioxidant status, thereby damaging the membrane. Membrane fluidity has a strong influence on important membrane functions such as the conformation and the activity of membrane associated enzymes [33]. Reduction in the activities of membrane-bound enzymes is due to the oxidative damage of membrane in atherosclerosis. In cardiac cells, ATPases plays a vital role in the contraction and relaxation cycles of the cardiac muscle by maintaining normal ion levels [$Ca^{2+}$, $Na^+$, $K^+$, $Mg^{2+}$] within the myocytes. Changes in the properties of these ion pumps affect the cardiac function.

The treatment of the Group V animals with CoRNS and EO might have stabilized the membrane property.Myocardial calcium-dependent ion channels have been stabilized by $CoQ_{10}$ which prevent the depletion of metabolites essential for ATP synthesis [34]. $CoQ_{10}$ decreases the viscosity of the blood. It also improves blood flow to cardiac muscle in ischemic heart disease [35]. The $Na^+/K^+$ ATPase and $Na^+$ gradient helps in the absorption of Selenate maintained by sulphate mechanism [36]. It has been studied that Selenium causes increase in reduced glutathione. [37] Peroxidative damage may be prevented by selenium [38]. All these cluster of properties may be responsible for the improvement of ATPases activities in CoRNS and EO treated animals. In the present study, the decrease in the activity of ATPases may be due to LPO caused by atherogenesis in the Group II animals. The combined effect of CoRNS and EO treatment could have significantly increased the activity of all the ATPases by means of its antioxidant property.

## 4. Conclusion

The cholesterol diet has altered the membrane integrity of erythrocytes which may be due the elevated levels of circulating lipids in the HCD fed animals. HCD fed animals showed decreased activity of lipid metabolizing enzymes which causes increased circulation of fatty substances. Treatment with CoRNS and EO would have stabilized the membrane integrity of the erythrocytes and channelized the movement of $Na^+/K^+$, $Ca^{2+}$ and $Mg^{2+}$ ions. The activity of the lipid metabolizing enzymes have been stabilized which in turn have maintained the erythrocyte membrane.

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