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Effect of Nicorandil: A Potassium Channel Opener against Experimentally-induced Hyperlipidemia

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Abstract: Hypercholesterolemia often occurs in conjunction with other metabolic risk factors including glucose intolerance, obesity, diabetes and metabolic syndromes. Nicorandil is a potassium channel opener and Nitric Oxide (NO) donor. The aim of study was to evaluate pharmacological effect of nicorandil (2 mg kg^{-1}, orally) on different lipid levels, enzyme-hydroxymethylglutaryl Coenzyme A (HMG-CoA) reductase and antioxidant enzymes. The lipid parameters, HMG-CoA reductase activity and antioxidant enzymes were evaluated in poloxamer-407 (acute model) and high-cholesterol diet-induced hyperlipidemia (chronic model) in Sprague dawley rats. The animals were divided into four group's viz., normal, high cholesterol diet (control), atorvastatin and nicorandil treated animals. In poloxamer-407-induced acute hyperlipidemia model, blood samples were collected at 15 and 24 h period. In high-fat diet-induced model, animals were given respective treatment for the period of twenty one days. Lipid parameters were commonly measured in both the models and compared with reference standard (atorvastatin; 50 mg kg^{-1}). In high fat diet model, antioxidant parameters were additionally measured in the terms of lipid peroxidation (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). High cholesterol diet and poloxamer-407 caused a significant increase in lipid parameters in rats. Nicorandil pre-treatment showed a significant decrease in total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C), very low density lipoproteins-cholestero l(VLDL-C) and atherogenic index (AI) in both the models. The results were comparable with that of atorvastatin treated animals. Further, nicorandil showed significant decrease in MDA and SOD along with significant increase in GSH and CAT against high-fat diet model. Based on our data, it is suggested that nicorandil possess hypolipidemic activity. The mechanism of action of this antihyperlipidemic activity of nicorandil could be attributed to its releasing nitric oxide property and inhibiting oxidative stress.

Key words: Hypercholesterolemia, lipid peroxidation, antioxidants, nicorandil, atorvastatin

INTRODUCTION

Hypercholesterolemia is a common risk factor for early atherosclerosis prior to the appearance of over atherosclerotic changes in the vascular wall; it induces vascular functional changes that may lead to local ischemia and vascular remodeling (Bentley *et al*., 2002). Clinical trials lane show that lowering lipids reduces the morbidity and mortality associated with cardiovascular complication (Amundsen *et al*., 2002).

Epidemiological studies have established a link between dyslipidemia and coronary artery diseases. Vascular endothelial dysfunction has been associated with various disorders such as hypertension, coronary artery disease, atherosclerosis and stroke (Balakumar *et al*., 2007). A higher level of plasma low-density lipoprotein (LDL) cholesterol is a key risk factor of atherosclerosis. Current hypothesis suggested that LDL oxidation, endothelial dysfunction and inflammation are involved in the pathogenesis of atherosclerosis (Steinberg and Witztum, 1999).

The oxidant systems are free radicals, molecules or molecular fragments containing one or more unpaired electron (Valko *et al*., 2007). Oxidative stress produced by free radicals has been linked to the development of several diseases such as cardiovascular, cancer and neurodegenerative diseases and also with ageing (Witzum, 1994; Southom and Powis, 1988). Oxidation of the lipid core of low-density lipoproteins leads to a change in the lipoprotein conformation. After this oxidation, LDL is better to enter into the monocyte/macrophage system of the arterial wall and develop the atherosclerotic plaques (Witzum, 1994). Lipids, DNA and proteins are oxidized by free radicals.

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Free radicals induced oxidation of lipids is controlled by a wide spectrum of enzymatic antioxidants and non-enzymatic antioxidants such as superoxide dismutase and glutathione peroxides (GSHPx), vitamin E and glutathione (Valko *et al*., 2007). Some non-enzymatic antioxidants such as vitamins C, vitamin E, carotenoids and phenolic compounds may be key factors in the pathogenesis of oxidative stress related disorders (Southom and Powis, 1988; Valko *et al*., 2007).

Nitric oxide (NO) is a crucial modulator of vascular damage. Indeed, NO has number of intracellular effects that lead to vasorelaxation, endothelial regeneration, reduction of oxidative mechanism, inhibit leukocyte chemotaxis and platelet adhesion (Napoli *et al*., 2001). Currently, Statin is the first choice for lowering cholesterol especially LDL cholesterol levels (Hasimun *et al*., 2011). They may also have anti-inflammatory, anti-proliferative and anti-oxidant effects (Elhaleem and Elsayed, 2011). Previous studies have demonstrated that statins regulate eNOS expression and subsequent NO synthesis and NO-mediated endothelium dependent relaxation (Prieto *et al*., 2008). Nicorandil [N-(2-hydroxyethyl) nicotinamide nitrate (ester)] is a potent orally administered vasodilator which is structurally a nitrate and nicotinamide (Fukunaga *et al*., 2010). The vasoactive effect of nicorandil on coronary arteries is well known. Nicorandil exerts its vasodilatative effect through cGMP formation and opening of K^+ channels in variety of cells (Brodmann *et al*., 2006).

In the light of above report, this study was aimed to evaluate the effects of nicorandil against experimentally-induced acute and chronic hyperlipidemia in Sprague dawely rats.

MATERIALS AND METHODS

Animals: Healthy rats (Sprague-Dawley strain) of either sex weighing 180-220 g were divided into four different groups having each of six (Table 1). Normal group received standard pellet diet (Pranav agroindustries, Vadodara, India). Animals were treated for 21 days. The animals were housed in a group of 3 rats per polypropylene cage under well- controlled conditions of temperature (22±2°C), humidity (55±5%) and 12 h/12 h light-dark cycle. Water was made available *ad libitum*. The study was approved by the institutional animal ethics committee established in accordance with committee for the purpose of supervision and control of experiments on animals (CPCSEA).

Chemicals and drugs: All chemicals and reagents used in present study were of analytical grade and were purchased from S.D. finechemicals Ltd., Bombay.

Table 1: Animal groups and respective treatments

Group	Treatment groups
I	1% carboxy methyl cellulose
II	Hyperlipidemic control
III	Nicorandil
IV	Atorvastatin

Atorvastatin and Nicorandil were obtained as gift samples from Astron Pharmaceuticals Ltd., Ahmadabad. Poloxamer-407 was obtained as a gift sample from Cadila pharma Ltd.

Drugs administration: Atorvastatin was suspended in 1% carboxy methyl cellulose solution in distilled water and administered in a dose of 50 mg kg^{-1} body weight, p.o. by gavage. Nicorandil was dissolved in distilled water and administered in a dose of 2 mg kg^{-1} body weight p.o. by gavage. These doses levels were selected on the basis of previous studies (Mansurah, 2011; Fukunaga *et al*., 2010). Drugs were administered to respective groups for once in case of poloxamer-407 induced hyperlipidemia model and once daily for 21 days in case of High-fat diet-induced hyperlipidemia model.

Poloxamer-407 induced hyperlipidemia: The acute hyperlipidemia was induced in rats using poloxamer-407 (Johnston and Palmer, 1997). Nicorandil and atorvastatin were administered orally 1 h before the i.p injection of 1 mL of 30% w/v of solution of p-407. Study design included following groups: Group 1: Normal (1% Carboxy Methyl Cellulose); Group 2: Hyperlipidemic control; Group 3: Nicorandil and Group 4: Atorvastatin.

High-fat diet-induced hyperlipidemia: Method of Blank *et al*. (1963) with modification was used to produce diet-induced hyperlipidemia. Briefly, normal group received standard chew diet and all other groups received high cholesterol diet consisting of standard pellet diet (92%), cholesterol (2.0%), cholic acid (1%) and coconut oil (5%) for 21 days. The standard pellet diet (Pranav Agro Industries, Vadodara) consisted of crude protein (22.06%), crude oil (4.04%), crude fiber (4.0%), Ash (10.0%) and sand silica (0.15%). The standard pellet diet supplies energy of 3620 kcal kg^{-1}. Study groups were kept same as that of acute model except poloxamer-407 treatment was replaced by high fat diet for 21 days.

Blood collection and biochemical estimation: The animals were treated for 21 days for high-fat diet model and once for polaxamer-407-induced model. At the end of experimental period, the rats in each group were deprived food overnight but not the water and sacrificed. The blood was collected by retro-orbital puncture technique

under ether anaesthesia and serum was separated. The serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) were estimated using commercially available kits (Span Diagnostic Ltd, India). Very low density lipoprotein-cholesterol (VLDL-C) was calculated as TG/5. Low density lipoprotein-cholesterol (LDL-C) levels were calculated using Friedewald (1972) formula. The Atherogenic index (AI) was calculated using formula Atherogenic Index (AI) = (VLDL-C + LDL-C)/HDL-C (Solanki and Bhatt, 2010). The liver tissues were collected, washed thoroughly in normal saline, bloated and preserved at -40°C for further analysis. The liver homogenates were prepared in tris-hydrochloride buffer. They were subjected to malondialdehyde (MDA) (Ohkawa *et al*., 1979), superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (Aebi, 1974) and reduced glutathione (GSH) (Beutler *et al*., 1963) estimation and HMG-CoA reductase activity (Rao and Ramakrishnan, 1975) is measured in case of high-fat diet induced hyperlipidemic model.

Statistical analysis: All the data were expressed as the Mean±SEM. The statistical analysis was performed using one way ANOVA followed by Tukey's multiple range tests using sigmastat 3.5 software. Value of $p<0.05$ was considered as statistically significant.

RESULTS

Poloxamer-407 induced hyperlipidemia: Single dose administration of poloxamer-407 produced significant increase in serum total cholesterol level (244.30±5.89, 254.19±3.36) at 15 and 24 h when compared with the normal group (66.15±2.35) at 15 and 24 h, respectively. Nicorandil had significant decrease in serum total cholesterol level (202.61±4.56) at 15 h study period when compared with the control group. This effect was comparable with the reference standard atorvastatin (193.14±7.47, 196.95±9.79) treated rats. Surprisingly, nicorandil did not show significant decrease in serum cholesterol level (249.98±9.18) at 24 h. Nicorandil had significant decrease in serum TG and VLDL-C levels when compared with the control group at the end of 24 h period. Reduction in serum LDL-C was found significant only at the end of 15 h study period (Table 2, 3).

High-fat diet-induced hyperlipidemia model

Lipid parameters: Serum cholesterol levels were significantly reduced in nicorandil (151.41±3.26) treated animals when compared with the high-fat diet group (208.51±11.99). Serum TG, VLDL-C, LDL-C levels and atherogenic index were also reduced in both atorvastatin and nicorandil treated groups when compared with the control group. Alongwith, increased serum HDL-C levels and HDL/LDL ratio were found in nicorandil treated group. HMG CoA/Mevalonate ratio is an index of the enzyme, which catalyzes the conversion of 3-hydroxy-3-methylglutaryl-CoA to mevalonate. HMG CoA/Mevalonate ratio was significantly raised in atorvastatin (4.35±0.32) as compared to the control group (1.27±0.03). However, it remained unaffected by nicorandil pretreatment (Table 4).

Table 2: Effects of various treatments on serum lipid profile of poloxamer 407-induced hyperlipidemic rats at the end of 15 h

Groups (n = 6)	1% CMC (control)	Poloxamer-407	Nicorandil	Atorvastatin
TC (mg dL^{-1})	66.150±2.35	244.30±5.89†	202.61±4.56**	193.14±7.47**
TG (mg dL^{-1})	54.560±1.71	312.58±8.9†	323.22±8.23	315.98±6.02
LDL-C	31.520±2.819	99.18±7.49†	64.63±1.64**	59.80±9.418**
HDL-C	23.710±0.92	82.60±4.14†	73.33±3.67	70.14±2.40
VLDL-C	10.910±0.343	62.51±1.78†	64.64±1.64	63.20±1.20
HDL/LDL ratio	0.793±0.09	0.86±0.08	1.25±0.22	1.55±0.5
Atherogenic index	1.810±0.15	1.99±0.176	1.79±0.15	1.78±0.184

Data are expressed as mean±SEM. The statistical significance of differences between the treated and control groups was determined using Tukey's test, † $p<0.05$, when compared with the normal group, ** $p<0.001$, when compared with the control group. TC: Triglycerides, TG: Triglycerides, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol

Table 3: Effects of various treatments on semm lipid profile of poloxamer 407-induced hyperlipidemic rats at the end of 24 h

Groups (n = 6)	1% CMC (control)	Poloxamer-407	Nicorandil	Atorvastatin
TC (mg dL^{-1})	66.15±2.35	254.190±3.36†	249.98±9.18	196.95±9.79**
TG (mg dL^{-1})	56.95±1.72	599.450±32.89†	494.66±15.30**	323.58±8.23**
LDL-C	29.53±2.193	95.088±5.93†	108.91±9.11	90.33±10.33**
HDL-C	25.22±0.94	39.210±2.56†	42.14±2.83	43.42±1.34
VLDL-C	11.39±0.343	119.890±6.58†	98.93±3.06**	63.19±1.204**
HDL/LDL ratio	0.83±0.08	0.880±0.07	0.70±0.07	0.84±0.12
Atherogenic index	1.63±0.09	5.630±0.482†	5.11±0.606	3.57±0.324**

Data are expressed as Mean±SEM. The statistical significance of differences between the treated and control groups was determined using Tukey's test, † $p<0.05$, when compared with the normal group, ** $p<0.001$, when compared with the control group. TC: Triglycerides, TG: Triglycerides LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol

Table 4: Effects of various treatments on serum lipid profile of high fat diet-induced hyperlipidemic rats

Groups (n = 6)	1% CMC (control)	High-Fat Diet	Nicorandil	Atorvastatin
TC (mg dL^{-1})	72.94±5.28	208.51±11.99†	151.41±3.26**	161.15±6.91**
TG (mg dL^{-1})	121.61±5.3	251.69±10.71†	164.04±8.29**	145.12±9.78**
LDL-C	26.15±4.95	143.98±14.16†	65.76±4.718**	111.35±7.63
HDL-C	30.05±1.52	14.18±0.85†	16.84±1.06	21.77±1.603**
VLDL-C	24.32±1.05	50.34±2.14†	32.80±1.66**	29.03±1.96**
HDL/LDL ratio	4.46±2.4	0.10±0.01†	0.26±0.03	0.31±06
Atherogenic index	1.45±0.21	14.16±1.63†	6.01±0.53**	6.65±0.67**
HMG CoA/Mevalonate ratio	1.27±0.03	1.30±0.10	1.20±0.09	4.35±0.39**

Data are expressed as Mean±SEM. The statistical significance of differences between the treated and control groups was determined using Tukey's test, † $p<0.05$, when compared with the normal group, ** $p<0.001$, when compared with the control group. TC: Triglycerides, TG: Triglycerides, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol

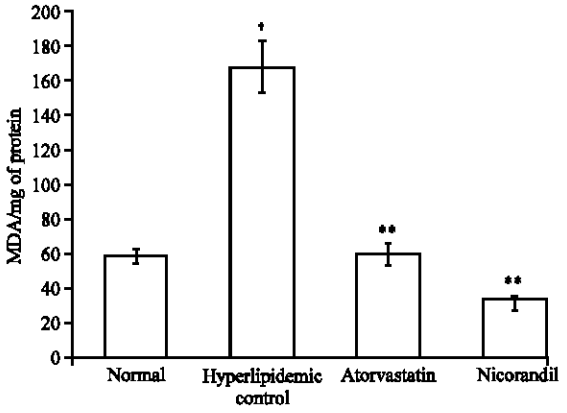

Fig. 1: Effect of Nicorandil on MDA level. Data are expressed as Mean±SEM. The statistical significance of differences between treated and control groups were determined using ANOVA followed by Tukey's test. †$p<0.05$, when compared with the normal group. **$p<0.001$, when compared with the control group

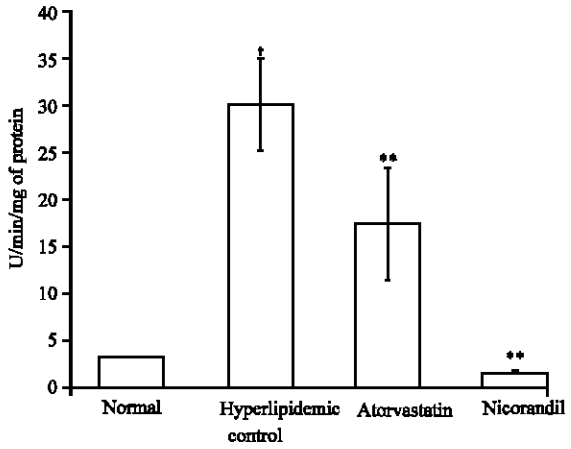

Fig. 2: Effect of Nicorandil on SOD level. Data are expressed as means±SEM. The statistical significance of differences between treated and control groups were determined using ANOVA followed by Tukey's test. †$p<0.05$, when compared with the normal group. **$p<0.001$, when compared with the control group

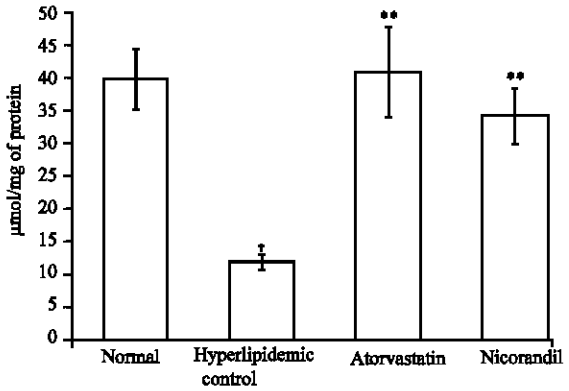

Fig. 3: Effect of Nicorandil on reduced glutathione level. Data are expressed as means±SEM. The statistical significance of differences between treated and control groups were determined using ANOVA followed by Tukey's test. †$p<0.05$, when compared with the normal group. **$p<0.001$, when compared with the control group

Antioxidant parameters: Nicorandil and atorvastatin treated rats showed significant decrease MDA and SOD levels as compared to hyperlipidemic control group (Fig. 1, 2). Paradoxically, reduced glutathione and catalase levels ($p<0.05$) were found increased significantly in nicorandil treated group when compared with the hyperlipidemic control group (Fig. 3, 4).

DISCUSSION

Coronary heart disease (CHD) is the most common cardiovascular disease and atherosclerosis is considered the most frequent cause of CHD (Yokozawa *et al*., 2006). It is well known fact that one of the major risk factors of atherosclerosis is hyperlipidemia. Hyperlipidemia and high cholesterol diet increase serum TC and LDL-C levels, resulting in an increased risk for the development of atherosclerosis. Thus regulating the serum cholesterol level is an important aspect in atherosclerosis prevention, as it has been shown that atherosclerosis could be

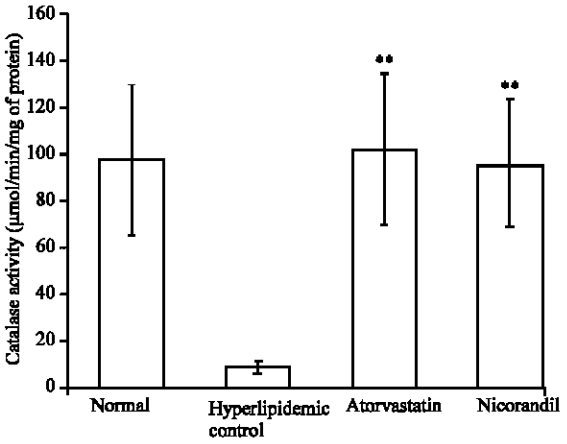

Fig. 4: Effect of Nicorandil on catalase. Data are expressed as means±SEM. The statistical significance of differences between treated and control groups were determined using ANOVA followed by Tukey's test. **p<0.001, when compared with the control group

suppressed by controlling the level of serum cholesterol. The cholesterol is an essential component of the cell membrane and starting material for the biosynthesis of bile acid, steroid hormones and vitamin D (Libby *et al*., 2000). In addition to this, TG is a major component of chylomicron and very low density lipoprotein (VLDL), both of which are energy substrates for liver and peripheral tissue, particularly, muscles. High levels of TG is a risk factor for atherosclerosis. Elevated LDL-C levels play a crucial role in the development of atherosclerotic lesions that progress from fatty steaks to ulcerated plaques. Thus, lowering the abnormally increased serum triglyceride, as well as the TC and LDLC levels reduces the incidence of atherosclerosis. HDL-C exerts an anti-atherogenic effect by counteracting LDL-C oxidation and facilitating the translocation of cholesterol from peripheral tissue such as arterial walls to the liver for catabolism. Besides, the atherogenic index, a ratio of LDL-C to HDL-C is commonly used as an index to evaluate the risk for atherosclerosis as a result of increased HDL-C levels (Fki *et al*., 2005). Therefore, elevating the level of HDL-C and lowering the atherogenic design are important measures in reducing the risk of atherosclerosis.

In the present study, we evaluated the effects of nicorandil on the serum lipid levels of hyperlipidemic rats induced by poloxamer 407 and the administration of a high-cholesterol diet. Poloxamer 407 is a hydrophillic, non-toxic, surface active agent with a low degree of toxicity that is being adapted for more specialized applications such as sealing permeabilized cell membranes and vascular occlusion procedures (Johnston and Palmer, 1997; Cogger *et al*., 2003; Ricci *et al*., 2005). It has been shown to cause significant elevations in the serum cholesterol and triglyceride levels after a single injection in rodent models (Wout *et al*., 1992). In support, our study results also showed similar results of TC and TG levels after the administration of poloxamer for 15 and 24 h duration. In previous study, nebivolol-a nitric oxide donor did not show any significant effect on plasma cholesterol in rabbits (De Nigris *et al*., 2008). In contrast, pre-treatment of nicorandil-a nitric oxide donor showed significantly reduced lipid levels in this acute model of hyperlipidemia at the end of 24 h study period.

The induction of hyperlipidemia by a high-cholesterol diet in experimental animals has long been used to assess the beneficial effect of hypolipidemic agents on the regulation of cholesterol (Yokozawa *et al*., 2006). Therefore, in this study, we also evaluated the anti-hyperlipidemic effects of nicorandil in a rat model in which hyperlipidemia was induced by high cholesterol diet. Oral administration of nicorandil for 21 days resulted in decreased concentration of TC, TG, VLDL-C, LDL-C as well as atherogenic index with raised level of HDL-C. These results suggests hypolipidemic activity of nicorandil. Our study results were contrasted with previous study results of nebivolol in rabbits (De Nigris *et al*., 2008).

An extensive range of antioxidant defenses, both the endogenous and exogenous are present to protect cellular components free radical-induced damage. These defenses include antioxidant enzyme like superoxide dismutase (SOD), catalase and chain breaking antioxidants Malondialdehyde (MDA) is the end product of lipid peroxidation. Therefore, measurement of MDA is an indirect method for assessing the extent of lipid peroxidation. High cholesterol diet induced a significant increase in MDA and SOD level, along with significant decrease in catalase activity when compared with the result of animals receiving standard diet. The increase in SOD may be due to the adaptive mechanisms because of oxidative stress. It has been also reported that oxidative stress increase SOD production (Mahfouz *et al*., 1997). The increased level of MDA could be due to significant lipid peroxidation and reduced catalase activity. The reduced catalase activity could be due to overproduction of superoxide anion which inactivated catalase by converting its resting ferric enzyme into poorly active ferro-oxy form.

As per our results, nicorandil showed significant decrease in MDA and SOD along with significant increase in GSH and CAT indicating protective mechanism of nicorandil against oxidative stress. Nicorandil has been

reported to possess antiapoptic property via NO/cGMP dependent mechanism and through activating mitochondrial K^+ATP channel (Hosseini-Tabatabaei and Abdollahi, 2008). According to this protective mechanism, we can hypothesize the protective role of nicorandil against oxidative nitrosative stress via lipid lowering activity. Further, it is the elevated cholesterol that leads to initial changes in the vascular wall. Hypercholesterolemia can produce profound effect on endothelium dependent function such as dilation of arterioles, fluid infiltration across capillaries and leukocytes recruitment. The endoplasmic reticulum stress induced by hypercholesterolemia also leads to oxidative stress and inflammation (Zhang, 2010).

Thus in conclusion, our results suggested antihyperlipidemic activity of nicorandil. The mechanism of its activity can be attributed to its inhibitory effect on oxidative stress as evident from its antioxidant activity and possibly through a NO/cGMP dependant mechanism and by activating mitochondrial K^+ATP channels. However, further studies are necessary to elucidate the molecular mechanism of nicorandil-induced protection against hyperlipidemia.

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