Effect of Isosorbide Dinitrate in Lipid Profile and Oxidant-Antioxidant Markers of Hypercholesterolemia Female Rats

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Received: 14-08-2019, **Revised:** 16-09-2019, **Accepted:** 30-10-2019, **Published online:** 28-11-2019

How to cite this article: Alyaa Majid, RanaTalib Al-Muswie and Khalid G Al-Fartosi (2019) Effect of Isosorbide Dinitrate in Lipid Profile and Oxidant-Antioxidant Markers of Hypercholesterolemia Female Rats, *Journal of International Pharmaceutical Research 46*(6): 114-118

Abstract

The present study aimed to investigate the effect of isosorbidedinitrate on lipid profile and oxidant-antioxidant markers of hypercholesterolemia female rats. Twenty four adult female albino rats weighting 170-190 g were divided randomly into four groups (six for each group), as follows: Group (1) : (Control group): Rats were fed on standard diet and supplied with tap water ad libitum for 30 days. Group (2) : (Cholesterol-treated group): Rats were treated with daily high cholesterol diet for 30 days. Group (3): (ISDN-treated group): Rats were administered a daily oral dose of ISDN (1.0 mg/kg BW dissolved in 0.5 ml distill water) for 30 days. Group (4): Rats were daily treated with (1 mg/kg dissolved in 0. 5 ml distill water) of ISDN besides high cholesterol diet for 30 days. Serum total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein, malondialdehyde, albumin and reduced glutathione were determined. The results showed a significant increase ($P \leq 0.05$) in the levels of total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and malondialdehyde; while there was a significant decrease in the levels of high density lipoprotein, albumin and reduce glutathione in cholesterol treated group in comparison with control group. In addition, ISDN administration caused a significant decrease in the levels of total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and malondialdehyde, with a significant increase in the levels of high density lipoproten, albumin and reduced glutathionein group(4) in comparison with group(2).

Keywords: Hypercholesterolemia, Isosorbidedinirate, Lipid Profile, Antioxidant.

Introduction

Being the main risk factor of cardiovascular mortality and acute coronary syndromes, the hypercholesterolemia also encourages the atherosclerosis. The multifactorial pathogenesis of hypercholesterolemia consists of the vascular superoxide's increased production, vascular inflammation and reactive oxygen species synthesis for instance, hydroxyl radicals, per-oxinitrite, and hydrogen peroxide [1,2]. The main concern of the increased vascular disease is that vascular oxidative stress is related to the diverse cardiovascular ailments for example hypertension, noninsulin dependent diabetes mellitus, heart failure and coronary artery disease [1]. The core mechanism of the improved bioavailability of vascular reactive oxygen species is multifactor and consists of augmented expression along with the enzyme activity producing oxygen radicals for instance, NADPH oxidase. While decreased expression with superoxide dismutase (SOD) enzyme activity also detoxifies the reduced cellular antioxidant's concentration e.g. glutathione, cysteine and other reactive species of oxygen [3]. Similarly, the bioavailability of vascular nitric oxide (NO) is reduced by oxidative stress while endothelial dysfunction is initiated [4]. Thus, this observation led to a hypothesis which stated that the imbalanced production of NO and superoxide subsidizes the cardiovascular disease pathogenesis.Therefore, both of these radicals are a significant biological mediator that stimulate the protective and pathogenic impacts on the vasculature [3, 5].

Being a structurally dissimilar compound's group, organic nitrates brig an aliphatic nitrogen moiety are reduced enzymatically due to the three electrons' floe to the nitrate nitrogen for generating NO [6,7]. The nitrogen moiety reduction happens in various cell types while comprising endothelial cells, and vascular smooth muscles. It has been displayed that treatment with organic nitrates upsurges the NO content in vein and arteries of rabbit [8]. However, the antioxidatve species impact the NO that has been in vitro established. but it is unknown that NO donors for instance organic nitrates are proficient in vascular oxidative stress reduction in hypocholesteremia.

Wever*et al*. and Jacobs *et al*. [9, 10] recommended that inborn LDL (low density lipoprotein) can obstruct the endothelium reliant relaxation which is

complete by NO in-activation. Likewise, the oxidized LDL forms were revealed to precisely damage the NO depending arterial relaxation with the help of various mechanisms such as the decline in the expression of endothelial nitric oxide synthase (eNOS) and reduction in eNOS substrate availability. Duplain *et al*. [11] reported that eNOS play an important role in the control of arterial pressure, glucose and lipid homeostasis. Deficiency of eNOS in mice resulted in hypertension, metabolic insulin resistance and hyperlipidemia. The declined bioactivity of NO seems to be a key provider to the vasoconstrictive remodeling and a main element of oxidative/nitrative stress incidence. Several risk elements of coronary heart diseases (CHD) are clinically acknowledged through endothelial dysfunction while containing hyperhomocysteinemia, tobacco usage, diabetes mellitus and hypercholesterolemia. All of them are linked with the reduced NO bioavailability which is demonstrated by an unusual coronary vasodilator's reaction to acetylcholine test [12]. The present study aims to investigate the role of ISDN as a NO-donor in theabnormal vascular responses induced by hypercholesterolemia on serum lipid profile, malondialdehyde and activity of antioxidants (albumin, uric acid and reduce glutathione).

Materials and Methods

The current study utilized the 24 albino female rats (*Rattusnorvegius*) having a weight of 170-190g. The rats were acquired from the Biology Department's animal house of the College of Science, University of Thi-Qar, Iraq. Animals were captivated in laboratory for ten days at 25±2 for twelve hours light/dark cycle afore the initiation of experiment. Rats were provided with standard food and *ad libitum* water all through this period.

Chemicals

Cholesterol, trichloro acetic acid (TCA) and thiobarbituric acid (TBA) was purchased from (BDH, England), sodium phosphate acidic NaH_2PO_4, sodium phosphate basic Na_2HPO_4 were purchased from FlukaGarnatie (Switzerland), Isosorbide dinitrate "ISDN" is the effective material in the commercial drug (Dinitra®) used for anginal syndromes. This drug is manufactured by the Egyptian International Pharmaceutical Industries Company (E.I.P.I.CO.).

Method for food (high cholesterol diet) preparation:

The 5% of the cholesterol diet was prepared by dissolving 50g of cholesterol in 200g of olive oil, heated on the water bath as cholesterol solubilized in oil it was further mixed with 1kilogram feed. Which was cut into the minor sized pieces that fitted in the iron lid holes of the boxes to assist the procedure used by the rats [13].

Experimental design

The animals were haphazardly distributed into four groups and each group consisted of seven rats. Such as:

Group1:(Control Group): the rats were fed with standard feed and provided the *ad libitum* tap water for thirty days.

Group 2: (Cholesterol treated group): rats were fed with a high cholesterol diet regularly for 30 days [13].

Group 3: (ISDN treated group): the oral dose of ISDN (1.0mg/kg BW dissolved in 0.5 ml D.W) was given to the rats for thirty days.

Group 4: the routine dose of ISDN (1mg/kg in 0.5 ml D.W) and high cholesterol diet as well was provided to the rats for 30 days.

Biochemical analysis

The measurement of the used serum for assessment of cholesterol (TC) was carried out according to the Allan & Dawson (1979) method [14]. The reagents used were provided by Biolabo, France for all and HDL (High Density Lipoprotein) was measured by following the Lopes-Virella (1977) methodology [16], the VLDL (Very low density lipoproteins) & the LDL (Low Density Lipoprotein) were estimated through Friedwald et al. (1972) process [17]. The calculation of antherogenic index was carried out by the equation (antherogenic index = LDL/HDL), furthermore, themalondialdehyde (MDA) and albumin were evaluated by Fong et al. [18] and Tietz technique (1999) respectively [19]. Moreover, the process of Ellmans (1959) was used for the estimation of reduced glutathione [20].

Statistical analysis

Software SPSS with version 15.0 was used to carry out the statistical examination. The outcomes were articulated as LSD and mean ± standard deviation (mean ±SD). The parameters of various study groups were compared by using one way ANOVA test. The P values were deliberated as statistically significant ($P \leq 0.05$).

Results

The outcomes indicated a significant upsurge ($p \leq 0.05$) in serum concentration of the low density lipoproteins (LDL), total cholesterol (TC), very low density lipoproteins (VLDL), triglycerides (TG) and group (2) antherogenic index in contrast to the group (1). However, a significant decrease of ($p \leq 0.05$) was observed in the LDL serum concentration of group (3) while comparing with group (1). Likewise, a substantial decrease was perceived in the antherogenic index of group 4 in comparison to the group 2 and in

the VLDL, TG, LDL and TC concentration as well. The concentration of high density lipoproteins' serum (HDL) was substantially decreased ($p \leq 0.05$) in group 2 as compared with group 1. Similarly the HDL serum concentration reduced for the group 3 when compared to the group1. However, the serum HDL concentration in group 4 showed an increase ($p \leq 0.05$) than that of group 2(Table 1).

Table- 1: Effect of daily administration of isosorbidedinitrate on serum lipid profile and atherogenicindex of hypercholesterolemia female rats

Parameters/ Groups	Group (1)	Group (2)	Group (3)	Group (4)	LSD
TC(mg/dL)	102.25 ± 2.50^{b}	140.70 ± 3.22^{a}	102.38 ± 2.27^{b}	103.59± 1.71^{b}	1.23
TG(mg/dL)	42.75± 1.59^{c}	62.28± 1.62^{a}	42.96± 1.63^{c}	45.13± 1.31^{b}	0.76
HDL(mg/DL)	39.95± 1.51^{a}	30.41± 1.43^{d}	37.04± 1.49^{b}	35.92 ±1.38^{c}	0.72
LDL(mg/dL)	58.05± 2.92^{b}	97.83± 2.42^{a}	56.76± 2.73^{c}	58.46± 1.90^{b}	1.25
VLDL(mg/dL)	8.55±0.32^{c}	12.45± 0.32^{a}	8.59± 0.33^{c}	9.02±0.26^{b}	0.15
Atherogenicindex	1.61± 0.25^{b}	3.22± 0.30^{a}	1.53±0.20^{b}	1.63± 0.21^{b}	0.27

Each value represents the mean± standard division of 6 rats.

- Same letters refer to non-significant difference at ($p \leq 0.05$)

- Different letter refer to a significant difference at ($p \leq 0.05$).

The observations specified an important upsurge ($p \leq 0.05$) in the malonadialdehyde (MDA) concentration in group 2 when compared to that of group 1. However, the MDA serum concentration indicated no substantial variance for group 3 when paralleled with group 1. The MDA serum in group4 showed a significant reduction of ($p \leq 0.05$) in its concentration when assessed with group 2. Further, more the reduced glutathione (GSH) and albumin concentration in serum deliberated a substantial reduction ($p \leq 0.05$) for group 2 while comparing it with group1. While the albumin concentration in serum exhibited no significant variance for group 3 when compared to group 2.Similarly, the GSH serum conc. in group 3 was non substantial in contrast to the group 1 but a major increase of ($p \leq 0.05$) was present in GSH and albumin of serum in group 4 when compared to group 2 (Table 2).

Table- 2: Effect of daily administration of isosorbidedinitrate on serum oxidant-antioxidants ofhypercholesterolemia female rats

Parameters / Groups	Group(1)	Group(2)	Group(3)	Group(4)	LSD
MDA(µmol/L)	1.54±0.17^{c}	4.23± 0.49^{a}	1.50± 0.08^{c}	3.44± 0.39^{b}	0.30
Alumine(g/dL)	4.33± 0.04^{a}	3.43± 0.28^{b}	3.62± 0.78^{b}	4.27± 0.64^{a}	0.58
GSH(µmol/L)	331.67± 31.7^{a}	216.93±13.16^{b}	328.67±26.54^{a}	316.38±9.19^{a}	24.49

Each value represents the mean± standard division of 6 rats

Same letters refer to non-significant difference at ($p \leq 0.05$)

Different letter refer to a significant difference at ($p \leq 0.05$)

Discussion

Total cholesterol level increased significantly after treatment with cholesterol. These increments may be explained by the fact that diets rich in fat content or cholesterol caused an increase in synthesis of lipids and lipoproteins in liver and serum. Fatty acids in diets may lead to increase the activity of the key enzyme Hydroxy-3- Methylglutaril-COA reductase (HMG-COA reductase) thus total cholesterol increased [21,22]. The higher levels of serum triglycerides have also been stated as a significant cause of risk as it impacts the mechanisms of clotting and deposition of lipids[23].ISDN administration caused a decrease in TC and TG levels this may be attributed to the effect of ISDN which acts as an NO donor [24].Treatment with ISDN induced a significant increase in serum HDL level. This increase was reported to help in transport of cholesterol from peripheral tissue to liver. ISDN led to an increase in lecithin cholesterol acyltransferase (LCAT) level which was associated with an increase in HDL-cholesterol concentration Nevin and Rajamohan (2004) [25]. Treatment with cholesterol caused an elevation in VLDL-C and LDL-C levels. Increased VLDL may be also attributed to decreased lipoprotein lipase activity [26].Cholesterol in diet may lead to down regulation of LDL-receptors thus LDL cannot influx into cells and its level rises. These results agree with Zulet*et al*. (1999) and Heibashy (2000)[21,22] who reported that diet rich in cholesterol and saturated fatty acids causes down regulation in LDL receptor in rats. In the present study, ISDN treatment induced a significant decrease in VLDL-C and LDL-C . This may be due to that ISDN acts as an NO donor. NO may increase the receptor of LDL C in cells, thus LDL-C inters the cells and reduced in circulation. LDL/HDL ratio is called (risk factor). It is very important in determining future risk to atherosclerosis

disease injury and that the increase in this ratio is due to abnormal high is LDL levels [27]. In the present study, ISDN treatment induced a significant decrease in atherogenic level. This may be due to the effect of ISDN on HDL and LDL where the taurine decreased LDL and increased HDL and this leads to a decrease in the level of atherogenic index[28].

The malondialdehyde (MDA) content, a measure of lipid peroxidation, is parallel with the degree of oxidative stress. Therefore, the assay of MDAis a maker of cell damage [29]. The level of MDA increases significantly in group (2) and this indicates a generation of free radicals and increasing in lipid peroxidation. [30]. It might be because of the balance loss relation amongst the anti-oxidation and lipid peroxidation, energy reduction & age acceleration in targeted organs for instance, brain, heart and kidney[31].Rats administered cholesterol exhibited a significant reduction in albumin and GSH levels. This reduction may be attributed to cholesterol which leads to increased oxidative stress, lipid peroxidation and production of $O^{-}{}^{2}$. These results agree with Kojda and Harrison (1999) and Gewalting and Kojda (2002) [1,32] who found that a variety of cardiovascular diseases which result from hypercholesterolemia associated with vascular oxidative stress. This may be due to increased production of $O^{-}{}^{2}$ and a decrease in antioxidant enzymes. In our study, GSH concentration was significantly increased in group(4) in comparison with group (2) The data of White et al.(1991)[33] supports our findings and also recommended the enzyme instigation in GSH production pathway's steps might be accountable for the elevation of GSH. Additionally, they specified a defensive role of NO all through the oxidative stress due to the GSH elevation. Concerning the NO role, several researchers tried to elucidate the NO mechanisms in the protection of cell. In which NO has a role of vasodilation and up keeping the blood flow of mucosa because of the decrease in adherence of leukocyte endothelium. Moreover, the protection is attained by the contribution of reactive oxygen species & decline in the parameters of reactive oxygen species viz. GSH activity upsurge and decline in MDA [34,35].

Conclusion

ISDN acted as NO donor that ameliorated most disturbances which resulted from hypercholesterolemia and it has no protective effect.

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