

RESEARCH ARTICLE

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EFFECT OF HYPERCHOLESTEROLEMIA AND ISOSORBIDE DINITRATE ADMINISTRATION ON SERUM NITRIC OXIDE AND SOME RELATED BIOCHEMICAL PARAMETERS IN MALE ALBINO RATS**ABSTRACT:**

Isosorbide dinitrate "ISDN" is an organic nitrate that generates nitric oxide "NO" that helps in treatment of the vascular disturbance which results from metabolic hypercholesterolemia. Forty two adult male albino rats weighting 170-190 g were divided randomly into six groups, each of seven rats, as follows: control group (standard diet), coconut oil treated group (0.5 ml daily), cholesterol treated group (400 mg/kg BW dissolved in 0.5 ml coconut oil), ISDN treated group (1.0 mg/kg BW dissolved in 0.5 ml H₂O), ISDN plus cholesterol treated group (ISDN + cholesterol) for 3 weeks and cholesterol followed with ISDN treated group (cholesterol for 3 weeks then ISDN for 3 weeks) by oral administration. Serum nitric oxide, antioxidant enzymes "superoxide dismutase (SOD) and glutathione peroxidase (GPx)" activities, lipid profile, glucose, uric acid, total protein, albumin and globulin percentage were determined. At the end of the experimental period, coconut oil treated group showed a significant increase in serum of both high density lipoprotein "HDL" and very low density lipoprotein "VLDL" and a significant decrease in serum SOD activity as compared to the control group. Cholesterol treated group showed a significant increase in total cholesterol "TC" and low density lipoprotein "LDL-C" and a significant decrease in NO level, SOD and GPx activity as compared to the control one. ISDN administration showed a significant increase in the triglyceride "TG", VLDL, and NO levels, but exhibited a significant decrease in LDL, albumin and GPx activity compared to control group. The ISDN plus cholesterol group showed a significant increase in LDL-C level and a significant decrease in albumin and GPx activity compared to the control one. The group treated with cholesterol followed with ISDN showed significant increase and decrease in HDL level and SOD activity, respectively compared to the control group. ISDN administration after cholesterol

induction helped in the return of most studied parameters to the control values.

KEY WORDS:

Hypercholesterolemia, Isosorbide dinitrate and Nitric oxide.

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ARTICLE CODE: 31.01.09**INTRODUCTION:**

Hypercholesterolemia is a major risk factor for cardiovascular diseases (Ross, 1999). It has been shown to promote a typical atherosclerotic remodeling of the vascular wall. One of the earliest manifestations is an inhibition of endothelium-dependent vasorelaxation often referred to as endothelial dysfunction. Most of the pathogenesis of the endothelial dysfunction involves either impaired generation or enhanced breakdown of NO (Busse and Flemin, 1996).

Organic nitrates preferentially dilate veins and this account for their use as vasodilators to treat angina and heart failure and for the unwanted effect of postural hypertension (Jiang *et al.*, 2001). New uses for NO donors are being explored, for example, ISDN has been widely used as vasodilator to treat acute myocardial ischemia. Their biological effect is due to the release of NO, which dilates blood vessels, making it easier for blood to flow and for heart to pump. Nitric oxide synthase (NOS) catalyzes the production of nitric oxide from the amino acid L-arginine and generates

citrulline as a product (Wu and Morris, 1998; Mori and Gotoh, 2000). L-arginine is an essential amino acid, supplied by the diet and actively transported into the cells.

Wever *et al.* (1998) and Jacobs *et al.* (1990) suggested that native low-density lipoprotein (LDL) might inhibit endothelium-dependent relaxation through in-activation of NO. Also oxidized forms of LDL were demonstrated to specifically impair NO-dependent arterial relaxation through a variety of mechanisms, including a decrease in endothelial nitric oxide synthase (eNOS) expression, and reduction in eNOS substrate availability. Duplain *et al.* (2001) 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.

Hypercholesterolemic rats exhibited significant increase in serum total lipid, TC, albumin, uric acid and glucose (Salem and Zaahkook, 2000) and low levels of HDL-C, hypertriglyceridemia, high fasting plasma glucose levels, and hyperuricemia (Nakanishi *et al.*, 2002).

Shaul (2003) showed that eNOS is normally targeted to cholesterol-enriched caveolae where it resides in a signalling module, and that oxidation of LDL "oxLDL" disrupts eNOS localization and action in caveolae by depleting caveolae cholesterol content. In contrast, HDL prevents the adverse effects of oxLDL and causes activation of eNOS.

Nevin and Rajamohan (2004) found that rats fed on diet contain virgin coconut oil showed a decrease in serum TC, TG, LDL, and VLDL levels, but there was an increase in HDL cholesterol.

Toshio *et al.* (2005) indicated that ingestion of certain NO-boosting substances, including L-arginine, L- citrulline, and antioxidants, can abrogate the state of oxidative stress and reverse the progression of atherosclerosis. This approach may have clinical utility in the treatment of atherosclerosis in humans. The use of the SOD inhibitor diethyldithiocarbamate (DETC) resulted in reduced endothelium-dependent and independent relaxation of the rabbit aorta. Treatment with DETC prevented nicotine-induced impairment of nitric oxide synthase-dependent arteriolar reactivity (Mugge *et al.*, 1991).

As postulated by Ohishi and Carmines (1995) suppressed SOD activity reduced the tonic influence of NO on renal arterioles during the early stages of diabetes mellitus. This occurred due to the accumulation of NO-scavenging superoxide anion. According to Asahi *et al.* (1995) the use of NO donors such as S-nitroso-N-acetylpenicillamine

(SNAP) inactivated bovine GPx and this inactivation was a dose and time dependent.

Ikenaga *et al.* (1999) found that a decrease in SOD activity leads to a decrease in NO level through the rapid formation of peroxynitrate. Kostic *et al.* (2000) reported that the injection of ISDN decreased the activity of catalase, glutathione peroxidase, glutathione transferase, and glutathione reductase in the interstitial compartment of testis. Gpx and SOD in both hemolysate blood and in liver tissue homogenates decreased significantly in cholesterol-fed diet (Heibashy, 2000).

Inal and Egüz (2004) found that reduced glutathione in blood levels were higher in rats treated with ISDN than in the control group, whereas the changes in SOD and GPx activities were not significant.

The present study aims to investigate the role of ISDN as a NO-donor in the abnormal vascular responses induced by hypercholesterolemia on serum NO, the activity of antioxidant enzymes (SOD & GPx), lipid profile, glucose, total protein, albumin, total globulin, and uric acid contents.

MATERIAL AND METHODS:

Forty two male albino rats (*Rattus norvegicus*) weighting 170-190 g were used in the present study. The animals were obtained from Helwan farm of Egyptian Organization for Vaccine and Biological Preparations.

Rats were caged in the laboratory for 10 days before the beginning of the experiment at 25±2°C for 12 hr light/dark cycle. Animals were given food (standard diet) and water *ad libitum* during this period.

Chemicals:

- Coconut oil was obtained from Pyramid Company for New Industry, Egypt.
- Cholesterol powder was obtained from El-Gomhoria Company, Egypt.
- 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.).

Experimental design:

Animals were randomly divided into six groups, each of seven rats as follows:

Group I: (Control group): The untreated group where rats were fed on standard diet and supplied with water *ad libitum*.

Group II: (Coconut oil-treated group): The rats were administered a daily (0.5 ml) oral dose of coconut oil by gastric intubation for three weeks.

Group III: (Cholesterol-treated group): Rats were administered a daily oral dose (400 mg/kg BW) of cholesterol (Dubey *et al.*, 2005) dissolved in 0.5 ml coconut oil for 3 weeks using gastric tube.

Group IV: (ISDN-treated group): Rats were administered a daily oral dose of ISDN (1.0 mg/kg BW dissolved in 0.5 ml H₂O) by gastric intubation for 3 weeks.

Group V: (ISDN plus cholesterol-treated group): Rats were administered a daily oral dose of ISDN (1.0 mg/kg BW dissolved in 0.25 ml H₂O) and cholesterol (400 mg/kg BW dissolved in 0.25 ml coconut oil) using a gastric tube for 3 weeks.

Group VI: (Cholesterol followed with ISDN-treated group): Rats were administered a daily oral dose of cholesterol (400 mg/kg BW dissolved in 0.5 ml coconut oil) by gastric intubation for 3 weeks after that they were administered a daily oral dose of ISDN (1.0 mg/kg BW dissolved in 0.5 ml H₂O) for 3 weeks using a gastric tube.

Biochemical analyses:

At the end of the experimental period blood samples were collected from the heart into small dry centrifuge tubes, left to coagulate and centrifuged at 3000 rpm for 15 minutes. The sera were separated and stored at -20 pending biochemical analysis.

Serum nitric oxide concentration was determined by the use of Quantichrom™ Nitric Oxide Assay Kit (DTNO-250) according to Ridnour *et al.* (2000). Serum SOD activity was determined spectrophotometrically by SOD Assay Kit-WST according to Goldstein and Czapski (1991). Serum GPx activity was estimated spectrophotometrically according to Paglia and Valentine (1967) using OXLtek total glutathione peroxidase kit.

Serum triglyceride concentration was determined spectrophotometrically according to Wahlefeld (1974) using Satnbio Liquid Color® Triglyceride kit, procedure No: 2100. Total cholesterol concentration was determined spectrophotometrically using the method described by Richmond (1973) using Stanbio Cholesterol Liquid Color® kit, procedure No: 1010. High density lipoprotein-cholesterol concentration was estimated spectrophotometrically according to the method of Warnick *et al.* (1983) using Satnbio HDL-cholesterol kit, procedure No: 0599.

Calculation of LDL and VLDL-cholesterol (mg/dL):

Low density lipoprotein (LDL)-cholesterol was calculated as follows:

$$\text{LDL-cholesterol} = \text{TC} - \text{HDL-cholesterol} - \text{TG}/5$$

Very low density lipoprotein (VLDL)-cholesterol was calculated as follows:

$$\text{VLDL-cholesterol} = \text{TG}/5$$

Glucose was determined spectrophotometrically by the use of El-Gomhoria company kit, according to Trinder (1969). Total protein was determined spectrophotometrically according to Gomal *et al.* (1949) using Satnbio Total Protein Liquid Color® kit, procedure No: 0250. Albumin was estimated spectrophotometrically by Satnbio Albumin Liquid Color® kit, procedure No: 0285 according to Rodkey (1964). Alpha (α)-globulin, beta (β)-globulin, and gamma (γ)-globulin were determined by electrophoresis according to the method of Nils (1983).

Quantitative enzymatic colorimetric determination of serum uric acid was carried out according to Fossati *et al.* (1980) by the use of Satnbio uric acid liquid Color® kit, procedure No: 1045.

Statistical analysis:

The data were as expressed as the mean of value ± standard deviation "SD" of 7 individual reading. The data were analyzed using one way analysis of variance (ANOVA) and two sample Student's *t* test (Snedecor 1971) on origin prolab (7.5) statistical program. The values of P>0.05 were considered statistically insignificant while those of P<0.01 were considered statistically significant.

RESULTS:

Nitric oxide, SOD and GPx:

Table 1 showed that the highest value of NO was found in ISDN treated group. Treatment with cholesterol caused a significant decrease in NO level. In groups treated with coconut oil and cholesterol followed with ISDN the levels of NO did not decrease significantly compared to that of the control group. In coconut oil, cholesterol and cholesterol followed with ISDN groups, SOD decreased significantly in comparison with the control group. ISDN and ISDN plus cholesterol treated groups exhibited a significant increase in SOD level at P<0.01 as compared to those of coconut oil, cholesterol and cholesterol followed with ISDN treated groups. In comparison to the control group, all treated groups showed significant decreases in GPx except the coconut oil and cholesterol followed with ISDN treated groups which showed non significant change in the GPx activity. Coconut oil treated group showed significant increase in GPx compared to cholesterol, ISDN and ISDN plus cholesterol treated groups. Cholesterol followed with ISDN treated group showed a significant increase in the GPx activity as compared to the cholesterol and ISDN treated groups.

Table 1. Effect of daily administration of isosorbide dinitrate; ISDN (1.0 mg/kg b.w. by gastric intubation for 3 weeks) on serum nitric oxide, superoxide dismutase and glutathione peroxidase of cholesterol treated male albino rats (400 mg/kg b.w. daily by gastric intubation dissolved in 0.5 ml coconut oil for 3 weeks) separately with or before ISDN treatments

parameter	Control	Coconut oil	Cholesterol	ISDN	ISDN with cholesterol	ISDN after cholesterol	P-value
Nitric oxide (ng/mL)	21.80 ± 2.51	17.44 ± 0.55 ^{de}	17.81 ± 0.42 ^{ade}	27.93 ± 1.99 ^{abcf}	23.72 ± 2.37 ^{bcf}	19.06 ± 1.97 ^{de}	***
Superoxide dismutase "SOD" (u/mL)	313.65 ± 16.09	246.77 ± 19.23 ^{acde}	217.73 ± 7.94 ^{abde}	324.94 ± 15.76 ^{bcf}	308.94 ± 15.76 ^{bcf}	220.56 ± 7.22 ^{ade}	***
Glutathione peroxidase "GPx" (u/L)	385.12 ± 10.70	371.13 ± 18.21 ^{cde}	282.90 ± 11.92 ^{abef}	276.85 ± 25.15 ^{abf}	319.04 ± 15.37 ^{abc}	353.98 ± 16.01 ^{cd}	***

Number of animals in each treatment = 7

Data presented as mean ± SD

P-value of ANOVA:

*** = significantly different at P < 0.001

Student's (t) test:

a = the difference between control and any other treated group significant at P < 0.01.

b = the difference between coconut oil and any other treated group significant at P < 0.01.

c = the difference between cholesterol and any other treated group significant at P < 0.01.

d = the difference between ISDN and any other treated group significant at P < 0.01.

e = the difference between ISDN with cholesterol and any other treated group significant at p < 0.01.

f = the difference between ISDN after cholesterol and any other treated group significant at p < 0.01.

Serum lipid profile:

Analysis of variance revealed that all parameters of serum lipid profile showed significant differences between groups (Table 2). The highest values of TC and LDL were found in cholesterol treated group compared to those of the control group and the other treated groups. High density lipoprotein increased significantly in coconut

oil treated group compared to the control one. In comparison to control and other treated groups, TG and VLDL were not significantly decreased in ISDN plus cholesterol and cholesterol followed with ISDN treated groups. The higher values of TG and VLDL were found in the ISDN treated group.

Table 2. Effect of daily administration of isosorbide dinitrate; ISDN (1.0 mg/kg b.w. by gastric intubation for 3 weeks) on serum lipid profile of cholesterol treated male albino rats (400 mg/kg b.w. daily by gastric intubation dissolved in 0.5 ml coconut oil for 3 weeks) separately, with or before ISDN treatments

Parameter	Control	Coconut oil	Cholesterol	ISDN	ISDN with cholesterol	ISDN after cholesterol	P-value
Triglycerides TG (mg/dl)	39.84 ± 6.41	47.49 ± 2.82 ^{def}	44.94 ± 5.85 ^{df}	79.99 ± 7.74 ^{abcfe}	35.13 ± 2.09 ^{bd}	26.88 ± 4.72 ^{bcd}	***
Total cholesterol TC (mg/dl)	50.00 ± 8.05	57.99 ± 1.45 ^{df}	67.58 ± 6.39 ^{adef}	47.48 ± 5.69 ^{bc}	51.44 ± 3.05 ^c	38.72 ± 5.81 ^{bc}	***
High density lipoprotein HDL (mg/dl)	20.59 ± 3.31	29.74 ± 2.73 ^{aef}	24.86 ± 1.42 ^{ef}	18.50 ± 2.66 ^b	16.06 ± 0.43 ^{bc}	15.40 ± 1.50 ^{bc}	***
Low density lipoprotein LDL (mg/dl)	21.44 ± 3.45	16.38 ± 4.97 ^{ce}	34.46 ± 5.33 ^{abdf}	15.39 ± 1.47 ^{acef}	28.66 ± 2.06 ^{abd}	23.75 ± 3.49 ^{cd}	***
Very low density lipoprotein VLDL (mg/dl)	7.91 ± 1.34	15.74 ± 2.82 ^{ac}	8.51 ± 1.40 ^{bd}	15.79 ± 1.47 ^{acef}	7.02 ± 0.41 ^d	6.56 ± 2.36 ^d	***

Number of animals in each treatment = 7

Data presented as mean ± SD

P-value of ANOVA:

*** = significantly different at P < 0.001

Student's (t) test:

a = the difference between control and any other treated group significant at P < 0.01.

b = the difference between coconut oil and any other treated group significant at P < 0.01.

c = the difference between cholesterol and any other treated group significant at P < 0.01.

d = the difference between ISDN and any other treated group significant at P < 0.01.

e = the difference between ISDN with cholesterol and any other treated group significant at p < 0.01.

f = the difference between ISDN after cholesterol and any other treated group significant at p < 0.01.

Glucose, serum proteins and uric acid:

All groups showed an increase in the glucose level in comparison with the control group except the coconut oil and cholesterol treated groups where no significant effect

was detected. There were non significant changes in the total protein levels between the control and any of the other treated groups. Coconut oil and cholesterol treated groups showed non significant increases in

serum albumin compared to the control one. In groups treated with ISDN and ISDN plus cholesterol, albumin decreased significantly compared to that of the control, but the decreases were non significant in the group treated with cholesterol followed with ISDN. The α -globulin showed non significant changes in all treated groups. In comparison to control group all treated groups showed non significant decreases in β globulin. Cholesterol followed by ISDN treatment

induced non significant increases in β globulin compared to that of the control, but the effect was significant when compared with the other treated groups. All the treated groups showed non significant increases in γ globulin compared to that of the control one except the ISDN-treated group where a significant increase was found in comparison with the control and other treated groups. The level of uric acid showed non significant changes in all treated groups (Table 3).

Table 3. Effect of daily administration of isosorbide dinitrate; ISDN (1.0 mg/kg b.w. by gastric intubation for 3 weeks) on serum glucose, total protein, albumin, total globulin and uric acid of cholesterol treated male albino rats (400 mg/kg b.w. daily by gastric intubation dissolved in 0.5 ml coconut oil for 3 weeks) separately, with or before ISDN treatments

parameter	Treatment						P-value	
	Control	Coconut oil	Cholesterol	ISDN	ISDN with cholesterol	ISDN after cholesterol		
Glucose (mg/dL)	109.50±21.12	107.91±9.93 ^{ce}	79.90±4.16 ^{bdef}	125.42±15.12 ^c	163.10±18.28 ^{bc}	116.59±19.19 ^c	***	
Total protein (g/dl)	6.79±1.08	7.18±0.54	6.67±1.18	7.61±1.15	6.79±0.90	7.04±1.16	N.S	
Albumin (g/dl)	5.22±0.81	5.98±0.66 ^{def}	5.46±0.93 ^{de}	4.14±0.60 ^{adc}	3.89±0.27 ^{abc}	3.80±1.33 ^b	***	
Total globulin (%)	Alpha " α "	12.91±1.15	13.08±0.44	14.35±1.33	11.78±0.88	12.10±1.20	N.S	
	Beta " β "	14.22±1.72	12.47±1.37 ^f	12.27±0.78 ^f	12.44±1.23 ^f	11.35±0.33 ^f	15.20±1.42 ^{bcdde}	***
	Gamma " γ "	17.15±2.28	19.23±1.14 ^d	19.70±1.14 ^d	23.32±1.28 ^{abcf}	20.70±1.71	18.12±1.07 ^d	***
Uric acid (mg/dL)	1.21±0.50	1.53±0.48	1.55±0.71	1.38±0.78	1.40±0.39	1.37±0.62	N.S	

Number of animals in each treatment = 7

Data presented as mean \pm SD

P-value of ANOVA:

N.S = non significantly different at P > 0.05

*** = significantly different at P < 0.001

Student's (t) test:

a = the difference between control and any other treated group significant at P < 0.01.

b = the difference between coconut oil and any other treated group significant at P < 0.01.

c = the difference between cholesterol and any other treated group significant at P < 0.01.

d = the difference between ISDN and any other treated group significant at P < 0.01.

e = the difference between ISDN with cholesterol and any other treated group significant at p < 0.01.

f = the difference between ISDN after cholesterol and any other treated group significant at p < 0.01.

DISCUSSION:

Cholesterol treatment induced a reduction in NO as compared to the control and other treated groups. Hypercholesterolemia was associated with an increase in LDL-C level. NOS normally targets cholesterol-enriched caveolae in cell membrane. LDL-C may cause an up-regulation of the structure protein caveolin and promoting its interaction with eNOS. This interaction may cause inactivation of eNOS, thus NO production decreased (Feron *et al.*, 1999). In addition, hypercholesterolemia was reported to induce disturbance in the NO-signal transduction pathway through initiating the expression of a dysfunction soluble guanylate cyclase "sGC". These changes caused by reactive oxygen species which increased in hypercholesterolemia (Francois and Kojda, 2004). These results agreed with earlier studies (Kausar *et al.*, 2000; Kuhlencordt *et al.*, 2001) which reported marked decrease of NO level in hypercholesterolemic rabbits and mice. Böger *et al.* (2004) showed that baseline NOS turnover rate was reduced in hypercholesterolemic rabbits. In addition, Zhang *et al.* (2008) showed that oxidized

LDL directly inhibited arginine uptake thus intracellular content decreased and NO production diminished.

ISDN treated group exhibited a higher NO level than those in the control and treated groups. ISDN is one of the organic nitrates which can convert to NO and endothelial and inflammatory cells have the ability to convert organic nitrate to NO through enzymatic and non-enzymatic bio-activation pathways. These results agree with Feelisch and Kelm (1991) who reported that glyceryl trinitrate "GTN" or ISDN were effective in the treatment or prevention of angina through the release of NO. Plotkine *et al.* (1991) found that ISDN has antithrombotic activity due to the generation of NO. ISDN helps in stimulating the release of endothelium-derived relaxing factor (Thaler *et al.*, 1996).

ISDN plus cholesterol-treated group showed a significant increase in NO level compared to that of cholesterol- and coconut oil-treated groups as well as in the group treated with cholesterol followed with ISDN

administration. This may be attributed to the effect of ISDN as an NO donor.

SOD activity decreased significantly with coconut oil treatment. This may be due to the used oil which might have led to increased oxidative stress and impaired antioxidant defense. These results are different from those of Nevin and Rajamohan (2006) and Nagaraju and Belur (2008) which reported that coconut oil helped in increasing the activity of antioxidant enzymes such as SOD by lowering the steady state of O_2^- .

Treatment with cholesterol caused significant reductions in SOD and GPx activity. These reductions may be attributed to hypercholesterolemia which increased oxidative stress and lipid peroxidation and might also caused an increase in production of O_2^- in arteries and increased the expression of oxidation-sensitive gene which led to decreased activity of antioxidant enzyme. Thus the antioxidant defense was impaired as previously reported by Heibashy (2000), Shah and Channon (2004), and Hayashi *et al.* (2005). However, these results disagreed with Devrim *et al.* (2008) who reported that high-cholesterol diet not induced changes in SOD and GPx activities in rats.

ISDN induced a significant reduction in GPx activity; this may be related to the ISDN property as an NO donor and NO may interact with the amino acid residue and thiol group to form nitroso-compounds. This may lead to inactivation of GPx enzyme. These results agree with Asahi *et al.* (1995) who reported that SNAP "NO donor" induced inactivation of bovine GPx in a dose and time dependent manner.

Treatment with ISDN plus cholesterol induced a significant decrease in GPx activity. This may be attributed to administration of ISDN with cholesterol on the same day and each of them caused a decrease in GPx activity. Thus the combination between them augmented the oxidative stress and subsequent activation of antioxidant enzyme.

Rats administered cholesterol followed with ISDN treatment exhibited a significant reduction in SOD activity. 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) 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.

ISDN administration caused an increase in TG level. This may be attributed to ISDN treatment which is not, by itself, protective against atherosclerosis. ISDN

increased the lipid value 4-8% in the ischemic heart disease and increased TG, TC, LDL-C, and VLDL-C (Sharma and Sharma, 1997).

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-Methylglutaryl-CoA reductase (HMG-CoA reductase) thus total cholesterol increased. These results agree with previous reports of Zulet *et al.* (1999) and Heibashy (2000) which revealed HMG-CoA reductase activity increased with high fatty acids diets and this was attributed to a high viability of acetyl-CoA which stimulate cholesterologenesis rate. Serum of rabbits fed a high cholesterol diet showed high level of TC and lipid peroxide levels and the ratio of cholesterol/phospholipids increased in erythrocyte (Balkan *et al.*, 2002). Similar results were recorded with other animals such as pigs and mice (Pond *et al.*, 1986; Kamata *et al.*, 1996).

Treatment with coconut oil induced a significant increase in serum HDL level. This increase was reported to help in transport of cholesterol from peripheral tissue to liver thus TC level kept constant. Coconut oil led to an increase in lecithin cholesterol acyltransferase (LCAT) level which was associated with an increase in HDL-cholesterol concentration Nevin and Rajamohan (2004). These results agree with the report of Tebib *et al.* (1994). Also the results agree with Nevin and Rajamohan (2008) who reported that virgin coconut oil reduced TC and TG levels and prevented LDL oxidation. ISDN plus cholesterol and ISDN administration following cholesterol induced a significant reduction in HDL-C compared to coconut oil and cholesterol treated group. This may be due to that cholesterol has a higher effect than ISDN. ISDN and cholesterol followed with ISDN caused a significant reduction in TC compared to the coconut oil group and this may be attributed to the effect of ISDN which acts as an NO donor.

Treatment with cholesterol caused an elevation in LDL-cholesterol level. 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) 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 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 enters the cells and reduced in circulation.

ISDN plus cholesterol treatment induced a significant increase in LDL-cholesterol level. This may be attributed to that treatment with cholesterol every day caused down regulation in LDL-receptor thus LDL-level increased. This agrees with Zulet *et al.* (1999) and Heibashy (2000) who reported that LDL receptors decreased in rats fed on diet rich in cholesterol. ISDN could not counteract the effect of cholesterol injected on the same day.

VLDL level increased significantly with coconut oil. The oil increased lipoprotein synthesis in liver and VLDL is the major lipoprotein synthesised in liver. These results agree with Driscoll *et al.* (1990) and Fungwe *et al.* (1992) who reported that there was an increase in plasma triglycerides and hepatic lipid in guinea pigs fed cholesterol in chow diets containing corn or coconut oil and in rabbits received coconut oil. Increased VLDL may be also attributed to decreased lipoprotein lipase activity (El-Gendy *et al.*, 2000). On the other hand, these results disagree with Nevin and Rajamohan (2004) who found that virgin coconut oil caused a decrease in TG and phospholipids in liver and other tissues and thus VLDL decreased.

Cholesterol administration induced a significant decrease in glucose level. This may be attributed to that hypercholesterolemia induced an increase in glucokinase activity. Glucokinase enzyme causes phosphorylation of glucose and facilitates glucose utilization by the liver, thus glucose level decreases. This result agrees with Zulet *et al.* (1999) who found that hypercholesterolemia induced

hypoglycemia and an increase in glucokinase activity.

ISDN and ISDN plus cholesterol treatment induced a significant decrease in serum albumin. ISDN caused an increase in NO level and NO is increased by stimulated activity of NOS. Albumin transport into the cells by endocytosis via caveolae has an important role in decreasing the eNOS signaling. Thus when NO increased more albumin transported into the cells. This agrees with Mani *et al.* (2006) who found that the lack of albumin was associated with a marked increase in the concentration of plasma nitrite/nitrate and increased NO synthesis. Cholesterol followed with ISDN induced a significant reduction in albumin compared to coconut oil group. This may be due to the effect of ISDN.

ISDN and cholesterol followed with ISDN treated groups showed significant increases in γ and β globulin respectively. This may be due to ISDN induced increase in WBC count and TG level which increase inflammation Nakanishi *et al.* (2002). Chiller and Weigle (1973) stated that serum globulins consists of different fractions, the main part of them is the gamma globulins which synthesized by plasma cells (mature B-lymphocytes) and these gamma globulins responsible for the specific immunological reaction.

In conclusion, ISDN administration after cholesterol is more effective than its administration with cholesterol in return of most measured parameters to control values. ISDN acted as NO donor that ameliorated most disturbances which resulted from hypercholesterolemia and it has no protective effect.

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تأثير ارتفاع الكوليستيرول في الدم وإعطاء الأيزوسوربيد ثنائي النترات علي أكسيد النيتريك وبعض القياسات الكيميائية الحيوية المتعلقة به في مصل ذكور الجرذان البيضاء

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قسم علم الحيوان ، كلية العلوم ، جامعة بنها

يعتبر الأيزوسوربيد ثنائي النترات احد النترات العضوية التي تنتج أكسيد النيتريك في الجسم والذي يساعد في علاج الخلل في الاوعية الدموية والذي ينتج من ارتفاع الكوليستيرول في الدم. تم تقسيم 42 من ذكور الجرذان البالغة التي تزن 170-190 جم عشوائيا إلي 6 مجموعات "7 حيوانات في كل مجموعة" علي النحو التالي: المجموعة الضابطة، المجموعة المعطاة زيت جوز الهند(0,5 مل يوميا)، المجموعة المعطاة الكوليستيرول (400 مجم/كجم مذاب في 0,5 مل زيت جوز الهند يوميا)، المجموعة المعالجة بالأيزوسوربيد ثنائي النترات (1 مجم/كجم مذاب في 0,5 مل ماء يوميا) و المجموعة المعالجة بالأيزوسوربيد ثنائي النترات مع الكوليستيرول لمدة 3 أسابيع و المجموعة المعالجة بالأيزوسوربيد ثنائي النترات بعد الكوليستيرول (الكوليستيرول لمدة 3 أسابيع ثم الأيزوسوربيد ثنائي النترات لمدة 3 أسابيع) و قد تمت جميع المعالجات عن طريق الفم. في نهاية التجربة تم تعين أكسيد النيتريك، الإنزيمات المضادة للأكسدة (سوبراوكسيد ديسميوتيز وجلوتاثيون بيروكسيداز)، صورة الدهون، الجلوكوز ، حمض البوليك، البروتين الكلي، الألبومين و الجلوبيولين الكلي. وقد أظهرت النتائج زيادة معنوية في الكوليستيرول عالي الكثافة و الكوليستيرول شديد الانخفاض في الكثافة وانخفاض معنوي في سوبراوكسيد ديسميوتيز في المجموعة المعطاة زيت جوز الهند منفردًا مقارنة بالمجموعة الضابطة. مجموعة الكوليستيرول أظهرت زيادة معنوية في الكوليستيرول الكلي و الكوليستيرول منخفض الكثافة

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