Original Article

Beneficial effects of combined administration of sodium molybdate with atorvastatin in hyperlipidemic hamsters

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ABSTRACT: This study aims to evaluate the benefit of combined administration of sodium molybdate with atorvastatin in management of hyperlipidemia. Hyperlipidemic male Serian golden hamsters were administered either atorvastatin (40 or 80 mg/kg, p.o.) sodium molybdate (100 mg/kg, p.o.) or combination of atorvastatin (40 mg/kg, p.o.) with sodium molybdate (100 mg/kg, p.o.) for 30 consecutive days. Blood lipids (total cholesterol, triglycerides, HDL-cholesterol, Non-HDL-cholesterol and anti-atherogenic index) in addition to the activities of liver transaminases (AST, ALT), as well as antioxidant status (lipid peroxidation, catalase, glutathione peroxidase) were estimated before and after 15 and 30 days of treatment. The results indicate that atorvastatin is effective in lowering the blood lipids with maximum effect achieved by the high dose (80 mg/kg, p.o.). However, this dose elevates the liver enzymes significantly after 15 and 30 days of treatment. Sodium molybdate lowers the blood lipids after 30 days from treatment without alteration in liver enzymes. Moreover, in this group, lipid peroxides were significantly reduced and activities of catalase as well as glutathione peroxidase were significantly elevated compared with the hyperlipidemic control group (saline). Combination of atorvastatin (40 mg/kg, p.o.) with sodium molybdate significantly reduced the elevated blood lipids in a similar degree as the high dose of atorvastatin. Meanwhile, in this group, the liver enzymes were not significantly elevated while, the antioxidants profile were significantly improved compared with that of control hyperlipidemic and atorvastatin groups. In conclusion, combination of sodium molybdate with atorvastatin is beneficial in management of hyperlipidemia as it allows maximum reduction in blood lipids, improves the antioxidant status with minimal disturbances in liver enzymes.

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1. Introduction

The elevation of plasma levels of low density lipoprotein-cholesterol, triglycerides, and reduction of high-density lipoprotein is often associated with the high incidence of atherosclerosis and coronary heart disease (*1*). Hyperlipidemia and accordingly atherosclerosis are correlated to the elevation of oxidative stress which is characterized by lipid and protein oxidation (*2*).

Statins or the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-Co-A) reductase inhibitors represent the main class of lipid lowering drugs (*3*). Statins are proved to be effective in reducing serum levels of total as well as low density lipoprotein-cholesterol, slowing the progression of atherosclerosis, reducing the incidence of cardiovascular events, and decreasing the mortality rate (*4*). The currently used statins are generally well tolerated and present a good safety profile (*5*). Nevertheless, many adverse effects of statins have been reported. The main reported adverse effects of statins may include hepatotoxicity, characterized by an increased level of transaminases (*6*), various forms of myotoxicity, myalgias and rhabdomyolysis (*3*). Other minor adverse effects such as generalized gastrointestinal discomfort and neuropathy are also known. These adverse effects may increase upon aging, physical exercise or when statins are given at high doses or when administrated with another medications that interfere with its metabolism (*7*). The most widely used statins are also suffered from limitations due to intolerance, partial effectiveness in lowering cholesterol levels, and the cost (*8*).

Atorvastatin is a long half-life, lipophilic statin metabolized by cytochrome P450 (CYP3A4) enzyme. Atorvastatin at doses of 10 to 80 mg is the leading prescribed statin in the world, providing an LDL reduction of 38% to 55%. However, the high doses

of atorvastatin have an incidence of liver enzyme elevation, approximately 2.5%, which is the highest of all the statins (*9*).

Syrian golden hamster has been used as a model for studying lipids as well as lipoprotein metabolism because of its similarities to humans with regards to cholesterol and bile metabolism (*10*), Non-HDL-cholesterol and triglycerides response to atherogenic diet (*11*), development of early atherosclerotic lesions (*12*), and exclusive hepatic production of apolipoprotein (apo) B-100 (*13,14*).

Molybdenum represents an important trace element involved in the structure of certain enzymes that catalyzing redox reactions. Although it can form complexes with numerous physiologically important compounds, it was supposed that this trace element is absorbed, transported and excreted in a simple molybdate form (*15*). Many simple as well as complex molybdenum compounds were found to significantly reduce the levels of blood glucose and free fatty acids (*16,17*). Moreover, sodium molybdate was proved to prevent oxidation of lipids and protects antioxidant systems in different experimental models (*17,18*).

Accordingly, the aim of the current study is to evaluate the effect of atorvastatin treatment either alone or in combination with sodium molybdate on blood lipids, liver enzymes, and antioxidant status in hyperlipidemic Syrian golden hamsters. The study also targets to improve the hypolipidemic effects of atorvastatin and to minimize its adverse actions in an experimental trial to suggest or recommend a safe and effective combined therapy to hyperlipidemia.

2. Materials and Methods

2.1. *Drugs and chemicals*

All chemicals used were of high analytical grade and were obtained from Sigma-Aldrich (St. Louis, Mo, USA). Atorvastatin was supplied from Parke-Davis, Germany. The selected drug doses were matched with that in the literature and were chosen after a preliminary experimental study in our laboratory. The dose of sodium molybdate was in accordance with that previously reported (*18*), and there were no toxic effects observed with this dose.

2.2. *Animals and experimental protocol*

Male Syrian golden hamsters weighing between 100-120 g, obtained from the animal facilities of King Saud University were used. The animals were randomly divided into groups and acclimatized for one week in colony cages (five hamsters per cage), at 22 ± 2ºC and under a 12-h light: dark cycle with free access to water and rodent chow. After acclimatization period, all hamsters were fed ad libitum with the hyperlipidemic diet consists of the standard diet containing 10% coconut oil and 0.2% cholesterol. The diet was prepared in the form of paste, and it was found to be effective for induction of hyperlipidemia in hamsters in our laboratory. After two weeks from hyperlipidemic diet blood samples were withdrawn from 16 h fasted animals, *via* the retro-orbital sinus into heparinized glass tubes, under light ether anesthesia. The separated plasma was used for estimation of total cholesterol (TC). The hamsters were then divided into five groups (n = 10) of matched blood cholesterol levels and body weights.

All groups were fed with the hyperlipidemic diet till the end of the experiment. Group 1 was administered saline for 30 days (control), and groups 2 and 3 were administered atorvastatin (40 or 80 mg/kg, p.o.), respectively. Group 4 was administered sodium molybdate (100 mg/kg, p.o.), group 5 was administered atorvastatin (40 mg/kg, p.o.) followed by sodium molybdate (100 mg/kg, p.o.) one hour latter. All treatments were continued daily for 30 consecutive days. Blood samples were withdrawn, into heparinized tubes, at 1, 15, and 30 days of treatment from hamsters deprived of food for 16 h. The separated plasma was used for determination of the biochemical parameters. All hamsters in each group survived the entire length of the study. All experiments were conducted according to the guidelines of the Animal Care and Use Committee Acts of King Saud University, and in accordance with the international guidelines of handling of laboratory animals.

2.3. *Measurement of biochemical parameters*

2.3.1. *Blood lipids determinations*

Total cholesterol (TC) and triglycerides (TG) were measured by CHOD-PAP and GPO-PAP methods, respectively, using commercial kits (Spinreact S.A., Sant Esteve de Bas, Spain). Measurement of high density lipoprotein (HDL) cholesterol in plasma was carried out in the supernatant after precipitation of low density, intermediate density and very low density containing lipoproteins (LDL, IDL and VLDL), with phosphotungstate reagent followed by centrifugation, using commercial kits (Spinreact S.A., Spain). Results were expressed as Non-HDL (VLDL + IDL+ LDL) cholesterol instead of LDL-cholesterol, because the Friedewald equation is not applicable to hamsters. The concentration of lipoprotein (Non-HDL) cholesterol was calculated by subtracting HDL-cholesterol concentrations from total plasma cholesterol. Anti-atherogenic index was calculated as ratio of high density lipoprotein cholesterol/total cholesterol of each sample.

2.3.2. *Estimation of plasma transaminases activities*

Plasma aspartate aminotransferase (AST) and alanine

aminotransferase (ALT) activities were determined using the commercial enzymatic kits (Bio Merieux, France) and following the instruction manual.

2.3.3. *Estimation of plasma lipid peroxides, glutathione peroxidase, and catalase activities*

Plasma lipid peroxides was assayed according to the method described by Ohakawa *et al.* (*19*), using thiobarbituric acid reaction. The results were expressed as malondialdehyde (MDA) in nmol/L. Glutathione peroxidase (GPx) activity was measured spectrophotometrically at 340 nm according to the method described by Paglia and Valentine (*20*) using a Cayman microplate assay kit (Cayman, Ann Arbor, MI, USA). GPx activity was expressed as nmol/min/mL. Catalase (CAT) activity was estimated in plasma following the method of Aebi (*21*). Catalase activities were measured at 240 nm and were expressed as µmol/L.

2.4. *Statistical analysis*

The data was analyzed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Results were presented as means ± SEM, n = 10 hamsters. The statistical significance of differences between data means were determined by using one way analysis of variance (ANOVA), followed by post hoc Tukey's test. Values of $P < 0.05$ were considered as statistically significant.

3. Results

As shown in Table 1, administration of hypercholesterolemic diet to hamsters for 30 days resulted in a significant elevation in plasma TC, Non-HDL-C, and TG levels compared with the initial value (day 1 of experiment after two weeks hyperlipidemic diet). This effect was accompanied by a significant reduction in the anti-atherogenic index. Meanwhile, the HDL-C level was not affected by the hypercholesterolemic diet.

Treatment of the hyperlipidemic hamsters with atorvastatin (40 or 100 mg/kg) for 30 days, resulted in a gradual, dose related and significant decrease in plasma TC levels (to 52% and 61% compared with the control hyperlipidemic hamsters at 30 days, respectively). A similar response was also observed for plasma Non-HDL cholesterol levels (to 61% and 74%, respectively). Additionally, atorvastatin treatment produced a significant and dose related decrease in triglycerides levels (to 40% and 53%, compared with the control hyperlipidemic hamsters at 30 days, respectively). HDL-C was significantly elevated after 30 days treatment with atorvastain (to 21% and 38%, compared with the control hyperlipidemic hamsters at 30 days, respectively). The anti-atherogenic index was significantly improved upon treatment by both doses of atorvastatin (to 2.5 and 3.7 folds compared with control hyperlipiemic hamsters at 30 days, respectively) (Table 1).

Table 1. Effect of treatment on plasma lipid parameters in hyperlipidemic hamsters

Item	Day 1				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
TC (mg/dL)	276.7 ± 12.33	313.0 ± 16.75	312.1 ± 21.82	318.7 ± 20.13	319.0 ± 19.35
HDL-C (mg/dL)	37.6 ± 2.40	38.9 ± 2.53	41.5 ± 2.04	38.7 ± 2.99	42.8 ± 2.39
Non HDL-C (mg/dL)	239.1 ± 10.22	273.9 ± 14.34	270.6 ± 19.86	279.9 ± 17.27	276.3 ± 17.05
TG (mg/dL)	194.2 ± 7.88	237.3 ± 12.05	238.5 ± 12.09	241.0 ± 10.45	233.8 ± 14.74
Anti-ath Ind	0.14 ± 0.005	0.13 ± 0.011	0.14 ± 0.003	0.13 ± 0.014	0.13 ± 0.003
Item	Day 15				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
TC (mg/dL)	332.7 ± 9.57$^{\#}$	251.6 ± 13.70$^{*\#}$	215.1 ± 16.62$^{*\#}$	256.6 ± 16.08$^{*\#}$	187.6 ± 7.16$^{*\#ab}$
HDL-C (mg/dL)	41.9 ± 2.32	45.5 ± 2.14	49.2 ± 2.61	48.9 ± 2.75	58.3 ± 1.48$^{*\#ab}$
Non HDL-C (mg/dL)	290.8 ± 7.75$^{\#}$	206.1 ± 12.13$^{*\#}$	165.9 ± 15.29$^{*\#}$	207.7 ± 14.68$^{*\#}$	128.9 ± 6.23$^{*\#ab}$
TG (mg/dL)	231.3 ± 10.40$^{\#}$	207.2 ± 11.15	179.6 ± 8.37$^{*\#}$	205.6 ± 10.69$^{\#}$	138.3 ± 6.31$^{*\#ab}$
Anti-ath Ind	0.13 ± 0.005	0.19 ± 0.015$^{*\#}$	0.24 ± 0.016$^{*\#}$	0.19 ± 0.016$^{*\#}$	0.31 ± 0.011$^{*\#ab}$
Item	Day 30				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
TC (mg/dL)	398.5 ± 7.75$^{\#}$	190.8 ± 4.09$^{*\#}$	153.7 ± 10.10$^{*\#b}$	198.9 ± 9.15$^{*\#}$	131.0 ± 3.01$^{*\#ab}$
HDL-C (mg/dL)	44.8 ± 2.13	54.0 ± 1.99$^{\#}$	61.9 ± 1.93$^{*\#}$	65.1 ± 3.13$^{*\#}$	76.4 ± 2.56$^{*\#ab}$
Non HDL-C (mg/dL)	353.8 ± 6.29$^{\#}$	136.8 ± 4.60$^{*\#}$	91.8 ± 9.24$^{*\#b}$	133.9 ± 7.34$^{*\#}$	54.7 ± 2.76$^{*\#ab}$
TG (mg/dL)	256.5 ± 10.28$^{\#}$	151.0 ± 6.20$^{*\#}$	121.7 ± 4.94$^{*\#b}$	164.3 ± 5.24$^{*\#}$	98.6 ± 4.86$^{*\#ab}$
Anti-ath Ind	0.11 ± 0.004$^{\#}$	0.28 ± 0.012$^{*\#}$	0.41 ± 0.022$^{*\#b}$	0.33 ± 0.023$^{*\#}$	0.58 ± 0.017$^{*\#ab}$

Abbreviations: HL, hyperlipidemic control group; At 40, atorvastatine (40 mg/kg, p.o.); At 100, atorvastatine (100 mg/kg, p.o.); Mol 100, sodium molybdate (100 mg/kg, p.o.); At 40 + Mol 100, atorvastatine (40 mg/kg, p.o.) + sodium molybdate (100 mg/kg, p.o.); TC, total cholesterol; HDL-C, high density lipoprotein; Non HDL-C, non-high density lipoprotein; TG, triglycerides; Anti-ath Ind, anti-atherogenic index.
Values are expressed as mean ± SEM of 16 h fasted hamsters (n = 10). All treatments were continued for 30 consecutive days.
* Significantly different from HL group at corresponding time intervals at $P < 0.05$.
$^{\#}$ Significantly different from the corresponding group value at day 1 at $P < 0.05$.
^{a} Significantly different from Mol 100 group value at corresponding time intervals at $P < 0.05$.
^{b} Significantly different from At 40 group value at corresponding time intervals at $P < 0.05$.

Treatment of the hyperlipidemic hamsters for 30 days with sodium molybdate (100 mg/kg) resulted in a significant reduction in TC (50%), Non-HDL-C (62%), TG (36%) and a significant elevation in HDL-C (45%) compared with hyperlipidemic hamsters at 30 days. Moreover, sodium molybdate produced a 3 fold improvement in the anti-atherogenic index (Table 1).

Combined administration of sodium molybdate (100 mg/kg) with atorvastatin (40 mg/kg) for 30 days resulted in a better protection against diet-induced hyperlipidemia in hamsters which was indicated by the significant reduction in TC (67%), Non HDL-C (85%), TG (62%), and the significant elevation in HDL-C (71%) compared with hyperlipidemic hamsters at 30 days. Moreover, the anti-atherogenic index was significantly improved by 5 folds. Interestingly, all these values were found to be significantly different from the corresponding values of either atorvastatin (40 mg/kg) or sodium molybdate alone groups at 30 days treatment (Table 1).

As shown in Table 2, Hyperlipidemic diet did not significantly affect the liver transaminases (AST and ALT) activities all over the experiment. Treatment of the hyperlipidemic hamsters with atorvastatin (100 mg/kg) for 30 days resulted in a significant elevation in plasma activities of both AST (34%) and ALT (36%) compared with the hyperlipidemic group at 30 days. On the contrary, sodium molybdate produced no effect on the activities of transaminases during treatment. Similarly combined administration of sodium molybdate (100 mg/kg) with atorvastatin (40 mg/kg) for 30 days did not affect the activities of transaminases (Table 2).

The results of Table 3 showed that administration of hyperlipidemic diet for 30 days produced a significant elevation in plasma lipid peroxides measured as MDA. On the contrary, Administration of atorvastatin (40 or 100 mg/kg), sodium molybdate (100 mg/kg), and combination of sodium molybdate (100 mg/kg) with atorvastatin (40 mg/kg) for 30 days resulted in a significant reduction in plasma lipid peroxides levels (to 55%, 60%, 56%, and 66%, compared with the control hyperlipidemic hamsters at 30 days, respectively).

As presented in Table 3, hyperlipidemic diet did not significantly affect the plasma glutathione peroxidase activity all over the experiment. Meanwhile, administration of atorvastatin (40 or 100 mg/kg), sodium molybdate (100 mg/kg), and combination of sodium molybdate (100 mg/kg) with atorvastatin (40 mg/kg) for 30 days produced a significant elevation in plasma glutathione peroxidase (GPx) activity (to 2.5, 2.8, 3.5, and 3.8 folds compared with the control hyperlipidemic hamsters at 30 days, respectively).

The present results showed that hyperlipidemic diet did not significantly affect the catalase activity during the experiment. Meanwhile, 30 days treatment of hyperlipidemic hamsters with atorvastatin (100 mg/kg) significantly increased the catalase activities (to 96%, compared with the hyperlipidemic group at 30 days). Similarly, treatment with sodium molybdate significantly elevated the catalase activity (to 111%, compared with the hyperlipidemic group at 30 days). Additionally, combined administration of sodium molybdate (100 mg/kg) with atorvastatin (40 mg/kg) for 30 days produced a significant elevation in plasma catalase activity (to 141% compared with the hyperlipidemic group at 30 days) (Table 3).

4. Discussion

The significant and progressive increase in hamsters blood lipids (TC, Non-HDL-C, and TG) induced by hypercholesterolemic diet which was observed in the current study is in agreement with a previous report

Table 2. Effect of treatment on plasma AST and ALT activities in hyperlipidemic hamsters

Item	Day 1				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
AST (U/L)	82.4 ± 3.32	84.9 ± 4.37	81.0 ± 3.49	79.8 ± 5.17	86.7 ± 4.63
ALT (U/L)	38.6 ± 4.03	39.9 ± 3.00	42.1 ± 3.68	40.8 ± 3.13	41.0 ± 3.31
Item	Day 15				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
AST (U/L)	84.2 ± 3.52	94.5 ± 4.66	104.1 ± 5.17*#	88.3 ± 4.97	95.7 ± 4.89
ALT (U/L)	45.1 ± 3.21	48.5 ± 3.01	58.9 ± 3.85*#	46.1 ± 2.89	49.1 ± 3.39
Item	Day 30				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
AST (U/L)	90.9 ± 3.97	103.1 ± 3.83#	121.4 ± 6.01*#	94.7 ± 5.28	102.5 ± 4.49
ALT (U/L)	49.2 ± 2.91	55.2 ± 2.74#	66.7 ± 3.85*#	51.0 ± 3.11	52.7 ± 3.89

Abbreviations: HL, hyperlipidemic control group; At 40, atorvastatine (40 mg/kg, p.o.); At 100, atorvastatine (100 mg/kg, p.o.); Mol 100, sodium molybdate (100 mg/kg, p.o.); At 40 + Mol 100, atorvastatine (40 mg/kg, p.o.) + sodium molybdate (100 mg/kg, p.o.); AST, Aspartate aminotransferase; ALT, Alanine aminotransferase.

Values are expressed as mean ± SEM of 16 h fasted hamsters (n = 10). All treatments were continued for 30 consecutive days.

* Significantly different from HL group at corresponding time intervals at $P < 0.05$.

Significantly different from the corresponding group value at day 1 at $P < 0.05$.

Table 3. Effect of treatment on plasma MDA, glutathione peroxidase and catalase activities in hyperlipidemic hamsters

Item	Day 1				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
MDA (nmol/L)	444.2 ± 39.13	460.9 ± 52.13	432.6 ± 50.17	418.9 ± 41.67	442.4 ± 48.27
GPx (nmol/min/mL)	51.0 ± 5.59	63.7 ± 7.47	50.7 ± 5.67	65.6 ± 7.54	59.1 ± 6.22
Catalase (µmol/L)	13.9 ± 1.32	12.9 ± 1.64	14.1 ± 1.29	15.0 ± 1.76	13.2 ± 1.64
Item	Day 15				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
MDA (nmol/L)	606.2 ± 48.30[#]	417.7 ± 50.17[*]	346.2 ± 34.95[*]	329.8 ± 32.69[*]	296.6 ± 30.10[*#]
GPx (nmol/min/mL)	49.8 ± 4.43	78.3 ± 8.35	99.2 ± 9.54[*#]	121.1 ± 14.78[*#]	128.5 ± 15.94[*#b]
Catalase (µmol/L)	12.2 ± 1.27	13.7 ± 1.62	15.7 ± 1.48	16.9 ± 1.95	15.5 ± 1.66
Item	Day 30				
	HL	At 40	At 100	Mol 100	At 40 + Mol 100
MDA (nmol/L)	659.9 ± 49.46[#]	294.9 ± 38.25[*#]	266.7 ± 28.07[*#]	289.0 ± 31.30[*#]	221.9 ± 28.58[*#]
GPx (nmol/min/mL)	47.7 ± 4.30	119.5 ± 10.72[*#]	135.1 ± 14.71[*#]	171.6 ± 14.37[*#]	183.4 ± 18.29[*#b]
Catalase (µmol/L)	10.5 ± 1.01	18.2 ± 1.85	20.5 ± 1.92[*#]	22.0 ± 2.32[*]	25.2 ± 2.45[*#]

Abbreviations: HL, hyperlipidemic control group; At 40, atorvastatine (40 mg/kg, p.o.); At 100, atorvastatine (100 mg/kg, p.o.); Mol 100, sodium molybdate (100 mg/kg, p.o.); At 40 + Mol 100, atorvastatine (40 mg/kg, p.o.) + sodium molybdate (100 mg/kg, p.o.); MDA, malonedialdehyde; GPx, glutathione peroxidase.
Values are expressed as mean ± SEM of 16 h fasted hamsters (n = 10). All treatments were continued for 30 consecutive days.
[*] Significantly different from HL group at corresponding time intervals at $P < 0.05$.
[#] Significantly different from the corresponding group value at day 1 at $P < 0.05$.
[b] Significantly different from At 40 group value at the corresponding time intervals at $P < 0.05$.

by Moghadasian (*22*). Similarly, the increase of lipid peroxidation in animals fed a hyperlipidemic diet has been previously reported (*23-25*). It is known that, the hypercholesterolemic diet can change the *in vivo* antioxidant status of blood by increasing the generation of oxygen free radicals these exert their cytotoxic effect by causing lipid peroxidation (*23*) which promotes the cellular consumption of glutathione and inactivates glutathione peroxidase (*26*). Meanwhile, the free radicals generated during lipid peroxidation could inactivate catalase, and reduce the effectiveness of the cells to protect themselves from damage (*27*) which is concordant with the present results.

In the current study, transaminases activity was estimated to evaluate the possible damage of the liver which may occur during feeding the hyperlipidemic diet or upon treatment with atorvastatin or sodium molybdate. AST and ALT levels remain the most useful tests for detection of hepatic cell damage. Both enzymes are present in high concentration in hepatocytes and leak into the circulation when hepatocytes or their cell membranes are damaged (*28*).

It was reported that treatment with a high concentration of cholesterol can cause liver damage (*29*). However, the results of the current study revealed that AST and ALT activities did not significantly elevated by administration of hyperlipidemic diet. Furthermore, hamsters treated with sodium molybdate did not present any alteration in plasma AST and ALT levels. On the contrary, treatment with atorvastatin (100 mg/kg) significantly elevated the levels of both enzymes at 15 and 30 days of treatment compared with hyperlipidemic group at the same corresponding time. Meanwhile, treatment of the hamsters with the combination of sodium molybdate with atorvastatin did not significantly affect the liver enzymes all over the experiment. The results indicate a possibility of liver injury by the high dose of atorvastatin.

It was reported that, some patients have to discontinue statins therapy due to liver transaminases levels exceeding three times the upper reference limit especially at high doses (*30,31*). Similarly, hepatotoxicity related to atorvastatin was also reported previously (*32-34*). The risk of significant rise in serum transaminases, while using atorvastatin, is thought to be dose dependent (*35,36*). Atorvastatin is significantly longer acting compared with other statins this could explain the increased risk of hepatotoxicity in comparison to other statins (*35*). The pathogenesis of atorvastatin-associated liver dysfunction is unclear. However, statins-induced transaminases elevation was explained by the direct inhibition of mevalonate synthesis (*37*). Another explanation is through interference as well as disturbance of cholesterol-bile acid pathways. In this context, statins are known to reduce levels of oxidized cholesterol and cholesterol substrate for the hydroxylase, the enzyme involved in bile acid synthesis (*31*). Additionally, Dujovne (*38*) postulated that the pronounced lowering in serum low-density lipoprotein induced by atorvastatin could influence the structure of hepatocellular membrane leading to greater leakage of cellular enzymes including transaminases. Furthermore, some authors suggested that the induction of the CYP450 system may be central to these adverse events (*34,39*). Furthermore, an immunoallergic basis for such hepatotoxicity was previously suggested (*40*).

The lack of effect of sodium molybdate on liver

enzymes observed in the current study is in agreement with the results of Van Reen (*41*) after excessive feeding of sodium molybdate to rats.

Results of the present study indicated that, treatment of the hyperlipidemic hamsters with atorvastatin resulted in a significant decrease in plasma total cholesterol and Non-HDL cholesterol levels. Similar response was observed for plasma triglycerides levels. These effects were accompanied by a significant increase in HDL-C level (specially, at the high dose) and a significant improvement in the anti-atherogenic index.

Atorvastatin usually reduces LDL-C, in human, by 30-70% depending on the dose used (*31*,*42*). Similarly, four weeks atorvastatin therapy was reported to cause a significant decrease in oxidative stress, and LDL-C with an increase in HDL-C levels in hypercholesterolemic rabbits (*43*). Similarly, atorvastatin was reported to produce a significant lowering in TC, TG, and LDL-C accompanied by a reduction in the oxidative stress in STZ-diabetic rats (*44*).

Statins are proved to inhibit HMG-CoA reductase, the rate-limiting enzyme for cholesterol biosynthesis, and subsequently reduce cholesterol synthesis in the liver. This inhibition induces hepatocytes to increase their surface expression of LDL-receptors, so as to increase uptake of LDL and reduce plasma cholesterol as well as LDL-cholesterol (*45*). The elimination half life of atorvastatin is considered to be long, compared with other statins, this may explain its greater efficacy for lowering the LDL-cholesterol. Additionally, atorvastatin is unique in that its metabolites are active as inhibitors of HMG-CoA reductase as well as being potentially antioxidants (*46*).

The observed atorvastatin-induced reduction in hamster plasma level of triglycerides is consistent with the observation of the previous reports by Mangalogu, *et al.* (*47*) and Funatsu, *et al.* (*48*), using fructose-fed hamsters and sucrose fed rats, respectively. The mechanism of triglycerides lowering effect of atorvastatin may involve the marked inhibition of cholesterol synthesis which suppresses the assembly and secretion of hepatic VLDL (*49*,*50*). Another plausible mechanism is the increase in hepatic LDL receptors induced by atorvastatin leading to increase clearance not only of plasma LDL but also of VLDL remnant particles, and resulting in the reduction of both cholesterol and triglycerides levels (*51*). Furthermore, reduction of hepatic fatty acids levels, induced by long term treatment with atorvastatin, is responsible for reduction of hepatic triglycerides synthesis and secretion (*52*).

The Mechanism behind the HDL raising effects of statins is likely to involve gene transcription and phosphorylation of peroxisomal proliferating activator receptor-α (PPAR-α) (*31*).

In the current study, atorvastatin significantly reduced lipid peroxidation and increased the activities of glutathione peroxidase and catalase in hyperlipidemic hamsters. These effects could be correlated to the protective antioxidant effect, and/or the significant lipid lowering effect of atorvastatin. Furthermore, the reduction in lipid peroxides could be a direct consequence of the significant decrease in LDL-C which is more prone to oxidation (*43*). Statins have been shown in animal models to act as antioxidants by decreasing LDL oxidation (*53*) and to modulate oxidation of lipoproteins (*54)*, superoxide generation (*55*), and scavenger receptor expression (*56*). Moreover, the metabolites of atorvastatin were found to be potent antioxidant (*46*). Additionally, atorvastatin may provide protection from oxidative damage, induced by hyperlipidemia, indirectly *via* upregulating expression of the free radical scavenging enzyme catalase (*9*) which is concordant with the present results.

In the present study, treatment of hyperlipidemic hamsters with sodium molybdate resulted in a significant decrease in TC, Non HDL-C, and TG levels with a substantial increase in the serum HDL-C levels. On the other hand, sodium molybdate produced a significant decrease in lipid peroxidation and a marked increase in activities of glutathione peroxidase and catalase in plasma. The obtained results are concordant with those by Panneerselvam and Govindasamy (*18*) who found that, oral administration of molybdate for thirty days significantly reduced the levels of lipids like cholesterol, triglycerides, phospholipids and lipid peroxidation and simultaneously increased the activities of antioxidants like superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione (GSH) in diabetic rats. Similarly, normalization of lipid levels in STZ-diabetic rats by orally administered sodium molybdate was corroborated (*16*,*17*).

The alterations in lipids and antioxidant status of hyperlipidemic hamsters can be attributed to the increase in peroxidative damage of lipids induced by the free radicals. Accordingly, the suppression of lipid peroxidation may result from the anti-free radical activities of sodium molybdate. Sodium molybdate was previously reported to have the ability to increase the activities of antioxidant enzymes, and decrease the availability of lipid substrates by acting as a free radical scavenger (*57*). This effect occur through trapping of the free radicals by the cationic molybdate compound leading to reduction in lipid peroxidation and a lipid lowering effect (*18*). Furthermore, the lipid lowering action of sodium molybdate can be also explained through its insulin like action (*18*,*58)*. In this context, sodium molybdate was shown to inactivate glycogen synthase and increase glycolytic flux in rat hepatocytes (*59*) and to display synergistic stimulation of glucose uptake in rat adipocytes in the presence of H_2O_2 (*60*,*61*).

Catalase and glutathione peroxidase are enzymes which destroy the peroxides and play a significant role in providing antioxidant defense to an organism. Both are involved in the elimination of H_2O_2. The functions

of these enzymes are interconnected and a lowering of their activities results in the accumulation of lipid peroxides and increases oxidative stress in tissues. The observed increase in activities of these enzymes after administration of sodium molybdate can be attributed to the potent antioxidant property of molybdate (*57*). Molybdenum is present in the active site of various molybdoenzymes, including a variety of oxidases (*62*). Additionally, molybdates were reported to increase the activities of antioxidant enzymes like superoxide dismutase (*63*) as well as the activities of enzymes like glutathione peroxidase and catalase (*64*).

The current study showed that combined administration of sodium molybdate with the low dose of atorvastatin (40 mg/kg) produced a maximum lowering in blood lipids, compared with atorvastatin (100 mg/kg) dose, provided a better antioxidant picture, compared with each drug alone, and kept the integrity of the liver enzymes in the same time. The results indicated that the combined therapy is able to modify the hyperlipidemia apparently by improving the efficiency of the antioxidant defense system in hamsters.

5. Conclusion

Atorvastatin is a potent hypolipidemic drug, especially at high dose, it effectively reduces the elevated blood lipids (TC, Non HDL-C, TG), improves HDL-C, anti-atherogenic index and strengthen the antioxidant status of hyperlipidemic hamsters. However, these effects are accompanied by deterioration in liver function and elevation in transaminases.

Sodium molybdate prevents oxidation of lipids, significantly reduces hyperlipidemia, increases HDL-C, anti-atherogenic index, and protects antioxidant systems in experimental hyperlipidemia. Moreover, it did not affect the liver transaminases.

Combination of sodium molybdate with atorvastatin lead to a maximum reduction in plasma total cholesterol, triglycerides, Non-HDL cholesterol and a pronounced increase in HDL cholesterol levels apparently by improving the activity of the antioxidant defense system. Meanwhile, this combined therapy produces no deleterious effect on the liver enzymes. Accordingly, sodium molybdate may be a useful candidate for combined therapy with atorvastatin to maximize the lipid lowering effect and affording a good antioxidant status with simultaneous protection of the liver. This is the first experimental study, after searching in the literature, to investigate the benefits of combination of sodium molybdate with atorvastatin in hyperlipidemic models. The obtained results greatly recommend combination of both drugs in management of hyperlipidemia.

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