Original Article

Evaluation of Anti Hyperlipidemic Effect of Zinc Sulfate Alone and in Combination with Atorvastatin in Diet Induced Hyperlipidemic Rats

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(This study was conducted while the authors at Sr. No 1, 2 4, 5 and 6 were working at King Edward Medical University, Lahore. During the publication process, they are promoted and current affiliation are as mentioned above)

Abstract

Background: Hyperlipidemia a lipid disorder that affects millions of people worldwide. It is a major contributor for developing cardiovascular diseases.

Objective: To assess the antihyperlipidemic activity of zinc sulfate alone and in combination with atorvastatin in diet induced hyperlipidemic rats.

Methods: Randomized clinical trial was conducted for 8 weeks in Post graduate Medical Institute, Lahore. Thirty -six healthy male albino rats weighing between 100-160mg were randomly divided into 6 groups each having 6 rats. Group A normal control (fed with rat chow) and Group B disease control (fed with high fat diet). Groups C,D,E and F, were experimental groups, fed with high fat diet during the initial four weeks to produce hyperlipidemia and during next four weeks, along with high fat diet (30 mg/kg zinc sulfate orally) was added to Group C, (40 mg/kg Atorvastatin orally) to Group D (30 mg/kg of zinc sulfate + 40 mg/kg atorvastatin) to Group E and (15 mg/kg zinc sulfate +20 mg/kg atorvastatin) to Group F. Blood samples were drawn at 0, 4 and 8 weeks and serum was assessed for lipid profile. Rats were sacrificed at the end of 8 weeks and liver samples examined for histopathological examination.

Results: Disease control group showed significant rise in total cholesterol from 80.17±17.82 at 0 week to 177.17± 15.96, low density lipoprotein from 32.62±17.15 to 102.03±10.88, and triglycerides from 58.17±23.79 to 159.00±39.83 with a p-value of <0.05. All the experimental groups improved lipid profile compared to disease control group a p-value of <0.001. All experimental groups increased high density lipoprotein compared to disease control group, most significant increase was seen in group E (63.00±5.29) with a p-value of 0.010. Histopathological examination showed, 66.7% of rats in disease control group had severe steatosis. Among the experimental groups, Group D had 33.3 % rats while Group C, E, and F had no animal with severe steatosis.

Conclusion: Zinc supplementation alone and in combination with atorvastatin has shown beneficial effects on lipid profile. Zinc sulfate also reduced the fatty alterations of hepatic architecture.

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Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality causing around 17.9 million deaths in 2019, a 25.1% increase compared to 2000[1] and are estimated to cause 23.6 million deaths annually

by 2030.[2] Hyperlipidemia is a modifiable risk factor for cardiovascular diseases, characterized by increase in total cholesterol, LDL and triglyceride levels while decrease in HDL concentration.[3]

Statins are a group of drugs used for treating hyperlipidemia with a recognized ability to decrease cardiovascular events. Despite all beneficial effects, statins can exhibit adverse effects like muscular pain leading to statin intolerance in many people[4] prompting researchers to look for alternative therapies with minimum adverse effects.

Hypercholesterolemia can be treated by nutraceutical supplements. They can be given either alone or as an adjuvant with conventional lipid lowering drugs. These substances have lesser side effects because of their natural origin.[5]

Zinc, an essential micronutrient and cofactor of various proteins, plays crucial part in various metabolic mechanisms.[6] Studies have shown that zinc deficiency may be associated with the risk for CVDs and development of non-alcoholic fatty liver disease and zinc administration might be beneficial for the prevention and treatment of these diseases.[7,8]

Few prior studies have examined the effects of zinc sulfate in hyperlipidemia but they were limited in comparing these effects with the conventional anti-hyperlipidemic drug; statins. The study aimed at fulfilling this gap by assessing the impact of zinc supplementation in hyperlipidemia, considering its potential as a safe, cost effective and efficient lipid lowering agent. It also assessed that whether zinc supplementation augments the lipid lowering efficacy of statin or not when administered co-currently.

Methods

Rats procured from University of Veterinary and Animal Sciences, Lahore were put in neat cages at Post graduate medical institute animal house, Lahore. Rats were exposed to natural day and night cycles at room temperature of 22 ± 1°C throughout the experiment. They were given free contact to water and rat chow ad libitum. Animals were marked for proper identification. A period of seven days was given to animals to get acclimatized before the start of experiment.

To induce hyperlipidemia, 2 gm. cholesterol powder, 1gm. sodium deoxycholate powder and 10 ml coconut oil were mixed with 100 gm. of normal rat feed. The diet was converted to small cakes and dried in shades at room temperature.

Rats in Group A (Normal Control) were given normal rat chow throughout the study duration. After 4 weeks, 1 ml/kg distilled water was given orally for the next 4 weeks.

Rats in Group B (Disease Control) were fed High fat diet throughout the study duration. After 4 weeks, distilled water was also given orally in a dose of 1 ml/kg for the next 4 weeks.

Rats in Group C,D,E and F were fed with high fat diet throughout the duration of study. After 4 weeks, along with the high fat diet, following drugs were given orally with the help of 1ml syringe.

Group C: 40 mg/kg of atorvastatin daily

Group D: 30 mg/kg zinc sulfate daily

Group E: 30 mg/kg of zinc sulfate and 40 mg/kg atorvastatin daily

Group F: 15 mg/kg zinc sulfate and 20 mg/kg atorvastatin daily

Rats were fed with high fat diet throughout the duration of study. After 4 weeks, 40 mg/kg of atorvastatin was also administered orally with the help of 1 ml syringe daily for the next 4 weeks.

High fat diet was fed to rats during entire study duration. After 4 weeks, 30 mg/kg zinc sulfate was also given orally with the help of 1 ml syringe daily along with HFD for the next 4 weeks.

High fat diet was given during the whole study duration. After 4 weeks, 30 mg/kg of zinc sulfate and 40 mg/kg atorvastatin were also given orally with the help of 1 ml syringe daily along with high fat diet for the next 4 weeks.

Rats were fed high fat diet during the whole the study period. After 4 weeks, 15 mg/kg zinc sulfate and 20 mg/kg atorvastatin were also given orally with the help of 1 ml syringe daily along with high fat diet for the next 4 weeks.

At the end of the eight weeks, the animals were fasted overnight, sacrificed under ether anesthesia and blood collected from the jugular vein for analysis. The blood samples were centrifuged for 5 min and the serum collected was stored in tubes at -20°C before analysis.

Liver piece of all rats was processed in an automatic tissue processor at Pathology Department of King Edward Medical University, Lahore. Tissues were then embedded in paraffin and thick sections of 5 micrometer were cut with microtome. The cut sections were placed on glass slides and stained with hematoxylin and eosin.

The slides were then examined under scanner (4×) of light microscope to look for nonalcoholic hepatic steato-

sis. The range of parenchymal involvement was examined by the occurrence of fat cells and graded according to NASH CRN system[9] that divides the tissue according to parenchymal involvement as follows.

Grade 1 = 0-5% parenchymal involvement by steatosis

Grade 2 = 5-33 % parenchymal involvement by steatosis

Grade 3 = 33-66% parenchymal involvement by steatosis

Grade 4 = above 66 % parenchymal involvement by steatosis

The data was analyzed by using statistical package for social studies (SPSS 20). P value <0.05 was taken as significant.

Results

At week 8, mean cholesterol level in disease control group was significantly higher than the normal control and all the experimental groups both having p values of (<0.001). When compared by one-way ANOVA, the difference among the groups was significant at week 8 with p value of (0.001). However, when groups were compared pairwise, the difference among the experimental groups themselves was insignificant.

Serum triglyceride levels in normal control and disease control increased from week 0 to week 8. In disease control group, the difference in triglyceride level was significant with p value of (0.008). When compared by one-way ANOVA, the difference among the groups was significant with a p value of (<0.001). However, when groups were compared pairwise, only group D showed reduction in triglyceride level with p value of <0.001.

Normal control and disease control group showed insignificant value from week 0 to 8. When compared by one-way ANOVA, the difference among the groups was statistically significant. However, when compared pairwise, all the experimental groups showed a significant increase in HDL level, most significant was seen in group E with a p-value of (0.001).

At week 8, LDL level in disease control group was significantly higher than the normal control group and all the experimental groups. When compared by one-way ANOVA, the difference in LDL levels among groups was significant. However, when compared pairwise, difference between the experimental groups was insignificant statistically.

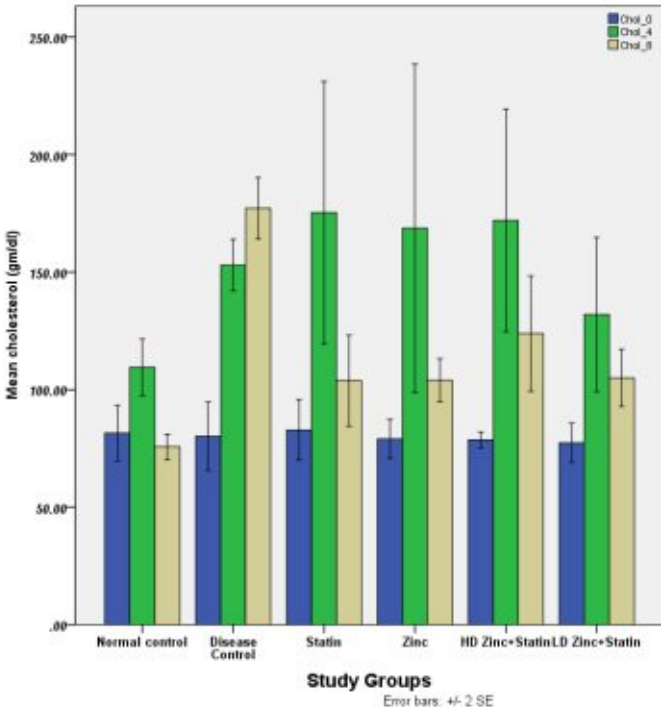

Figure 1a. *Effect of zinc sulfate and atorvastatin on cholesterol level in HFD induced hyperlipidemic rats*

Histopathological examination

Estimation of percentage of fatty alteration in hepatic parenchyma was used for histological examination of liver slides

Table 1: *The values are expressed as mean ± SE. *Mean value of high fat diet is significantly different from normal control (p≤0.05). °Mean values are significantly different compared to high fat diet group (p≤0.05)*

Group	Cholesterol	Low Density Lipoprotein	Triglyceride	High Density Lipoprotein
A	75.67±6.65	49.60±11.74	75.33±27.39	41.00±11.29
B	177.17±15.96*	102.03±10.88*	159.00±39.83*	43.33±9.24
C	103.83±23.71°	27.63±26.69°	131.00±22.89	50.00±13.08
D	104.00±11.35°	34.03±20.17°	69.83±13.60°	56.00±10.79
E	123.83±30.06°	40.43±20.47°	102.00±31.62°	63.00±5.29°
F	105.00±14.89°	43.17±24.61°	88.33±41.94°	44.17±13.14
p-value	**<0.001**	**<0.001**	**<0.001**	**0.010**

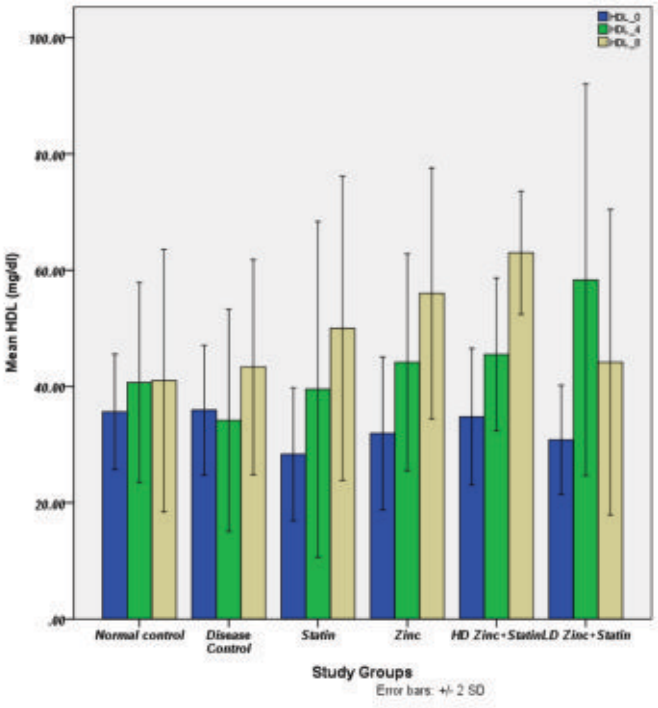

Figure 1b. *Effect of zinc sulfate and atorvastatin on HDL levels in HFD induced hyperlipidemic rats*

Table 2: *Percentage of Rats according to Hepatic Steatosis Grading in each Group (n=6)*

Study Groups	Hepatic Steatosis			
	Nil	Mild	Moderate	Severe
Group A	83.3%	16.7%	0.0%	0.0%
Group B	0.0%	0.0%	33.3%	66.7%
Group C	0.0%	66.7%	33.3%	0.0%
Group D	0.0%	33.3%	33.3%	33.3%
Group E	0.0%	66.7%	33.3%	0.0%
Group F	0.0%	16.7%	83.3%	0.0%

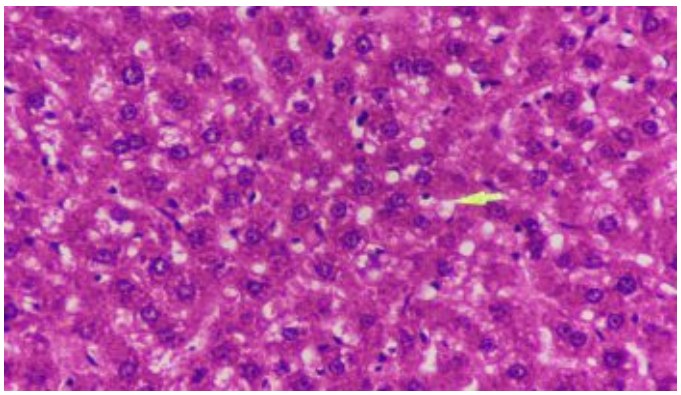

Figure 1. *H&E stained section of rat liver at 40X magnification in Disease control group showing fat globule pointed by arrow.*

By applying Mann Whitney U test for pair wise comparison of hepatic steatosis, we found that disease control group and all the experimental groups developed statistically significant hepatic steatosis as compared to normal control group. The difference in hepatic steatosis in all experimental groups was significantly less than the disease control group while the difference among the experimental groups themselves was insignificant.

Discussion

In this study, male albino rats were chosen as experimental model and hyperlipidemia was induced by giving high fat diet which is the most common composition used to induce hypercholesterolemia in experimental models.[10] High fat diet was given for two months which induced hyperlipidemia and hepatic steatosis.

The parameters of study comprised of serum analysis of total cholesterol, triglyceride, LDL, HDL and liver histology for the presence of hepatic steatosis. Lipid profile parameters varied throughout the study period in normal control group.

All experimental groups, showed significant reduction in cholesterol level as compared to disease control group. Among the combination groups, group E had numerically better cholesterol lowering effect compared to group F. However, it was statistically insignificant. Studies done by Berman and Ali on diabetic rats also showed that zinc supplementation improved oxidative stress and serum cholesterol levels.[11,12]

All the experimental groups showed significant reduction in LDL level compared to disease control. This reduction was almost similar in all experimental groups with no statistically significant difference among them. A recent study carried out in Pakistan also showed that Zinc supplementation improved glycemic control and decreased levels of serum cholesterol, LDL and triglyceride.[13]

Among the experimental groups, only group D showed significant reduction in triglyceride levels. A study conducted on overweight people who were given zinc supplementation also showed reduction in triglyceride level.[14]

Serum HDL levels in the present study did not show significant reduction after induction of hyperlipidemia. This may be because of various underlying factors. Firstly, the duration for induction of hyperlipidemia is short and in previous studies on rodents, it was shown that cholesterol is mainly found as HDL and it is less likely to be affected by this short period of HFD induction[(11)]. Secondly, studies have shown that coconut oil present in HFD increases HDL cholesterol.[15] At the end of week 8, all experimental groups increased HDL

level except group F. Maximum increase was seen in group E. A previous study conducted on effect of zinc supplementation in hyperlipidemic male rabbits showed no significant effect of zinc supplementation on HDL levels.[16]

After completion of study, rats were sacrificed and livers were dissected out for histopathological examination. Hepatic slides were examined for alteration in hepatic parenchyma. The normal control group had no fatty alteration and showed normal parenchyma. The disease control group had significant fatty infiltration and steatosis. Experimental groups had significantly less steatosis in comparison to disease control but normal liver architecture was not observed at the end of the study. A study investigating role supplemental zinc taken concurrently with a high fat diet show reduction in hepatic steatosis.[17] Another study established that zinc deficiency is associated with the development liver injury and hepatic steatosis.[18] Similarly, patients with non-alcoholic fatty liver disease showed significantly reduced serum zinc concentrations compared with controls.[19] In another study on diabetic rats, it was also observed that zinc administration resulted in reversal of hepatic steatosis features.[20] Total duration of study was short (8 weeks) and only four weeks were given to reverse the hyperlipidemic changes induced by high fat diet consumption which might be insufficient. Longer duration studies are needed to assess the long-term effect of zinc on lipid profile. More studies are also needed to illustrate the correct mode of action of zinc sulfate as a lipid lowering agent and also to determine the maximum therapeutic dose of zinc sulfate.

Conclusion

Zinc sulfate has shown anti-hyperlipidemic effect which is comparable in many aspects to atorvastatin, the conventional current treatment for hyperlipidemia. The combination groups also showed lipid lowering activity but is was not significant compared to the individual supplementation of zinc and atorvastatin.

Ethical Approval: The Institutional Review Board, King Edward Medical University, Lahore approved this study vide letter No. 204/ RC/ KEMU.

Conflict of Interest: The authors declare no conflict of interest.

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Authors' Contribution:

HT: Conception and design, or acquisition of data, or analysis & interpretation of data. Drafting the article or revising it critically for important intellectual content.

RA: Analysis & interpretation of data, final approval of the version to be published

SH: Analysis & interpretation of data

SHK: Conception and design, Acquisition of data

HP: Final approval of the version to be published

WS: Acquisition of data, drafting of article

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