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Original Article

Anti-Hyperlipidemic Effect Of Zinc Complex Of Betulinic Acid In High Fat Diet- Induced Hyperlipidemia

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Abstract

Objective: This study evaluated the anti-hyperlipidemic and comparative effects of the Zinc complex of Betulinic acid (Zn+BA) with simvastatin (SIM), on high-fat diet-induced hyperlipidemia in rats, and the safety profile of the two treatments was also assessed.

Methodology: Hyperlipidemia was induced by giving a high-fat diet. BA +Zn 10 mg/kg and SIM 20 mg/kg were given orally for four weeks. On the final day terminal sampling was done and serum lipid profile (TG, TC, LDL, HDL) and hepatic enzymes (ALT) for assessing hepatotoxicity were estimated. Results: Our results showed that BA+Zn significantly increased HDL levels and significantly reduced serum TC, TG, and LDL ($p<0.001$) as compared to Simvastatin. Correspondingly serum ALT levels also showed a significant reduction ($p<0.001$) in comparison with Simvastatin.

Conclusion: Our study suggests that BA+Zn effectively attenuates high-fat diet-induced hyperlipidemia while preserving hepatic function and could serve as a better alternative to simvastatin in treating hyperlipidemia.

Keywords: Zinc, Betulinic acid, High Fat Diet, Hyperlipidemia, Simvastatin

Introduction

Hyperlipidemia is a metabolic disorder that results from an impaired lipid metabolism indicated by an elevated level of plasma lipids in the blood[1]. Hyperlipidemia has been acknowledged as a risk factor for atherosclerotic cardiovascular disease (ASCVD), coronary heart disease (CHD), transient ischemic attack (TIA), and ischemic heart disease (IHD). These diseases have proven to be the leading cause of mortalities worldwide[2]**.** In 2013 a study revealed that about 31% of all deaths in the world resulted from CVD, which is likely to increase by 26% by 2030[3].

Data from different research depicts that modifying lifestyle preferences or initiating lipid-lowering therapy can remarkably reduce the incidence of atherosclerosis and CVD [4]. Among lipid-lowering drugs, Statins provide the key support for treating hyperlipidemia by inhibiting the hepatic synthesis of cholesterol and consequently reducing other lipid fractions[5] **.** Despite the statins demonstrating agreeable results, patient compliance has been reported to be only 40%–60% [6], the reason being adverse effects such as digestive system disturbances, myalgia, myositis, and rhabdomyolysis [7], raised levels of glucose, glycosylated hemoglobin (HbA1C) [8] and elevation of transaminases, thereby limiting the statins usefulness in acute and chronic liver diseases [9].

During the last couple of decades, phytochemicals derived from squalene and terpenoids have received the most attention due to their vast range of biological actions[10]. Among these, Betulinic acid (BA), a pentacyclic triterpenoid, (3β-hydroxy-lup-20-en-28-oic) has proven to exhibit several biological properties, including antioxidant, anti-inflammatory, anti-hyperlipidemic, antihyperglycemic, antiviral [10], and hepatoprotective action [11]. BA can be isolated from the bark of birch trees (*Betula sp.,* Betulaceae*)*, Jujube (*Ziziphus*), Gebang palm (*Corypha taliera*), Black plum (*Syzygium* spp. Myrtaceae), Date plum (Diospyros) and Peony plant (*Paeonia*)[12]. Its hypolipidemic effect is attributed to a variety of actions including reduced lipid absorption from the small intestine, decreased lipogenesis and accumulation of lipids by inhibiting sterol regulatory element-binding protein1c (SREBP-1c) and its nuclear translocation through AMP-activated protein kinase (AMPK) pathway. BA also stimulates lipolysis and plays an important role in reversing obesity [10].

Trace elements, such as zinc and copper are involved in numerous physiological processes including, lipid metabolism, inflammation, and oxidative stress[13]. Multiple studies have suggested that zinc is an essential component of the regulation and expression of Proliferator-activated receptors (PPARs) and zinc depletion could lead to impaired PPAR function causing endothelial dysfunction and leading to diseases like diabetes and CVD[13]. Recent studies have suggested that zinc supplementation up-regulates PPAR and SREBP1 amount. Sub-groups of PPAR regulate processes like lipid storage, adipocyte differentiation, and fatty acid oxidation consequently leading to decreased total cholesterol, LDL, and reduced leptin levels.[14],[15].

Recent research has been aiming at developing newer treatment options by identifying active ingredients of various food extricates, one of the novel compounds being betulinic acid. Accordingly, there is a rising concern about the role of zinc as a nutritional supplement and its anti-hyperlipidemic activity as a potential treatment regimen for metabolic disorders.

Numerous undesirable effects attributed to Statins have been reported during the treatment of hyperlipidemia.[8] Establishing better alternatives to deal with the shortcomings of the standard has always posed a challenge for the researcher. The development of a drug with better or similar efficacy, which is safer and less toxic is the need of the hour. The present study aimed to investigate the antihyperlipidemic effects of BA+Zn and compare its effects with simvastatin on high-fat diet-induced hyperlipidemic rats. Subsequently, the safety profile of the two treatments was also compared in terms of adverse effects.

Materials and Methods

Study Design

The experimental work had a duration of eight weeks and was performed at the Department of Pharmacology, a postgraduate research laboratory at the Al-Mizan campus.

Preparation of animals

Forty adult male rats Sprague Dawley were procured from NIH, Animal House Islamabad. Under standard laboratory conditions, the temperature was controlled at 20–25°C with relative humidity maintained at 50 ± 5%. Photoperiod was kept constant at a 12-hour light and dark cycle, [16] The rats received standard rodent

chow and a high-fat diet (HFD) containing 60% fat-, 20% carbohydrate, and 20% protein [17], which was prepared at NIH and served with standard food pellets. They had free access to tap water through 200ml inverted bottles. Rodent chow was purchased and approved by the NIH, Islamabad. One week acclimatization preceded the experiment to ensure normal behavior and growth.

Preparation of drugs

Research-grade Simvastatin, Betulinic acid, and zinc were demanded from the German company SIGMA-ALDRICH. BA+Zn were prepared at –removed for blind review----

SIM 20 mg/kg was given by oral route OD for 30 days[18]

BA+Zn 10 mg/kg was given by oral route OD for 30 days [11],[19].

Methods

Study design

We made four equal sub-groups of rats through balloting method 10 rats in each. Following a week of acclimatization, baseline levels were checked through biochemical parameters. Healthy male rats weighing 300-350 grams(g), without any evident visible physical abnormality and normal lipid profile were included, while rats weighing less than 300 g and greater than 350 g, with any evident visible physical abnormality and deranged lipid profile were excluded from the study.

Group 1 was named Negative control (NC) group and received standard rodent chow and tap water for 56 days. Group 2 was named Disease control (DC) group and received HFD with normal tap water for the first 28 days and then standard animal diet from day 29 to day 56[20]. Group 3 was named (BA+Zn) group and received HFD for the first 28 days and then treated with Zinc complex of Betulinic acid and fed standard rodent chow from 29th day till 56th. Group 4 was named (SIM) group and received HFD from day 1 to day 28 and then administered along with SIM and given standard animal diet from the said period.

Blood sample collection

Blood samples were taken via cardiac puncture on day 0 to establish parameters within normal range, on day 29 to confirm induction of hyperlipidemia, and on day 57 to confirm the results of the intervention. Samples collection was done after anesthetizing the rats in a closed lid glass jar with cotton wool soaked in chloroform and the blood was stored in SST (serum separating tube with clot activating gel). Centrifugation of the sample tubes was performed at speed of 2500 rpm consuming ten minutes. Later serum was stored for biochemical analysis [16].

Biochemical analysis

Estimation of triglycerides (TGs), total cholesterol (TC), LDL, HDL, and serum ALT was done by using Erba Chem 7 Clinical Chemistry Analyzer.

Statistical analysis

SPSS version 25 was used for data analysis. Quantitative variables were calculated as Mean ± SEM. To observe group, mean differences between the 4 groups (Total cholesterol, TGs, HDL, and LDL), One-way ANOVA was applied, and a p-value of < 0.05 was considered significant. Paired t-test was applied to analyze the differences between BA +Zn and simvastatin for variable ALT. A p-value of ≤ 0.05 was significant.

Results

Our findings showed, a significant rise in serum total cholesterol in the DC group in contrast NC group ($p<0.001$). BA+Zn and SIM group depicted a significant reduction in total cholesterol levels in contrast to DC group ($p<0.001$) (Table I). A significant increase in serum triglycerides was seen in the DC group when compared with the NC group ($p<0.001$). Significant reduction of triglycerides, in the BA+Zn group, was similar to that of the SIM group, when compared with the DC group ($p<0.001$) (Table I). Significant increase in serum LDL was detected in DC group in contrast to NC group ($p<0.001$). Following BA+Zn treatment significantly reduced serum LDL, in contrast to DC group ($p<0.001$). The effect of treatment in the SIM group was like that of the BA+Zn group. (Table I). Significantly lower values of serum HDL were observed in the DC group when compared with the NC group. BA+Zn group and SIM group depicted a significant improved serum HDL levels in contrast to DC ($p<0.001$). (Table I). Significant difference was found in serum ALT between BA+Zn group and SIM group ($p<0.001$). This hepatoprotective effect of BA+Zn is shown in figure 1.

Acute physiopathological effects

Our study also aimed to investigate the safety profile of the novel compound in terms of adverse effects in comparison with Simvastatin. For this purpose, the safety profile study was carried out under the guidelines of "OECD guidelines for testing of chemicals"[21].

Four healthy non-pregnant Sprague Dawley female rats, 12 weeks old weighing approximately 0.3kg were

made available from the NIH, animal house, Islamabad. The animals were retained in well-ventilated metallic cages. Under standard laboratory conditions, the temperature was controlled at 20–25°C with relative humidity maintained at 50 ± 5%. The photocopier was kept constant at a 12-hour light and dark cycle. They received a normal standard diet which was prepared at the NIH and served with standard food pellets. They had free access to tap water through 200ml inverted bottles fixed on top of the cages. One week of acclimatization was allowed before the experiment to ensure normal behavior and growth.

Preparation and administration of doses

A two-fold dose (20 mg/kg/day) of BA + Zn was given during the first week which then gradually increased during the subsequent weeks. The dose increased three-fold (30 mg/kg/day) during the second week, four-fold (40 mg/kg/day) during the third, and five-fold (50 mg/kg/day) during the fourth week.

Observations

The rats were observed individually after dosing for any signs of toxicity or toxic reactions daily. They were assessed in terms of differences in food plus water intake, body weight, alertness, and changes in skin color or fur, and the findings were noted by the end of four weeks. According to our observations, the novel compound was very well tolerated, and no noticeable toxicities or toxic reactions were seen even after increasing the dose up to five times the original dose.

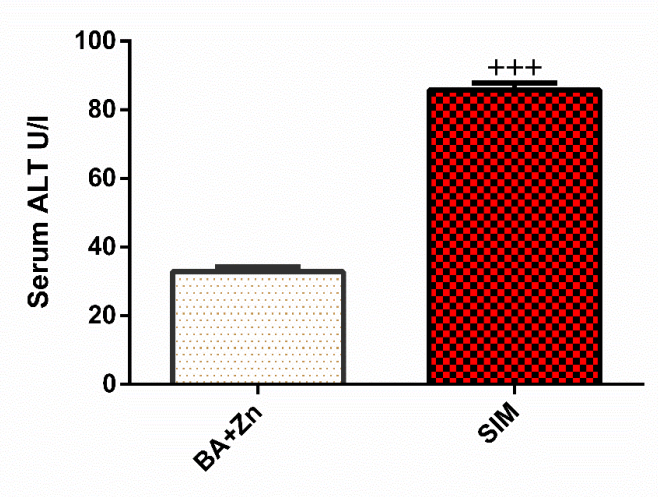

Figure-1: Applied paired t-test on serum ALT between two treated groups. Data presented as Mean ± SEM. BA+Zn, Betulinic acid + Zinc, SIM, Simvastatin, +++ p<0.001, significant difference when BA+Zn compared with SIM, which shows the significant hepatoprotective effect of the compound as compared to SIM.

Table-1: Multiple comparisons of Total cholesterol, Triglycerides, LDL, and HDL values between experimental groups. Data presented as Mean ± SEM. NC, Normal control, DC, Disease control, BA+Zn, Betulinic acid + Zinc, SIM, Simvastatin

n = 10	*NC*	*DC*	*BA + Zn*	*SIM*
Serum Total Cholesterol (mg/dl)	114.8 ± 4.719	164.7 ± 5.153###	104.8 ± 2.272 ***	123.0 ± 3.790 ***
Serum Triglycerides (mg/dl)	85.00 ± 3.202	221.1 ± 7.436###	102 ± 4.017 ***	114 ± 2.31 ***
Serum LDL (md/dl)	63.10 ± 4.363	124.0 ± 1.915###	50.30 ± 2.161 ***	69.10 ± 1.233 ***
Serum HDL (mg/dl)	52.10 ± 3.375	22.80 ± 1.153###	48.80 ± 2.250 ***	26.40 ± 1.424 ***

P < 0.001 when disease group compared to normal control group, ***p<0.001 when drug-treated groups compared to disease group

Discussion

Despite favorable results, data from recent studies have established the link between Statins and numerous adverse effects [22,23]. Hence, ACC/AHA has issued guidelines for managing hyperlipidemia and urged the need for additional research for developing newer treatment options may be required [24]. We, therefore, used the lipid profile, in the study to evaluate the efficacy of BA and Zinc in comparison with simvastatin on high fat diet-induced hyperlipidemia and compared the safety profile of the novel compound with that of simvastatin in terms of adverse effects.

In our HFD model, it was observed that BA + Zn, significantly reduced serum Cholesterol levels in the hyperlipidemic group. Similar findings were reported by Ahangarpour, Shabani, & Farbood[25], Zainub *et al.* [16] They observed that plasma cholesterol level was significantly lowered in hyperlipidemic mice treated with BA. Correspondingly a study by Naito, Yoshikawa, Yoshizawa, Takenouchi, & Yasui[26] reported a decrease in the raised serum cholesterol levels after treatment with zinc in high fat diet-induced hyperlipidemic mice.

We also observed that treatment with BA + Zn complex for four weeks significantly reduced serum TG levels in hyperlipidemic rats. Our results were in accordance to Ajala-Lawal *et al*. [11] and Wei *et al* [27] findings who suggested treatment with BA and zinc reduced serum TGs and might be useful in the treatment and prevention of atherosclerosis and hepatocellular lipid accumulation respectively.

Likewise, serum LDL level in the group treated with BA+Zn was markedly reduced when compared with that of the disease control and simvastatin group. Our results were in concurrence with Yoon *et al* [28] Choi, Liu, & Pan, [29] who proved that administration of BA reversed the HFD-mediated rise in serum TGs. Sadri *et al*. [19] reported similar results regarding the lipid-lowering effect of zinc supplementation.

Treatment with BA+Zn showed significantly raised serum HDL levels comparable with those of the control group. This is depicted in Fig. 4. These findings are backed by the study of Ahangarpour *et al*. [25] and Zainub *et al*. [16] who reported similar findings, also Asbaghi *et al* [30] observed similar results in their study about zinc supplementation. Oriakhi *et al*. [31] observed that BA ameliorated hepatic damage by significantly decreasing serum ALT and AST levels while Zhang *et al* revealed that supplementing a high dose of zinc significantly decreased serum ALT activity. These findings agree with our observations. Moreover, our study proved that treatment with BA+Zn at 10 mg/kg/day alleviated the rise and maintained serum ALT within the normal range as compared to simvastatin thus proving the hepatoprotective effect of the novel compound.

Conclusion

In conclusion, this study has revealed that BA+Zn effectively and synergistically ameliorates high-fat diet-induced hyperlipidemia and has comparable effects with simvastatin in improving hyperlipidemia. It was also observed that this novel compound preserves hepatic function and thus could be a better and safer alternative to simvastatin.

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