Protective effects of alendronate in Triton X-100-induced hyperlipidemia in rats

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Cite this article as: *Parwin A, Najmi AK, Ismail, MV, Kaundal M, Akhtar M. Protective effects of alendronate in Triton X-100-induced hyperlipidemia in rats. Turk J Gastroenterol 2019; 30(6): 557-64*

ABSTRACT

Background/Aims: *The aim of the present study was to evaluate the protective effects of alendronate (used in osteoporosis disease) in Triton X-100 (a polyethylene glycol-based non-ionic surfactant)-induced hyperlipidemia in rats.*

Materials and Methods: *The animals were randomized into seven groups receiving different treatments for 21 days, and alendronate was administered (1.5 and 3 mg/kg body weight, per orally (p.o.) by oral gavage). On day 21, the rats were anesthetized and decapitated, blood samples were extracted, and the livers were dissected for various biochemical tests and histopathological examinations.*

Results: *The biochemical parameters, total cholesterol (TC), triglycerides (TGs), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C), thiobarbituric acid reactive substances (TBARS), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and atherogenic index, were increased, and reduction in high-density lipoprotein-cholesterol (HDL-C) levels was observed following Triton X-100 treatment to rats. Alendronate (1.5 and 3 mg/kg) produced a dose-dependent reduction in serum TC, VLDL-C, TGs, ratio of TC/HDL-C, ALT, AST, and TBARS. It significantly increased the HDL-C and superoxide dismutase levels but did not cause a significant decrease in serum LDL-C and/or an increase in catalase levels. Histopathological examinations of alendronate showed beneficial effects with lower capsular thickening, slight enlargement of the hepatocytes at the margin, and lower inflammatory cell infiltration.*

Conclusion: *Alendronate showed dose-dependent antihyperlipidemic and hepatoprotective effects. It may serve a dual purpose as anti-osteoporotic and hypolipidemic by reducing blood cholesterol and TG synthesis and offering hepatic protection*

Keywords: *Alendronate, triton X-100, hyperlipidemia, LDL-C, VLDL-C, HDL-C*

INTRODUCTION

Hyperlipidemia and associated lipid disorders are considered as the major causes of atherosclerotic cardiovascular diseases, ischemic heart disease, stroke, and cerebrovascular diseases (1). The body needs cholesterol to build cell membranes, make certain hormones, and produce compounds that aid in fat digestion. Too much cholesterol, however, increases an individual's risk of developing heart diseases. Liver dysfunction and alteration in cholesterol synthesis is one of the predominant causes of hyperlipidemia in various pathologies (2,3). The rate of dyslipidemia is a great concern in most developing countries with diet and other lifestyle changes (4).

Hyperlipidemia results in increased serum total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) and decreased high-density lipoprotein (HDL) levels, which are the risk factors for coronary heart diseases (5). Farnesyl pyrophosphate synthase is a key enzyme that plays an important role in the mevalonate pathway, which is responsible for cholesterol synthesis (6,7).

Most of the medicine available for the treatment of hyperlipidemia includes statins, fibrates, and bile acid binding agents. Various antioxidants are also used as a major injury is caused by free radicals generation, so it forms an important therapeutic base. Therapeutic agents, such as β-carotene, vitamin C, vitamin E, selenium, and other synthetic drugs, are also used. Angiotensin-converting-enzyme inhibitors and β-adrenergic receptor blockers are drugs of choice in the therapy, but none of the compounds provide a satisfactory prevention against hyperlipidemia; therefore, a target-specific therapy is needed (8,9).

Bisphosphonates (BPs) are a class of nonhydrolyzable analogs of pyrophosphate that have high affinity for bone mineral and the ability to inhibit osteoclast-mediated bone resorption (10). Alendronate is a nitrogen-containing second generation BP, which was first used to treat Paget's disease in 1971 (http://drug.report/Alendronate/interactions/5/) (11). It acts as a potent inhibitor of bone resorption. It binds rapidly to bone hydroxyapatite and

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Received: ***February 1, 2018*** *Accepted:* ***August 30, 2018*** *Available online date:* ***January 21, 2019***

DOI: ***10.5152/tjg.2019.18076***

then released and internalized by bone-resorbing osteoclasts. This drug acts on the mevalonate pathway and inhibits farnesyl pyrophosphate synthetase, one of the enzymes involved in producing isoprenoid compounds that are essential for post-translational modification of small guanosine triphosphate-binding proteins, such as Rho, Ras, and Rab. Inhibition of this process interferes with osteoclast function and survival (7).

Since alendronate potently acts on the mevalonate pathway and inhibits farnesyl pyrophosphate synthase enzyme, which is a key enzyme responsible for the formation of cholesterol, it can be hypothesized that alendronate can help in reducing the cholesterol level and act as a hypolipidemic drug. Thus, alendronate may show dual therapeutic effects, targeting hyperlipidemia and osteoporosis, and prevent unnecessary use of multiple drugs.

There are various experimental animal models through which hyperlipidemia can be induced, such as Triton X-100-induced hyperlipidemia, Triton WR-1339-induced hyperlipidemia, cholesterol-induced hyperlipidemia, poloxamer 407-induced hyperlipidemia, and methionine-induced hyperlipidemia. Triton X-100-induced hyperlipidemia is a well-known model to induce cholesterol-induced hyperlipidemia (12). Triton X-100 is a non-ionic surfactant that accelerates hepatic cholesterol synthesis and increases intestinal lipid absorption by the emulsification process (13). It suppresses the action of lipoprotein lipase and blocks the uptake of lipoproteins from circulation by the extrahepatic tissues, resulting in increased blood lipid concentrations (14). Therefore, the present study was designed to investigate the antihyperlipidemic activity of alendronate against Triton X-100-induced hyperlipidemia in rats.

MATERIALS AND METHODS

Experimental animals

The Institutional Animal Ethics Committee Jamia Hamdard approved the experimental protocol (Protocol Number: 1220). Male Wistar albino rats weighing 250-300 g (average mean weight 269.83±6.9) were obtained from the Central Animal House Facility. They were housed four animals per cage and maintained at 20°C-30°C and 50%-55% humidity in a natural light/dark cycle with free access to food and water.

Experimental groups

Animals were randomized into seven groups with six animals in each group. All drugs were administered per orally (p.o.) by oral gavage for 21 days except toxic control in which Triton X-100 was injected as single intraperitoneal (i.p.) injection on day 21 only. The groups are as follows: Group I normal saline treated (1 mL/kg body weight, p.o.), Group II toxic control (Triton X-100, 100 mg/kg body weight, i.p. as single injection on day 21), Group III atorvastatin per se (10 mg/kg body weight, p.o.), Group IV atorvastatin (10 mg/kg body weight, p.o. as pretreatment daily for 21 days) and Triton X-100 (100 mg/kg body weight, i.p. as single injection on day 21), Group V alendronate per se (1.5 mg/kg body weight, p.o. pretreatment daily for 21 days), Group VI alendronate (1.5 mg/kg body weight, p.o. as pretreatment daily for 21 days) and Triton X-100 (100 mg/kg, i.p. as single injection on day 21), and Group VII alendronate (3 mg/kg body weight, p.o. as pretreatment daily for 21 days) and Triton X-100 (100 mg/kg body weight, i.p. as single injection on day 21.

The present study converted therapeutic human dose of alendronate as sodium salt into animal dose at two dose levels, that is, alendronate 1.5 mg/kg body weight and 3 mg/kg body weight, p.o.

Biochemical estimations

a) Blood lipid parameters

Blood samples were collected in a sterile centrifuge tube 24 h after the last dose from the rat's tail vein of all groups of overnight fasted rats. Serum was separated for biochemical estimation of HDL-cholesterol (HDL-C) (15), TC (16), triglyceride (TG) (17), aspartate aminotransferase (AST) (18), and alanine aminotransferase (ALT) (18) levels as per the methods previously described.

The following parameters were calculated by using different formulas:

LDL-C levels

LDL-Cholesterol=Total cholesterol−[High-density lipoprotein-cholesterol (HDL-cholesterol)+Triglycerides] (19).

VLDL-C levels

VLDL-Cholesterol=Triglycerides/5 (19).

Atherogenic index (AI)

AI=TC/HDL-C.

b) Oxidative stress parameters in the liver tissue

After blood collection, all the animals were sacrificed by decapitation under light ether anesthesia, and the livers

were dissected. The liver tissue was washed with ice-cold saline, and the homogenate was made in distilled water. All the biochemical estimations were performed as per the already standard procedures of thiobarbituric acid reactive substances (TBARS) (20), superoxide dismutase (SOD) (21), catalase (22), and protein estimation (23).

Histopathology of the liver tissue

On day 21, the animals were sacrificed after blood samples were extracted, and the liver was collected. The liver tissues were fixed in 10% formalin, routinely processed, and embedded in paraffin wax. A paraffin section (5 µm) was cut on a glass slide, stained with hematoxylin and eosin (H&E), and examined under a light microscope by a pathologist blinded to the group studies.

Statistical analysis

All experimental results were expressed as mean±SEM. Comparisons between the experimental and control groups were performed by one-way analysis of variance followed by Dunnett's t test for post hoc comparison, when appropriate. A p value of <0.05 was considered as significant, whereas p>0.05 was not significant. All statistical tests were performed using the Prism software package (version 4; GraphPad, San Diego, CA, USA).

RESULTS

Effect of alendronate on serum TC levels (mg/dL) in Triton X-100-induced hyperlipidemia in rats

In the Triton X-100 (100 mg/kg body weight, i.p.)-treated groups, there was a significant increase in serum TC levels when compared with the Group I rats (p<0.01). Alendronate (1.5 and 3 mg/kg/day body weight, p.o. for 21 days) treatment followed by Triton X-100 administration showed dose-dependent reduction in serum TC. Alendronate (3 mg/kg) showed more significant (p<0.01) reduction than alendronate (1.5 mg/kg) (p<0.05). Atorvastatin (10 mg/kg/day body weight, p.o.) treatment followed by Triton X-100 injection showed a highly significant reduction (p<0.01) in serum TC levels (Table 1).

Effect of alendronate on serum LDL-C levels (mg/dL) in Triton X-100-induced hyperlipidemia in rats

Triton X-100-treated rats (Group II) showed a highly significant increase in mean serum LDL-C as compared with control rats (p<0.01). Both doses of alendronate showed a significant decrease in serum LDL-C levels. Similar observations were shown by atorvastatin (10 mg/kg body weight) (p<0.01) (Table 1).

Effect of alendronate on serum VLDL-C levels (mg/dL) in Triton X-100-induced hyperlipidemia in rats

The mean serum VLDL-C levels were significantly (p<0.01) higher in the toxic control group than in the control animals. Atorvastatin treatment significantly reduced the increased VLDL-C levels. Treatment of both doses of alendronate showed significant (p<0.01) reduction of VLDL-C level (Table 1).

Effect of alendronate on serum HDL-C levels (mg/dL) in Triton X-100-induced hyperlipidemia in rats

The mean serum HDL-C levels were significantly lower in Triton X-100-treated rats than in vehicle control rats (p<0.01). Treatment with alendronate (3 mg/kg body weight) significantly (p<0.05) increased the HDL-C levels as compared with the toxic control group (Table 2).

Table 1. Effect of alendronate on serum TC, LDL-C, and VLDL-C levels in Triton X-100-induced hyperlipidemia in rats

S.NO.	Groups (n=6)	Total cholesterol (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
1	Vehicle control (normal saline 1 mL/100 g, p.o.)	77.99±3.89	27.94±3.36	14.78±0.43
2	Triton X-100 (100 mg/kg, i.p. given on day 21)	106.92±2.88**	48.13±2.49**	30.82±0.50**
3	Atorvastatin per se (10 mg/kg body weight, p.o.)	79.80±1.89	30.64±2.98	15.35±0.21
4	Atorvastatin (10 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	70.45±1.25##	24.10±1.47##	18.41±0.22##
5	Alendronate per se (1.5 mg/kg body weight, p.o.)	80.92±1.82	36.64±2.33	14.39±0.33
6	Alendronate (1.5 mg/kg, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	91.94±3.24#	39.61±2.98#	23.12±0.97##
7	Alendronate (3 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	87.26±4.81##	37.77±3.94#	17.57±0.79##

All values were expressed as mean±SEM, n=6, analyzed by ANOVA followed by Dunnett's t test

**p<0.01; when compared with the vehicle control; #p<0.05; ##p<0.01; when compared with the Triton X-100 group

Table 2. Effect of alendronate on serum HDL-C, TG, and AI levels in Triton X-100-induced hyperlipidemia in rats

S.NO.	Groups (n=6)	HDL-C (mg/dL)	TG (mg/dL)	AI (TC/HDL-C)
1	Vehicle control (normal saline 1 ml/100 g, p.o.)	35.29±0.98	73.94±2.16	2.20±0.06
2	Triton X-100 (100 mg/kg body weight, i.p. given on day 21)	27.97±0.68**	154.15±2.52**	3.82±0.14**
3	Atorvastatin per se (10 mg/kg body weight, p.o.)	33.81±1.05	76.80±1.05	2.35±0.12
4	Atorvastatin (10 mg/kg, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	29.94±0.46#	91.98±1.09##	2.34±0.03##
5	Alendronate per se (1.5 mg/kg body weight, p.o.)	34.57±2.24	71.99±1.69	2.67±0.09
6	Alendronate (1.5 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	29.21±0.84#	115.61±4.89##	3.15±0.19#
7	Alendronate (3 mg/kg, p.o.)+Triton X-100 (100 mg/kg, i.p.)	31.92±0.88#	87.89±3.97##	2.72±0.098##

All values were expressed as mean±SEM, n=6, analyzed by ANOVA followed by Dunnett's t test
**p<0.01; when compared with the vehicle control; #p<0.05; ##p<0.01; when compared with the Triton X-100 group

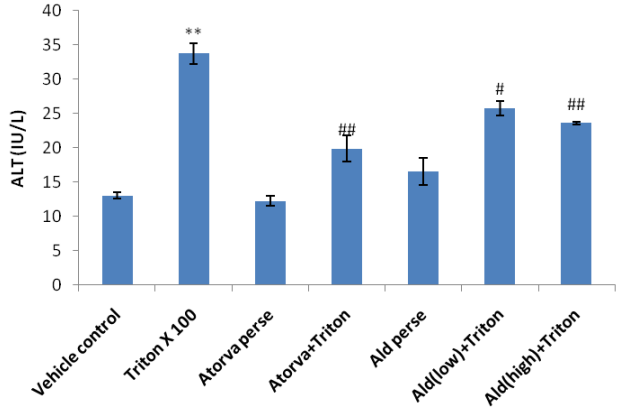

Figure 1. All values were expressed as mean±SEM, n=6, analyzed by ANOVA followed by Dunnett's t test. **p<0.01, when compared with Group I. #p<0.05, when compared with Group II. ##p<0.01, when compared with Group II. Triton X-100 (100 mg/kg body weight, i.p. single injection on day 21), Atorva per se: atorvastatin (10 mg/kg body weight, p.o.), Atorva+Triton: atorvastatin (10 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.), Ald per se: alendronate (1.5 mg/kg body weight, p.o.), Ald (low)+Triton: alendronate (1.5 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.), Ald (high)+Triton: alendronate (3 mg/kg body weight)+Triton X-100 (100 mg/kg body weight)

Effect of alendronate on serum TG levels (mg/dL) in Triton X-100-induced hyperlipidemia in rats

There was a significant increase in serum TG levels (p<0.01) in Triton X-100-treated rats. Both doses of alendronate showed a significant (p<0.01) reduction in serum TG levels when compared with the toxic control rats (Table 2).

Effect of alendronate on AI in Triton X-100-induced hyperlipidemia in rats

The mean TC/HDL-C levels, i.e., AI, were significantly increased in the toxic control rats (p<0.01). Alendronate treatment showed dose-dependent reduction in the AI. A higher dose of alendronate (3 mg/kg) was more potent than a lower dose (1.5 mg/kg) in reducing TC/HDL-C levels (Table 2).

Effect of alendronate on serum ALT levels (IU/L) in Triton X-100-induced hyperlipidemia in rats

The mean serum ALT levels were significantly increased in the Triton X-100-treated group (p<0.01). Alendronate showed dose-dependent reduction in serum ALT levels. A higher dose (3 mg/kg) of alendronate treatment produced more significant (p<0.05) results than a lower dose (1.5 mg/kg) in reducing serum ALT levels (Figure 1).

Effect of alendronate on serum AST levels (IU/L) in Triton X-100-induced hyperlipidemia in rats

The mean serum AST levels were significantly higher in Group II rats than in normal control rats (p<0.01). It was observed that both doses of alendronate (1.5 and 3 mg/kg body weight) treatment showed significant (p<0.01) reduction in serum AST levels (Figure 2).

Effect of alendronate on TBARS levels (nmol MDA/mg protein) in Triton X-100-induced hyperlipidemia in rats

The mean TBARS levels were significantly higher in the toxic control rats than in the normal control rats (p<0.01). Alendronate (1.5 mg/kg) treatment showed significant (p<0.05) reduction in tissue TBARS level, but higher dose of alendronate (3 mg/kg body weight) did not produce any reduction in TBARS level as compared with the levels in the toxic group rats (Table 3).

Effect of alendronate on SOD levels (U/mg) in Triton X-100-induced hyperlipidemia in rats

SOD activity was significantly decreased in the Triton

Table 3. Effect of alendronate on tissue TBARS, SOD, and CAT in Triton X-100-induced hyperlipidemia in rats

S.NO.	Groups (n=6)	TBARS (nmol MDA/mg protein)	SOD (U/mg)	CAT (μmol of H_2O_2/min/mg)
1	Vehicle control (normal saline 1 mL/100 g, p.o.)	0.21±0.01	1.79±0.07	127.5±1.63
2	Triton X-100 (100 mg/kg body weight, i.p. given on day 21)	0.73±0.09**	1.02±0.13*	96.75±0.51**
3	Atorvastatin per se (10 mg/kg body weight, p.o.)	0.20±0.01	1.69±0.19	119.22±5.52
4	Atorvastatin (10 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	0.30±0.02##	2.13±0.11##	131.13±0.84##
5	Alendronate per se (1.5 mg/kg, p.o.)	0.23±0.02	1.92±0.03	95.07±2.54
6	Alendronate (1.5 mg/kg, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	0.46±0.06##	2.26±0.12#	97.23±1.00
7	Alendronate (3 mg/kg, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.)	0.77±0.05	2.95±0.03##	90.57±0.76

All values were expressed as mean±SEM, n=6, analyzed by ANOVA followed by Dunnett's t test

**p<0.01; when compared with the vehicle control; #p<0.05; ##p<0.01; when compared with the Triton X-100 group

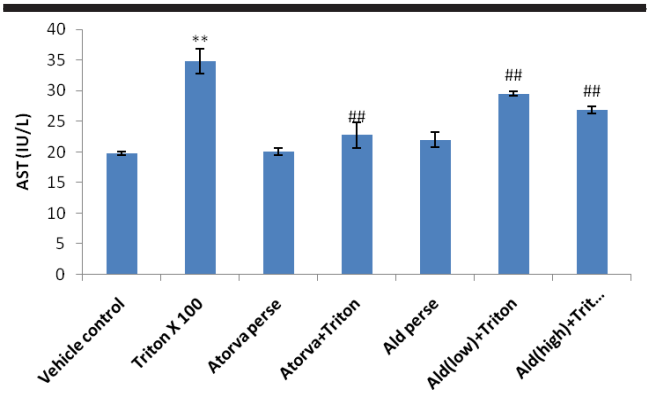

Figure 2. All values were expressed as mean±SEM, n=6, analyzed by ANOVA followed by Dunnett's t test. **p<0.01, when compared with Group I). ##p<0.01, when compared with Group II. Triton X-100 (100 mg/kg body weight, i.p. single injection on day 21), Atorva per se: atorvastatin (10 mg/kg body weight, p.o.), Atorva+Triton: atorvastatin (10 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.), Ald per se: alendronate (1.5 mg/kg body weight, p.o.), Ald (low)+Triton: alendronate (1.5 mg/kg body weight, p.o.)+Triton X-100 (100 mg/kg body weight, i.p.), Ald (high)+Triton: alendronate (3 mg/kg body weight)+Triton X-100 (100 mg/kg body weight)

X-100-treated group (p<0.01). Both doses of alendronate treatment showed significant (p<0.05) elevation in SOD, but the highly significant elevation in SOD activity was observed with a higher dose when compared with Group II rats (Table 3).

Effect of alendronate on catalase (U/mg) in Triton X-100-induced hyperlipidemia in rats

Catalase activity was significantly decreased in the toxic control group (p<0.01). None of the dose of alendronate showed a significant improvement in catalase activity as compared with the pathogenic control group (Table 3).

Histopathological examination

Microscopic section of normal control group liver showed the normal architecture and arrangement of the liver cells around the central vein and portal vein (Figure 3a). The Triton X-100-treated toxic group showed peribiliary inflammatory cells of inflammation, apoptosis of the hepatocytes, capsular thickening in the capsular region, and proliferation of fibrous connective tissue (Figure 3b). The atorvastatin per se (10 mg/kg) group showed the normal architecture and arrangement of the liver cells around the central vein and portal tract and enlargement of the hepatocytes at the margin (Figure 3c). Atorvastatin (10 mg/kg)-treated rats showed severe vacillation of the hepatocytes and hemorrhage in the cells as compared with the normal control rats (Figure 3d). The alendronate per se (1.5 mg/kg)-treated groups showed normal hepatocytes and normal arrangement of the cells around the central vein and portal tract. Low abnormality was detected (Figure 3e). The alendronate (1.5 mg/kg)-treated group showed less proliferation of liver cells and slight enlargement of the hepatocytes. The arrangement of the hepatocytes around the central vein and portal tract was normal (Figure 3f). The alendronate (3 mg/kg)-treated groups showed lower capsular thickening, slight enlargement of the hepatocytes at the margin, and lower inflammatory cell infiltration as compared with the toxic group (Figure 3g).

DISCUSSION

The current study was designed to assess the effect of alendronate against Triton X-100-induced hyper-

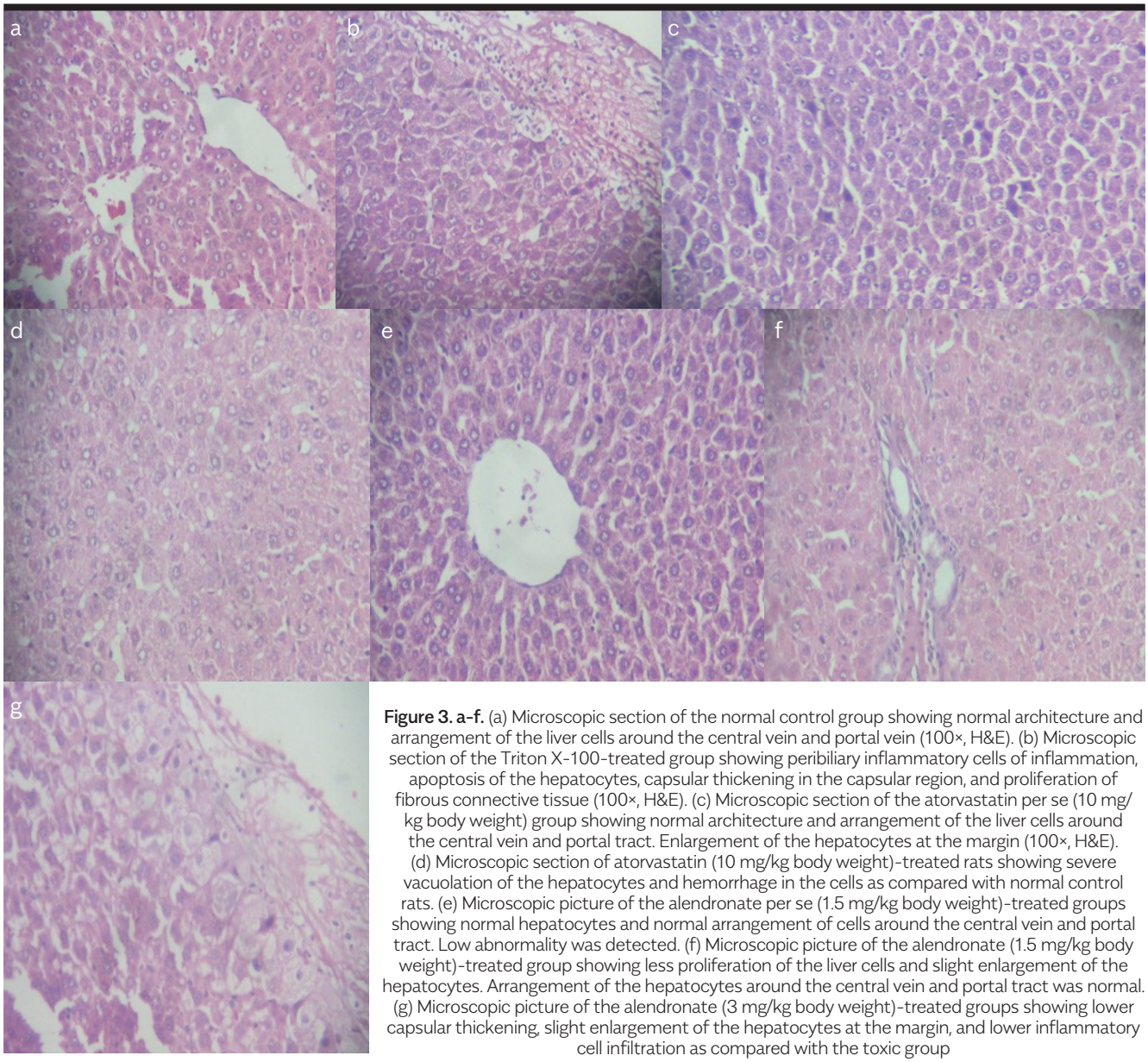

Figure 3. a-f. (a) Microscopic section of the normal control group showing normal architecture and arrangement of the liver cells around the central vein and portal vein (100×, H&E). (b) Microscopic section of the Triton X-100-treated group showing peribiliary inflammatory cells of inflammation, apoptosis of the hepatocytes, capsular thickening in the capsular region, and proliferation of fibrous connective tissue (100×, H&E). (c) Microscopic section of the atorvastatin per se (10 mg/kg body weight) group showing normal architecture and arrangement of the liver cells around the central vein and portal tract. Enlargement of the hepatocytes at the margin (100×, H&E). (d) Microscopic section of atorvastatin (10 mg/kg body weight)-treated rats showing severe vacuolation of the hepatocytes and hemorrhage in the cells as compared with normal control rats. (e) Microscopic picture of the alendronate per se (1.5 mg/kg body weight)-treated groups showing normal hepatocytes and normal arrangement of cells around the central vein and portal tract. Low abnormality was detected. (f) Microscopic picture of the alendronate (1.5 mg/kg body weight)-treated group showing less proliferation of the liver cells and slight enlargement of the hepatocytes. Arrangement of the hepatocytes around the central vein and portal tract was normal. (g) Microscopic picture of the alendronate (3 mg/kg body weight)-treated groups showing lower capsular thickening, slight enlargement of the hepatocytes at the margin, and lower inflammatory cell infiltration as compared with the toxic group

lipidemia in rats. Triton X-100 is reported to block the clearance of TG-rich lipoproteins and produces acute hyperlipidemia in animal models (24). Plasma TG and cholesterol levels were elevated due to Triton X-100 injection as an increase of VLDL secretion by the liver, followed by reduction of VLDL and LDL catabolism (25). In the present study, Triton X-100 significantly increased the serum TC, LDL-C, VLDL-C, TGs, AST, ALT, and AI and decreased HDL-C levels as also reported in a previous study (13).

Previously, alendronate reported an antihyperlipidemic effect in patients with osteoporosis (26). In the current study, alendronate was able to reverse the Triton X-100-induced levels of serum TC, LDL-C, VLDL-C, TG, AI, AST, and ALT and decreased the level of HDL-C toward normal limits. The effects of alendronate on the markers of hyperlipidemia were comparable to atorvastatin, which is mostly prescribed as a standard antihyperlipidemic drug (27). Thus, based on the markers of hyperlipidemia, it may be concluded from the present study that

alendronate may inhibit the synthesis of cholesterol via the mevalonate pathway, and its efficacy was similar to atorvastatin. In the present study, it was established that AI increased in Triton X-100-administered rats as reported in a previous study (13). AI, which is a measure of the extent of atherosclerotic lesion, was significantly reduced by the oral treatment of alendronate and atorvastatin, thus confirming previous findings (28). Moreover, pretreatment with both doses of alendronate showed decreased AI, thus confirming previous findings.

Reactive oxygen species are responsible for causing atherosclerosis. Lipid peroxidation induced by reactive oxygen species is a key factor for atherosclerosis (29). In the present study, there was a significant increase in the levels of lipid peroxide in the liver tissue during Triton X-100 administration as expressed in the MDA formation. Alendronate (1.5 mg/kg) attenuated the MDA formation significantly, but the higher dose of alendronate (3 mg/kg) showed a more inhibitory effect on the MDA formation. The Triton X-100-treated animals were under severe stress as it was shown by the reduction in liver enzymes and markers of oxidative stress. The lower dose of alendronate reduced the MDA formation to a lower extent as compared with the higher dose that has marked tendency of MDA reduction. Therefore, the higher dose showed a more inhibitory effect than the lower dose on the MDA formation. These observations further strengthen that pretreatment with alendronate significantly reduced the level of TBARS in the liver tissue, confirming that during oxidative stress, reactive oxygen species were formed.

One study reported that fenofibrate reduced the formation of free radicals in vitro, lipid peroxides, and elevated antioxidant enzymes, thus demonstrating antioxidant effects in hyperlipedemic mice (30).

The levels of antioxidant enzymes, such as catalase and SOD, are decreased during cellular inflammation. These enzymes remove oxygen free radicals, thus decreasing inflammatory conditions (31). We observed that alendronate treatment increased the SOD levels more than the vehicle control group, and the per se treatment with alendronate did not have such profound effects. A similar pattern of observation with atorvastatin treatment resulted in increased SOD levels more than the vehicle control group. The reason for such effect may be the system was under severe stress, and some compensatory mechanisms may be operative to detoxify or cleavage the production of reactive oxygen species.

The effect of drug treatment on catalase activity was not consistent in our study because there was a tendency of reduced catalase activity in the per se group. While the reason for such an effect observed by alendronate per se is not clear, one possible explanation for an insignificant reduction could be due to a delayed decline of such antioxidant enzymes following treatment with alendronate.

Histopathological studies are the most precise evidence for the protective effect of drug as protectants (32). Simultaneous treatment of alendronate with Triton X-100 exhibits less damage to the hepatic cells as compared with rats treated with Triton X-100 alone. The Triton X-100-treated group showed peribiliary inflammatory cells of inflammation, apoptosis of the hepatocytes, capsular thickening in the capsular region, and proliferation of fibrous connective tissue. Moreover, the alendronate-treated group showed less proliferation of liver cells and slight enlargement of the hepatocytes in histopathological examinations. Alendronate was able to reverse the arrangement of the hepatocytes around the central vein and the portal tract toward normal. Moreover, alendronate in hyperlipidemic rats showed beneficial effects by maintaining the normal architecture of the liver tissues, which was altered in hyperlipidemia as evidenced by histopathological studies (32). Almost negligible damage to a few hepatocytes present in the close vicinity of the intralobular vein was observed in alendronate-treated rats.

The results of a histopathological study further support the results of biochemical parameters. Thus, the histopathological evidences again confirm the hepatoprotective and antihyperlipidemic effects of alendronate. Further research is needed to confirm the molecular pathways and potential therapeutic use of alendronate in hyperlipidemia and hyperlipidemia associated with osteoporosis in different animal models with multiple doses.

In conclusion, alendronate may serve a dual purpose in patients with hyperlipidemia and osteoporosis by reducing serum cholesterol and along with reducing osteoblastic cells and also improve liver function tests. Therefore, these patients can be prescribed, and the doctors may avoid antihyperlipidemic drug along with BPs.

Ethics Committee Approval: Ethics Committee Approval was received for this study from the Institutional Animal Ethics Committee Jamia Hamdard (Protocol Number: 1220).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - A.P., M.A.; Design - A.P., A.K.N., M.V.I., M.K., M.A.; Supervision - M.A.; Materials - A.P., M.K.; Analysis and/or Interpretation - A.P., A.N.K., M.V.I., M.K., M.A.; Literature Search - A.P.; Writing Manuscript - M.K., M.A.; Critical Review - A.P., A.N.K., M.V.I., M.K., M.A.

Conflict of Interest: Ashiyana Parwin received the financial assistance from the University Grants Commission (UGC), New Delhi, India. The other authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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