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Article

Alirocumab in Dexamethasone-Induced Metabolic Syndrome: Therapeutic Benefits and Potential Hepatic Risks through RXR-α / p-AKT Modulation

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Abstract

Background: Metabolic syndrome (MetS) elevates the risk of developing cardiovascular diseases and type 2 diabetes., driven by insulin resistance (IR), inflammation, and metabolic dysregulation. The PI3K/AKT pathway shows a key part in glucose and lipid metabolism. Alirocumab, a PCSK9 inhibitor, lowers LDL-C and improves IR but may cause hepatotoxicity. **Aims:** This study examines alirocumab's effects on dexamethasone-induced MetS in rats, focusing on its metabolic benefits and potential liver toxicity. **Methods:** Over 30 days, 40 male Albino rats were distributed into control, Alirocumab, Dexamethasone, and Dexamethasone+Alirocumab groups, with the latter receiving Dexamethasone to induce metabolic syndrome followed by Alirocumab administration. **Results:** Dexamethasone significantly reduced body weight and HDL-C while increasing insulin, glucose, insulin resistance, and cholesterol levels. Alirocumab mitigated dexamethasone-induced dyslipidemia, lowering LDL-C and total cholesterol. However, it elevated ALT and AST levels, suggesting potential hepatotoxicity. Reactive oxygen species and inflammatory markers (IL-4, IL-6) increased with alirocumab and dexamethasone. Histopathological analysis revealed immune cell migration and decreased RXR-α with increase in p-AKT expression in Dexamethasone group. A significant rise in RXR-α and p-AKT expressions were observed in the Dexamethasone/Alirocumab group compared to the Dexamethasone group. These findings indicate that alirocumab may counteract dexamethasone-induced metabolic syndrome but has liver-related effects. **Conclusions:** Alirocumab mitigates dexamethasone-induced metabolic syndrome by improving lipid profile and insulin sensitivity; however, its use is associated with elevated hepatic enzyme levels and altered RXR-α and p-AKT expression, indicating potential hepatotoxic effects.

Key words: Metabolic Syndrome X; Dexamethasone; Hepatotoxicity Alirocumab; RXR-α and p-AKT

Introduction

Metabolic syndrome (MetS) is a cluster of disorders that occur simultaneously and heighten the hazard of cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM), and dyslipidemia (1)(2). There is no underlying cause for this syndrome, but there are several leading causes, including insulin resistance (IR), chronic inflammation, and neurohormonal activation (3)(1).

Dexamethasone (DEXA), a synthetic glucocorticoid widely used for its potent anti-inflammatory and immunosuppressive effects (4). But they can also lead to significant side effects, such as insulin resistance (IR). This metabolic disturbance mimics the pathophysiology of human MetS and are frequently used in animal models to study its underlying mechanisms and evaluate therapeutic interventions. This ability to provoke IR makes glucocorticoids a useful tool for studying the mechanisms underlying metabolic diseases and testing potential therapeutic interventions (5).

PI3K/AKT is the key pathway that regulates lipid metabolism and glucose (6), and its imbalance contributes to the progress of obesity and type 2 diabetes mellitus (6) Akt helps keep glucose and lipid metabolism in check, together with other cellular responses (7). These processes include apoptosis, proliferation, growth, secretion, and chemotaxis. The PI3K/AKT pathway plays a key role in numerous liver diseases, such as chemical-induced liver damage, and is closely correlated with oxidative stress and inflammation (8).

Retinoic acids (RAs) exert their physiological effects through two classes of nuclear receptors, the retinoic acid receptors (RARs) and retinoid X receptors RXR. (9) RXRs function as pivotal regulators of gene networks controlling cell proliferation, differentiation, survival, and apoptosis. (10) Notably, the accumulation of insoluble RXRα-containing droplets in hepatic tissue has been proposed as a potential biomarker of liver injury, particularly in the context of hepatic stellate cell activation (11).

Alirocumab is a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor (12). The PCSK9 inhibitor lowers elevated LDL-C levels and regulates glucose metabolism and IR. Patients with MetS have a range of metabolic characteristics related to PCSK9, including high LDL-C levels, high blood glucose, and IR (13). However, the use of PCSK9 inhibitors has been related to hepatocyte injury, which may result in elevated serum transaminase levels (14). This study hypothesizes that alirocumab may attenuate dexamethasone-induced metabolic syndrome (MetS) in rats by improving lipid profiles and modulating key metabolic pathways, particularly the PI3K/Akt signaling pathway, which is thought to play a central role in its therapeutic effects. However, emerging evidence indicates that PCSK9 inhibitors, including alirocumab, may be associated with hepatocyte injury and elevated serum transaminase levels, highlighting the need to assess potential hepatotoxicity. Unlike previous research that primarily focused on lipid-lowering effects of alirocumab in hyperlipidemia or atherosclerosis models, our study is the first, to our knowledge, to evaluate the protective role of alirocumab against dexamethasone-induced metabolic syndrome. Moreover, this work provides novel mechanistic insights by exploring the involvement of RXR-α and p-AKT signaling pathways in mediating alirocumab's metabolic and hepatoprotective effects. These findings extend the potential therapeutic applications of alirocumab beyond dyslipidemia to glucocorticoid-induced metabolic disturbances.

2. Materials and Methods

2.1 Ethical Approval:

This study was accepted by the Medical Ethics Committee at Qassim University and followed to international standards and regulations for laboratory animal research. The Research Ethics Committee reviewed and accepted the research protocol under approval number (24-02-07). All animals' procedures were conducted in agreement with the National Research Council's guidelines for the attention and usage of laboratory animals (15).

2.2 Drugs, Chemicals and Antibodies:

Dexamethasone sodium phosphate ampoules 8mg/2ml: (manufactured by Egyptian int. pharmaceutical industries co., egypt). alirocumab: (manufactured by Regeneron pharmaceuticals inc. and sanofi). praluent prefilled pen 75mg/1ml. All drugs were dissolved in normal saline. Mouse monoclonal antibodies against RXR-α (Cat # sc–515929) and p-AKT (Cat # sc-514032) were purchased from Santa Cruz Biotechnology (TX, USA)

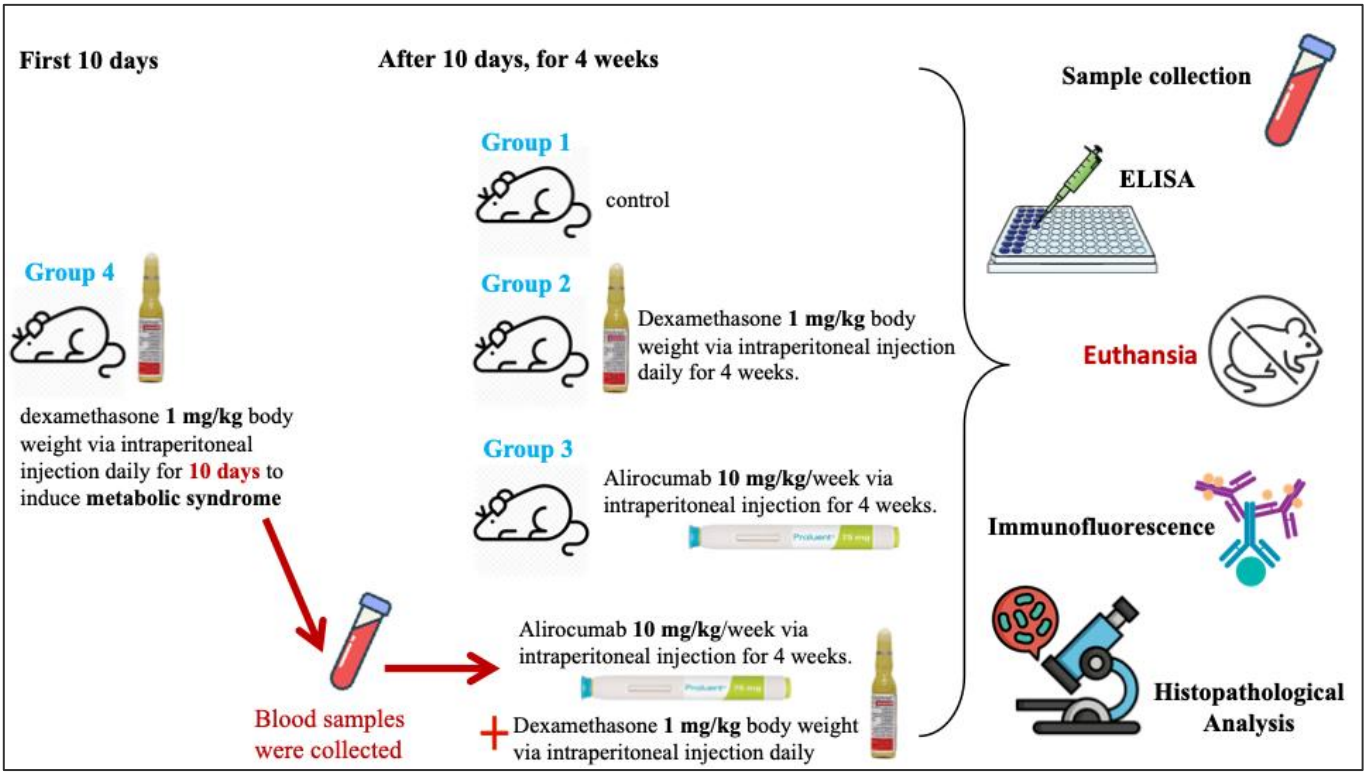

Figure (1) Schematic representation of the experimental design and treatment protocol.

2.3 Animals

Forty male Albino rats of the Sprague-Dawley strain, weighing among 190 and 230 grams, were used in this study. The animals were taken from the Animal House at Qassim University. They were kept at room temperature under a normal light-dark cycle in appropriately sized cages, with five rats per cage, and provided standard laboratory food along with allowed access to water. All processes involving the animals were carried out in agreement with the Institutional Ethics Committee's guidelines, as well as the recommended standards for the attention and usage of laboratory animals. The experiments were guided in full agreement with NIH guidelines and Saudi Arabian regulations (16). Sample size calculated using G-Power© software version 3.1.7 (Institute of experimental psychology, Heinrich Heine University, Dusseldorf, Germany). Sample size was (10) rats in each group. Depending on previous research results. Two sided (two tails) type I error 0.05 and power of 80% with effect size 1.10

2.4 Methods and Experimental Design

Animals were stratified by body weight and then randomly assigned to four main groups using a random number table to ensure comparable weight distribution across groups.

Group 1 [control group]: Rats received ordinary diet and distilled water daily by oral route and normal saline via intraperitoneal injection for 4 weeks; group 2 [Dexamethasone group]: Rats were treated with dexamethasone sodium phosphate 1 mg/kg body weight via intraperitoneal injection daily for 4 weeks (17). Group 3 [Alirocumab group]: Rats were treated with alirocumab 10 mg/kg/week via intraperitoneal injection for 4 weeks (18). Group 4 [Dexamethasone and Alirocumab Group]: Dexamethasone sodium phosphate (1 mg/kg/day, i.p.) was administered to induce metabolic syndrome and continued throughout the experimental period concurrently with alirocumab (10 mg/kg/week, i.p.) for four weeks. Figure (1) Drugs injection volumes were adjusted weekly based on measured weights to ensure accuracy. Metabolic syndrome was induced by 10 days of dexamethasone administration. Measurements obtained on day 10 were considered baseline values prior to the initiation of alirocumab treatment. This study was designed as an induction–treatment model, with dexamethasone representing the induction phase and alirocumab representing the treatment phase.

2.5 Measurement of body weight:

The body weight of all animals was measured at the start and the end of the experiment.

2.6 Sample collection:

Blood Sampling was performed at two points during the experiment: on day ten and at the end of the study. On day ten the experimental rats were anesthetized via intraperitoneal injection of thiopental sodium at a dose of 50 mg/kg. Blood samples were collected from facial veins into tubes and allowed to coagulate for 20 minutes. Serum was then separated by centrifugation at 3000 rpm for 15 minutes and stored at -80°C until biochemical analysis (19)(20)(21). At the end of the experiment, animals were humanely euthanized by intraperitoneal injection of thiopental sodium overdose (150 mg/kg). Blood samples were collected immediately via cardiac puncture into plain tubes (22) For histological assessment, liver specimens were excised immediately after collection and immersed in 10% neutral buffered formalin for 48 hours to ensure proper fixation. Following tissue collection, animal carcasses were disposed of by incineration at the College of Veterinary Medicine, Qassim University.

2.7 Biochemical analysis:

The serum levels of the lipid profile (total cholesterol, triglycerides, HDL, and LDL) as well as liver function tests (ALT, AST, ALP, total protein, and albumin) were measured using commercially available diagnostic kits. The kits were provided by the Crescent Diagnostics Test, KSA and Diagnostica mbH, Wiesbaden, Germany LOT: 23010, 23007, 23006, 23002 through a spectrophotometric method, following the manufacturers' guidelines. Blood glucose levels were measured using a glucose meter (accu-check, Roche Diabetes Care). Comparison of insulin resistance measured by HOMA (Homeostasis model analysis) (23).

2.8 Enzyme-Linked Immunosorbent Assay (ELISA) for Blood Analysis:

Measuring IL-6, IL-4, ROS were analyzed using the ELISA technique for their quantitative determination agreeing to the manufacturer's instructions and the samples absorbance was measured at wavelength 450 nm. The concentration of the samples was determined using the standard curve. The determination of IL-6 and IL-4 levels were measured using sandwich enzyme immunoassays from Cloud-Clone Corp (TX, USA). Finally, the serum level of ROS levels was measured using a sandwich enzyme immunoassay from ABclonal (MA, USA). Mouse monoclonal antibodies against RXR-a and p-AKT were obtained from Santa Cruz Biotechnology (TX, USA). Goat anti-mouse Cyanine red (Cy3) or Alexa 488 conjugated goat anti-mouse secondary antibodies were purchased from Invitrogen Thermo Fisher Scientific (San Francisco, CA, USA) LOT: L131205128, L131205125.

2.9 liver sectioning and staining for histopathological Analysis:

The liver tissues were immersed in formalin 10 % solution and fixed in paraffin blocks. Tissues imbedded in paraffin were dehydrated using gradient alcohol concentrations (50%, 70% for 24 hours, 90%, and 100% for 12 hours) and then defatted in xylene for two hrs. Serial sections of tissues, each 4 μm thick, were cut out for H&E and immunofluorescence techniques. Slides were kept in oven (60℃) for 20 min to melt paraffin, deparaffinized by dipping in xylene and gradually hydrated using graded alcohol concentration and finally in distilled water. The sections were boiled with Dako® citrate buffer (pH 6.0) in the microwave at 500 Watt for 20 min. to retrieve the antigens and allowed to cool down to room temperature. Slides were rinsed with washing solution (0.05% twin in phosphate buffered saline) and fixed with methanol for 10 min using dark humidified chamber. After washing, slides were blocked using blocking solution (1% BSA, 10% horse serum and 1% in PBS) for 1 hour at room temperature. Sections were incubated for 3 hours at 37°C followed by overnight at 4°C with the appropriate primary antibodies including mouse monoclonal antibody against RXR-□ (dilution 1:200) and p-AKT (dilution 1:150). After washing, the slides were incubated at 37ºC for 30 min with goat anti-mouse Cyanine red (Cy3) or Alexa 488 conjugated goat anti-mouse secondary antibodies. The slides were washed again and incubated with 4',6-diamidino-2-phenylindole (DAPI) for nuclear counterstaining. The slides were washed again and mounted with coverslips using fluoromount® mounting solution (DAKO, Carpinteria, CA, USA). Tissue sections were analyzed, and the images were captured using Leica fluorescence microscope (Model: Leica DM 5500B, Leica Microsystems, Wetzlar, Germany) using blue, green, and red channels. A minimum of 6 fields of each mice

Table (1): Comparison between Control and Dexamethasone groups on 10th day (n=10).

Variables (Mean ± SD)	Control Group	Dexamethasone Group on 10th day
Body weight	209.8 ± 12.19	176.6 ± 17.06 [a]
Insulin (mIU / ml)	2.96 ± 0.62	24.2 ± 1.01 [a]
Blood glucose (mg/dL)	81.8 ± 11.51	141.7 ± 24.35 [a]
HOMA-IR	0.6 ± 0.18	8.44 ± 1.35 [a]
Total Cholesterol (mg / dl)	45.73 ± 6.4	110.12 ± 22.62 [a]
Triglycerides (mg/ dl)	109.44 ± 18.39	126.7 ± 30.58
High-density lipoprotein -C (mg/ dl)	37.42 ± 5.28	22.92 ± 3.04 [a]

SD: Standard deviation, **a**: Significant at $P < 0.05$ as compared to control group

section were used in fluorometric analysis of the results using Image-J software (NIH, USA) (24).

2.10 Statistical Analysis:

Data was coded and inserted using SPSS (Statistical Package for the Social Sciences) version 27. The data were summarized as Mean ± Standard Deviation (Mean ± SD). Comparisons among groups were performed using Analysis of Variance (ANOVA) followed by a specific post-hoc test (Tukey's multiple comparisons). P-values less than 0.05 were considered statistically significant. The Independent Student's t-test was used to measure the differences between the means of two continuous variables in unpaired groups.

3- Results

3.1 Comparison between Dexamethasone and Control groups on 10th day:

This study evaluated changes in body weight, insulin level, blood glucose, insulin resistance, cholesterol, and high-density lipoprotein cholesterol (HDL-C) among the study groups, with statistical significance set at $p < 0.05$. The results demonstrated a significant decrease in body weight and HDL-C levels in the dexamethasone group compared to the control group. In the dexamethasone group, there was a noteworthy increase in the level of insulin, blood glucose, insulin resistance, and cholesterol compared to the control group. However, no significant difference in triglycerides level was detected between the studied groups. (Table 1)

3.2 Percentage change from initial to final body weight among the Studied Groups:

The results demonstrated a statistically significant reduction in the final body weight percentage in the dexamethasone group compared to its initial body weight ($p < 0.05$). Likewise, a significant decrease was detected in the dexamethasone/Alirocumab group relative to its initial body weight ($p < 0.05$) (Figure 2).

3.3 Results of Dexamethasone, Alirocumab, and Dexamethasone/Alirocumab groups in Lipid Profile:

On day 30, total cholesterol level was significantly elevated in the dexamethasone group compared to the control and alirocumab groups, however the cholesterol was a markedly decrease in dexamethasone/alirocumab treated group compared to the dexamethasone group. The serum level of triglycerides was also significantly greater in the dexamethasone group relative to the control and alirocumab groups, with the dexamethasone/alirocumab group showing an additional increase.

HDL-C level was elevated in the alirocumab group compared to the control group, whereas the dexamethasone group showed a substantial decrease. Although HDL-C level in the dexamethasone/alirocumab group was lesser than in the alirocumab group, it stayed significantly greater than in the dexamethasone group.

LDL-C level was markedly elevated in the dexamethasone group compared to the control and alirocumab groups. The dexamethasone/alirocumab group showed a significant reduction in LDL-C level compared to the dexamethasone group, though level remained greater than in the control and alirocumab groups. These findings indicate that dexamethasone induces significant dyslipidemic effects, which are mitigated, at least partially, by alirocumab co-administration. (Table 2)

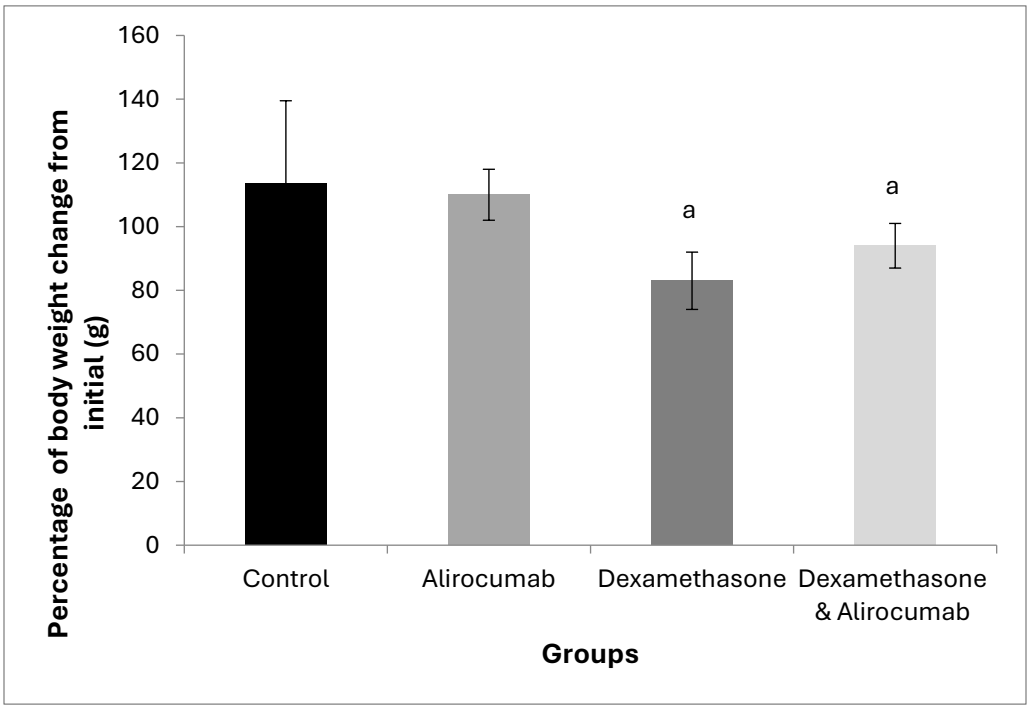

Figure (2) Percentage of body weight change from Initial a: Significant at P < 0.05 as compared to initial

Table 2: Effects of dexamethasone, alirocumab, and their co-administration on serum lipid profile parameters (Mean ± SD)

Variables (Mean ± SD)	Control Group	Alirocumab Group	Dexamethasone Group on 30th day	Dexamethasone and Alirocumab Group
Total Cholesterol(mg/dl)	45.73 ± 6.4	46.92 ± 4.52	167.717 ± 10.6 [a,b]	53.05 ± 8.91[c]
Triglycerides (mg/dl)	109.44 ± 18.39	93.79 ± 11.86	160.66 ± 29.57 [a,b]	170.73 ± 19.45 [a,b]
High-density lipoprotein (mg/dl)	37.42 ± 5.28	45.16 ± 5.95 [a]	16.54 ± 4.73 [a,b]	38.39 ± 5.59 [b,c]
Low-density lipoprotein (mg/dl)	29.5±6.6	23±5.2	96.6±12.3 [a,b]	70.3±16.7 [a,b,c]

SD: Standard deviation, a: Significant at P < 0.05 as compared to control group. b: Significant at P < 0.05 as compared to Alirocumab group. c: Significant at P < 0.05 as compared to Dexamethasone on 30th day.

3.4 Impact of Dexamethasone, Alirocumab, and Dexamethasone/Alirocumab on Liver Function Tests:

On day 30, significant differences were observed in alanine transaminase (ALT), aspartate transaminase (AST), total protein, and albumin levels among the study groups, while alkaline phosphatase (ALP) levels remained unchanged (Table 3). ALT level was significantly elevated in the alirocumab and dexamethasone/alirocumab groups compared to the control group, with the dexamethasone/alirocumab group also still showing a significant rise relative to the dexamethasone group. Similarly, AST level was significantly greater in the alirocumab, dexamethasone, and dexamethasone/alirocumab groups compared to the control group.

AST levels in the dexamethasone group were lesser than in the Alirocumab group, while the Dexamethasone/Alirocumab group displayed a significant increase compared to the Dexamethasone group. Total protein levels were significantly elevated in the Alirocumab, Dexamethasone, and Dexamethasone/Alirocumab groups compared to the Control group, with the Dexamethasone/Alirocumab group showing a further increase relative to the Dexamethasone group. Albumin levels were significantly greater in the Dexamethasone and Dexamethasone/Alirocumab groups compared to the Control group. These findings suggest that dexamethasone and alirocumab impact liver function and protein metabolism, with their combined administration leading to pronounced alterations in these biochemical parameters.

Table (3): Effect of Dexamethasone, Alirocumab, Dexamethasone and Alirocumab Groups on Liver Function tests (n=10).

Variables (Mean ± SD)	Control Group	Alirocumab Group	Dexamethasone Group on 30th day	Dexamethasone and Alirocumab Group
ALT (IU / L)	29.46 ± 7.97	46.94 ± 12.39[a]	40.38 ± 10.85	57.38 ± 17.98 [a,c]
AST (IU / L)	59.04 ± 14.08	113.85 ± 28.88[a]	80.11 ± 18.79 [a,b]	100.78 ± 19.83[a,c]
ALP (IU / L)	12.86 ± 3.02	16.31 ± 7.56	17.29 ± 3.36	16.27 ± 4.55
Total Protein (g / dl)	4.56 ± 0.69	5.927 ± 0.6[a]	5.73 ± 0.45[a]	6.59 ± 1.04 [a,c]
Albumin (g / dl)	3.00 ± 0.42	3.34 ± 0.31	3.44 ± 0.26[a]	3.61 ± 0.72 [a]

ALT: Alanine transaminase; **AST**: Aspartate Transaminase; **ALP**: Alkaline phosphatase, **SD:** Standard deviation, **a**: Significant at P < 0.05 as compared to control group. **b**: Significant at P < 0.05 as compared to Alirocumab group. **c**: Significant at P < 0.05 as compared to Dexamethasone on 30th day.

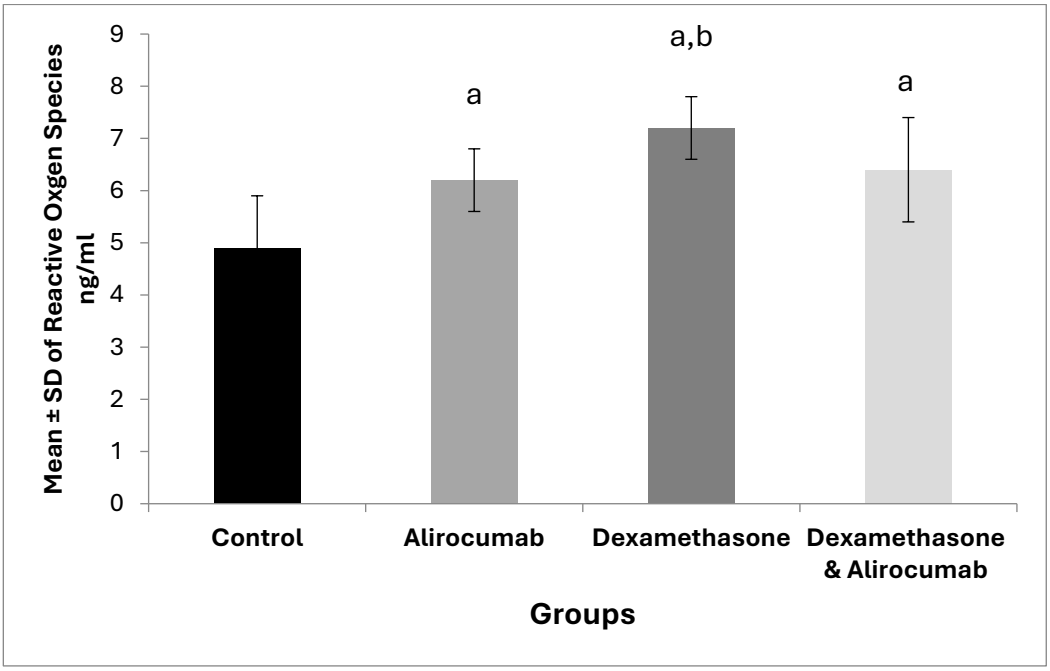

Figure (3) Mean Changes in Reactive Oxygen Species (ROS pg / ml) within Treatment Groups.

a: Significant at P < 0.05 as compared to control group. b: Significant at P < 0.05 as compared to Alirocumab group

3.5 Changes in Reactive Oxygen Species (ROS) Among the Treated Groups:

The analysis identified significant variations in reactive oxygen species (ROS) levels among the studied groups. Notably, ROS levels were significantly elevated in the Alirocumab, Dexamethasone, and Dexamethasone/Alirocumab groups compared to the Control group. (Figure 3).

3.6 Variations of Anti \ pro-Inflammatory Markers (IL-4, IL-6):

The serum level of Interleukin-4 (IL-4) levels revealed statistically significant differences among the study groups. Specifically, IL-4 levels were significantly higher in the Alirocumab and Dexamethasone/Alirocumab groups compared to the Control group. The Dexamethasone group displayed a significant decrease in IL-4 levels relative to the Alirocumab group. Furthermore, the Dexamethasone/Alirocumab group demonstrated a significant rise in IL-4 levels compared to the Dexamethasone group. (Figure 4A). Similarly, the analysis of Interleukin-6 (IL-6) levels indicated statistically significant increase in the Alirocumab and

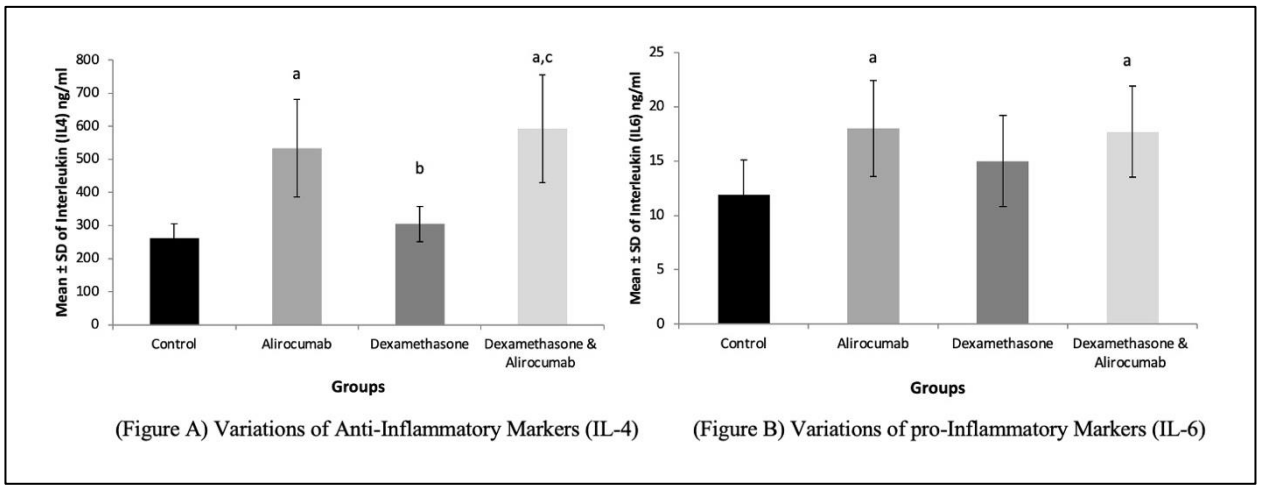

Figure (4) (A) Interleukin4 (IL4 pg / ml) among the Studied Groups (B) Interleukin6 (IL6 pg / ml) among the Studied Groups

a: Significant at $P < 0.05$ as compared to control group. b: Significant at $P < 0.05$ as compared to Alirocumab group. c: Significant at $P < 0.05$ as compared to Dexamethasone group.

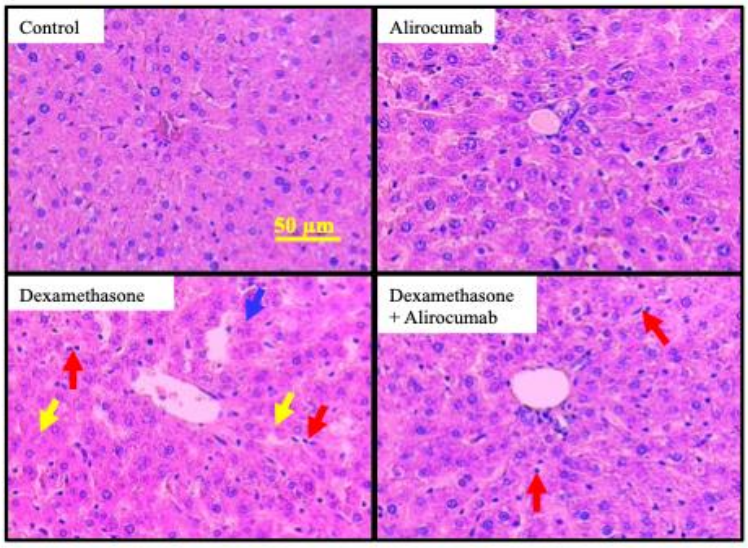

Figure (5): Effect of Dexamethasone with or without Alirocumab treatment on histopathological architectures in rats' livers. Mild apoptotic nuclei (yellow arrows), Necrotic pericentral nucleus (blue arrows), interstitial immune migration (red arrows). Scale bar = 50 µm

Dexamethasone/Alirocumab groups compared to the Control group. (Figure 4B).

3.7 Histopathological finding (H&E) stain:

The control group exhibited a normal liver architecture, while dexamethasone treatment induced mild apoptotic nuclei (yellow arrows), necrotic pericentral nucleus (blue arrows), and interstitial immune cell migration (red arrows). Liver sections from animals co-treated with Alirocumab and Dexamethasone also demonstrated interstitial immune migration (red arrows) (Figure 5).

3.8 Immunofluorescence results:

3.8.1 Expression of p-Akt in livers tissue:

Immunofluorescence analysis (Figure 6A) demonstrates p-AKT protein expression represented with fluorescence intensities quantified in the graph (Figure 6B) and shown as a histogram (Figure 6C). Rats treated with Alirocumab exhibited stronger p-AKT protein

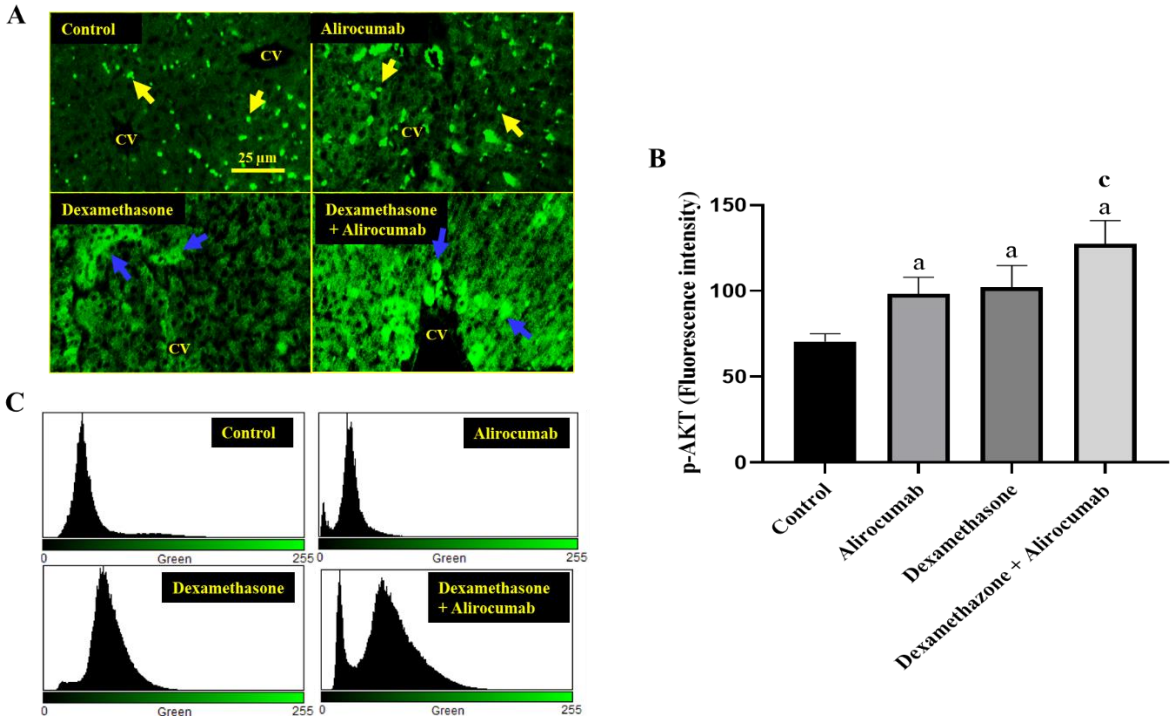

Figure (6) Protein expression of p-AKT in Dexamethasone and/or Alirocumab treatment in sections of rat livers as shown in immunofluorescence figure (A), fluorescence intensities graph (B) or shown as histogram (C). p-AKT protein expression in activated hepatic stellate cells of interstitial sinusoidal in pericentral area (yellow arrows), p-AKT protein expression in pericentral hepatocytes (blue arrows). Scale bar = 25 µm. a: Significant at $P < 0.05$ as compared to control group. c: Significant at $P < 0.05$ as compared to Dexamethasone group.

expression in activated hepatic stellate cells within the interstitial sinusoidal region of the pericentral area (yellow arrows) compared to the control group. In contrast, the Dexamethasone-treated group exhibited diminished p-AKT protein expression in pericentral hepatocytes (blue arrows) relative to the Control group. Liver tissues from rats co-treated with Alirocumab and Dexamethasone demonstrated enhanced p-AKT protein expression in pericentral hepatocytes (blue arrows) compared to the Dexamethasone-only group. Quantitative analysis indicated a significant rise in p-AKT expression in all experimental groups compared to the Control group, with a significantly greater rise observed in the Dexamethasone/Alirocumab group compared to the Dexamethasone group ($p < 0.05$).

3.8.2 RXR-a protein expression:

Immunofluorescence analysis (Figure 7A) illustrates RXR-α protein expression, with fluorescence intensities quantified and plotted in a bar graph (Figure 7B) and presented as a histogram (Figure 7C). The Control group exhibited constitutive RXR-α protein expressions in activated hepatic stellate cells within the interstitial sinusoidal region of the pericentral area (yellow arrows). In contrast, the Dexamethasone-treated group displayed a reduced RXR-α protein expression (yellow arrows) compared to the Control group. Liver tissues from rats co-treated with Alirocumab and Dexamethasone demonstrated RXR-α protein expression in pericentral hepatocytes (blue arrows). Quantitative analysis shown a significant reduction in RXR-α expression in the Dexamethasone group compared to the Control group, whereas a significant rise was observed in the Dexamethasone/Alirocumab group compared to the Dexamethasone group ($p < 0.05$).

4- Discussion

Alirocumab is PCSK9 inhibitor lowers LDL-C levels and regulates glucose metabolism and IR in Metabolic syndrome (13) (1). This study suggests that alirocumab may counteract dexamethasone-induced MetS in rats, with the PI3K/AKT pathway playing a key part in its therapeutic effects, while potentially causing hepatotoxicity.

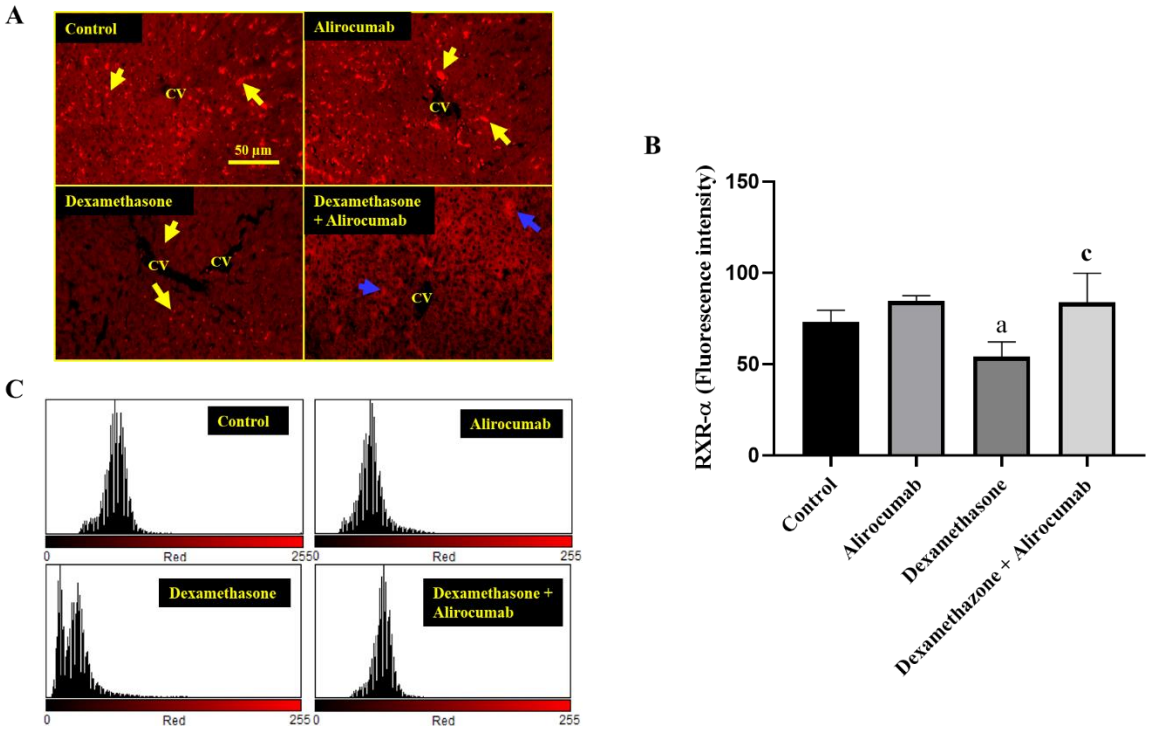

Figure (7) Effect of Dexamethasone in the presence or absence of Alirocumab treatment on RXR-a protein expression in sections of rats' livers as shown in immunofluorescence figure (A), fluorescence intensities were calculated and blotted (B) or shown as histogram (C). RXR-a protein expression on activated hepatic stellate cells in interstitial sinusoidal of pericentral area (yellow arrows). RXR-a protein expression in pericentral hepatocytes (blue arrows). Scale bar = 50 µm. a: Significant at $P < 0.05$ as compared to control group. c: Significant at $P < 0.05$ as compared to Dexamethasone group

The results of the present study exhibit that dexamethasone administration significantly altered multiple metabolic parameters on the 10th day of treatment. Specifically, dexamethasone induced a marked increase in insulin levels, blood glucose, insulin resistance, and total cholesterol, while concurrently leading to a reduction in HDL-C levels. These alterations collectively indicate the development of metabolic syndrome because of dexamethasone exposure. These outcomes are consistent with prior research by Shittu et al., who reported that dexamethasone treatment led to elevated blood glucose levels, insulin resistance, and a significant rise in total cholesterol, accompanied by a decrease in HDL-C levels in experimental animals. The observed metabolic disturbances reinforce the role of dexamethasone in inducing metabolic syndrome through its effects on glucose and lipid metabolism. (25). In support of these findings, a study by Malkawi et al. reported that dexamethasone administration was associated with several metabolic abnormalities. (26). Such metabolic disruptions provide potential mechanisms by which dexamethasone may contribute to the development of metabolic syndromes, including insulin resistance and dyslipidemia.

The data from the present study indicate a decrease in body weight in both the dexamethasone-treated group and the dexamethasone-alirocumab co-treatment group compared to their initial body weight. This outcome is consistent with the study of Shittu S et al., which demonstrated that dexamethasone administration in experimental animals significantly reduces body weight (25). However, this observation contrasts with the primary characteristic of metabolic syndrome in humans, which is typically associated with weight gain (27). Sivabalan et al., reported that the result of dexamethasone on body weight is dose-dependent, with high doses (250 µg/kg/day) leading to weight reduction, while lower doses (50 µg/kg/day) stimulate appetite and promote weight gain. (28)

In line with our findings, previous studies have reported a correlation between dexamethasone administration and reduced body weight, suggesting that plasma leptin may contribute to dexamethasone-

induced anorexia and represent a potential mechanism underlying the observed weight loss in rats (29).

The current study's results on lipid profiles demonstrated a decrease in high-density lipoprotein (HDL) levels and a rise in total cholesterol, triglycerides (TG), and low-density lipoprotein (LDL) levels in the dexamethasone-treated group compared to the control and alirocumab-treated groups. These findings align with those reported by Dolatabadi AA et al., who observed similar lipid alterations following dexamethasone administration (30). Conversely, alirocumab treatment led to a rise in HDL levels and a reduction in cholesterol, TG, and LDL levels, consistent with the results of Kühnast S et al. (31).

Researchers made a similar observation, reporting that alirocumab significantly improved lipid profiles, especially by lowering LDL-C, total cholesterol, and triglycerides, in comparison to inclisiran. The study reported that alirocumab effectively improved the lipid profile, particularly by reduction LDL-C, total cholesterol, and triglycerides, compared to inclisiran (32). Furthermore, a study relating patients with type 2 diabetes mellitus (T2DM) and mixed dyslipidemia showed that alirocumab significantly reduced LDL-C, non-HDL-C, and total LDL particles, while increasing HDL-C (33). Another study reported that alirocumab significantly reduced LDL-C levels by 61.0% at 24 weeks compared to placebo. In addition, it resulted in a modest but notable increase of 4.6% in HDL-C levels over the same period (34). These findings highlight alirocumab's efficacy not only in lowering atherogenic lipids but also in slightly enhancing cardioprotective lipid fractions.

The cholesterol-lowering result of alirocumab is attributed to its capability to bind circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) and inhibit its interface with low-density lipoprotein receptors (LDLR), thereby enhancing LDL clearance (35) .

Furthermore, the present study confirms that dexamethasone induces hypercholesterolemia, insulin resistance, hypertension, and dyslipidemia, as documented by M Askar MM et al. (27). The dexamethasone-alirocumab co-treatment group demonstrated a reduction in cholesterol, LDL, and TG levels compared to the dexamethasone-alone group, though these levels remained elevated relative to the control and alirocumab-only groups. HDL levels increased in the co-treatment group and alirocumab group compared to the dexamethasone-alone group. These findings suggest that while alirocumab mitigates dexamethasone-induced dyslipidemia, it does not fully restore lipid parameters to normal levels.

A notable observation was the rise in liver enzymes, particularly alanine transaminase (ALT) and aspartate transaminase (AST), in the alirocumab-treated and dexamethasone-alirocumab co-treatment groups. The elevation was extra pronounced in the co-treatment group compared to the alirocumab-alone group, while the dexamethasone-treated group exhibited lower ALT and AST levels than the other two groups. No significant differences were observed in alkaline phosphatase (ALP) levels among the groups. These findings suggest a potential hepatocellular effect associated with alirocumab, which may be exacerbated by dexamethasone co-administration. This aligns with the results of Zafar et al., who reported that PCSK9 inhibitors negatively affect hepatocytes, leading to increased serum transaminases.(14).

Additionally, dexamethasone has been known as a contributing influence on liver dysfunction, consistent with findings by Alkot et al., and Nkono et al., who found that dexamethasone treatment led to a significant increase in liver enzyme activity. (36) (37) Likewise, Barale et al., reported that rats treated with dexamethasone exhibited a marked rise in ALT, AST, and ALP levels compared to the control group. (38) Collectively, these studies indicate that dexamethasone induces liver injury, and its combination with alirocumab may further exacerbate hepatocellular damage.

In contrast to the findings of the present study, research conducted on mice treated with dexamethasone demonstrated no significant increases in serum glutamate dehydrogenase (GLDH), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) levels at either 24- or 72-hours post-treatment.(39) This suggests that, under certain conditions or time points, dexamethasone may exert a limited effect on these specific liver enzymes.

Clinical cases have also linked PCSK9 inhibitors to liver injury as Ibrahim et al., who recorded A 61-year-old woman progressed autoimmune hepatitis shortly after initiating alirocumab treatment, suggesting a potential causal link. The temporal association raises the possibility of a drug-induced autoimmune reaction.

(40) Similarly, in October 2022, reported A 66-year-old patient progressed idiosyncratic drug-induced liver injury (DILI) three days after receiving inclisiran, a synthetic small interfering RNA (siRNA) that targets PCSK9 expression in hepatocytes (41), this was one of the earliest documented cases of hepatic injury associated with PCSK9 inhibitors (42).

However, other studies suggest that alirocumab and evolocumab exhibit hepatic safety profiles comparable to placebo (43). A study by Vujkovic, who assessed the hepatic safety of PCSK9 inhibitors by evaluating liver damage biomarkers, including bilirubin and aminotransferases. Their results showed that prolonged PCSK9 suppression effectively lowered LDL cholesterol without significantly impacting circulating markers of liver injury across diverse populations (44). The ODYSSEY OUTCOMES trial showed that alirocumab effectively reduced recurrent cardiovascular events and overall mortality through sustained LDL-C lowering, with a safety profile similar to placebo except for minor injection-site reactions, supporting its long-term use even in high-risk patients (45). These findings emphasize the requirement for extra research to better understand the hepatic safety profile of PCSK9 inhibitors, especially when used alongside other potential hepatotoxic agents like dexamethasone.

Total protein levels increased across all groups, with the most pronounced elevation in the dexamethasone-alirocumab co-treatment group. Albumin levels were elevated in both the dexamethasone-treated and dexamethasone-alirocumab groups, consistent with previous study, who attributed this effect to upregulated hepatic albumin synthesis. Elevated serum albumin levels have been linked with an augmented risk of acute kidney injury (AKI), as high concentrations may contribute to renal tubular epithelial and glomerular podocyte damage (46).

Reactive oxygen species (ROS) play a critical part in inflammatory liver diseases (47)(48). The present study demonstrated increased ROS levels across all experimental groups compared to the control, suggesting oxidative stress involvement in liver dysfunction. However, these findings contrast with those of D'Onofrio et al., who found that PCSK9 inhibitors reduce ROS-induced damage, highlighting their antioxidative properties (49). The increase in ROS levels in dexamethasone-treated groups aligns with previous research indicating that dexamethasone promotes ROS production, causative to oxidative stress and hepatic injury (50).

Pro-inflammatory interleukins (ILs), for example IL-6, play a crucial role in inflammation, however anti-inflammatory ILs, for example IL-4, regulate immune responses (51). The present study observed a rise in IL-6 and IL-4 levels in the alirocumab-treated and dexamethasone-alirocumab co-treatment groups. These findings contrast with studies reporting reduced IL-6 concentrations following PCSK9 inhibitor administration (52). This goes in hand with Marques et al., found that IL-4 and IL-6 plasma levels remained unchanged after alirocumab treatment. (53).

These results may be clarified by the fact that serum and intrahepatic IL-6 levels are significantly raised in patients with acute and chronic liver diseases (54). Additionally, the increase in anti-inflammatory cytokines, for example IL-4, may be part of the compensatory anti-inflammatory response syndrome (CARS), which is characterized by immune suppression and excessive cytokine production (55).

Histopathological analysis revealed that dexamethasone treatment resulted in mild apoptotic nuclei, necrotic pericentral space, and interstitial immune migration, while dexamethasone-alirocumab co-treated sections also exhibited interstitial immune migration. These results align with Jasim et al., who reported that dexamethasone can lead to liver necrosis and inflammation (56).

The PI3K/Akt pathway plays a vital role in mitigating liver fibrosis. (57) also, increased hepatic Akt expression is associated with the amelioration of glucose intolerance, attenuation of liver injury, and reduction of hyperlipidemia (58). The present study observed strong p-AKT protein expression in hepatic stellate cells (HSCs) in alirocumab-treated rats, suggesting a function in HSC activation (59). Conversely, the dexamethasone-treated group displayed lower p-AKT protein expression in pericentral hepatocytes, whereas the dexamethasone-alirocumab co-treatment group showed stronger p-AKT expression compared to the dexamethasone-alone group.

Scheving et al. reported that prolonged dexamethasone exposure may suppress AKT signaling in liver cells, (60) whereas other studies suggest that dexamethasone can stabilize or activate the PI3K/AKT pathway, leading to increased p-AKT levels and enhanced cell

survival (61). Similarly, Sun et al., observed that PCSK9 overexpression elevated AKT phosphorylation, although alirocumab treatment did not significantly alter p-AKT levels in hepatocellular carcinoma (HCC) cells (62). In contrast, Zhang et al., demonstrated that lipid accumulation upregulated AKT phosphorylation, while alirocumab treatment markedly reduced p-AKT expression (63).

The present findings suggest that p-AKT expression plays a dual role in dexamethasone-induced metabolic alterations. Increased p-AKT in hepatocytes appears to mediate protective effects by enhancing insulin sensitivity and supporting metabolic balance, whereas its upregulation in hepatic stellate cells may indicate activation and a potential risk of fibrosis. Thus, while alirocumab treatment partially restored p-AKT expression and improved metabolic outcomes, its influence on stellate cell activation underscores the need for cautious interpretation and further studies to clarify whether this response reflects adaptive protection or progression toward hepatic injury (64).

Furthermore, this study revealed that dexamethasone administration decreased RXR-α protein expression compared with controls. However, liver tissues from rats co-treated with alirocumab and dexamethasone showed RXR-α expression in pericentral hepatocytes, while no significant alterations were detected in the alirocumab-only group. These findings suggest that the combination of alirocumab and dexamethasone may contribute to hepatic stress or injury, given that RXR-α expression has been associated with liver damage (65). In line with this, Steineger et al. reported that both dexamethasone and fatty acids independently induced RXR-α expression (66).

In the present study, both p-AKT and RXR-α expression were altered by dexamethasone administration, reflecting disruption of interconnected signaling and transcriptional pathways that regulate hepatic glucose and lipid metabolism. Reduced p-AKT indicated impaired insulin signaling and metabolic stress, while decreased RXR-α expression suggested suppression of nuclear receptor–mediated transcriptional control of lipid homeostasis and inflammation. It is worth noting that Alirocumab co-treatment enhanced the expression of both markers, supporting their collaborative role in restoring metabolic balance through AKT phosphorylation and RXR-dependent transcription. However, the increased p-AKT signal in stellate cells and the stress-associated reappearance of RXR-α also suggest that these changes may reflect not only protective adaptation but also potential activation of fibrogenic or injury-related responses (67) (68) (69).

Overall, alirocumab mitigates dexamethasone-induced metabolic disturbances but may cause hepatic stress, as evidenced by elevated liver enzymes and alterations in RXR-α and p-AKT expression.

Conclusion

This study demonstrates that alirocumab effectively improves lipid profiles and partially mitigates dexamethasone-induced metabolic disturbances, likely via modulation of the PI3K/AKT pathway. However, its administration is also associated with elevated liver enzymes, increased oxidative stress, and alterations in RXR-α expression and AKT activation, indicating potential hepatocellular stress. Further investigations are warranted to confirm these findings, elucidate the underlying mechanisms, and assess their translational relevance in the context of glucocorticoid-induced metabolic dysfunction.

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