Original Article

Access this article online
Quick Response Code:

Website:
www.carcinogenesis.com
DOI:
10.4103/jcar.jcar_22_01_03

Effect of Apremilast in Experimentally Induced Hyperlipidemic Mice

Shaimaa S Abdullah[1*], Ahmed R Abu Raghif [1]

Abstract

Background: Hyperlipidemia is a chronic condition defined by unusually high amounts of lipids and lipoproteins in the blood, including total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). It is a major cause of arteriosclerosis, coronary heart disease, myocardial infarction, cerebral stroke, and renal failure. Both inherited and acquired hyperlipidemia can often be included in the classification of hyperlipidemia (1). The objective: To evaluate the antihyperlipidemic effect of apremilast in Swiss albino male mice induced by a high-fat diet HFD. Method: Thirty-two healthy male albino mice were divided randomly into four groups, each with eight mice (n=8). The Control (apparently healthy) group 1 was neither induced nor treated and fed with a standard diet and tap water. Groups 2, 3, and 4 were fed HFD for 28 days to induce hyperlipidemic models. After induction of hyperlipidemia, group 2 acted as an induction group, whereas group 3 received atorvastatin as a standard drug at 10 mg/kg/day orally for 28 consecutive days. apremilast was administered orally at 20 mg/kg/day to group 4 for 28 consecutive days to evaluate its effect on hyperlipidemia in mice. The lipid profile, liver enzymatic activity, and oxidative stress parameters were measured and the histopathological changes in the liver was measured. Results: lipid profile, hepatic enzyme activity, and oxidative stress parameters show a significant reduction ($p < 0.05$) with significant improvement in the liver histology compared to those induced non-treated groups. Conclusion: apremilast has a therapeutic effect on hyperlipidemia in mice induced by HFD through its ability to reduce lipid profile, liver enzyme activity, and oxidative stress parameters and improve the liver histopathological changes.

Keywords:

Hyperlipidemia, Apremilast, HFD, Statin.

Introduction

Hyperlipidemia is a metabolic disease characterized by abnormal serum levels of total cholesterol, triglycerides, or both, involving abnormal levels of related lipoprotein species. The most commonly associated clinical consequence of hyperlipidemia is atherosclerosis and related cardiovascular diseases [1].

One of two types of hyperlipidemia exists, depending on the underlying reasons: primary and secondary. Treated hyperlipidemia by changing lifestyles and following healthy behaviors or using medications. The type of lipid problem determines the medication that should be used to treat it, whereas statins (HMG-CoA reductase inhibitors), niacin, resins, PCSK9 inhibitors, and ezetimibe are used to lower LDL cholesterol, while omega-3 polyunsaturated fatty acid derivatives (PUFA), fibric acid derivatives, and nicotinic acid are used to decrease levels of TG and VLDL and increase the level of HDL cholesterol [2]. The fact that many of these medications have adverse side effects, such as symptoms connected to the muscles Consequently, it is necessary to develop potential new lipid-lowering medicines as an alternative to allopathic medications [3, 4]. Apremilast is a selective phosphodiesterase 4 (PDE4) inhibitor and was approved by United States Food and Drug Administration in 2014 for oral administration in the treatment of moderate to severe psoriasis and psoriasis arthritis. The inhibition of the PDE4 enzyme leads to an increase in cyclic adenosine monophosphate(cAMP), The increase in the cAMP resulted in the anti-inflammatory effects of apremilast by reducing inflammatory cytokines [5].

[1] Department of Pharmacology- College of Medicine-Al Nahrain University/Iraq.

Address for correspondence:
Shaimaa S Abdullah, Department of Pharmacology- College of Medicine-Al Nahrain University/Iraq.

Submitted: 10-Dec-2022
Revised: 18-Jan-2023
Accepted: 25-Mar-2023
Published: 04-Apr-2023

How to cite this article: Abdullah S S, Abu Raghif A R. Effect of Apremilast in Experimentally Induced Hyperlipidemic Mice. J Carcinog 2023;22(1):20-27

Material and Methods

Experimental animals:

Thirty-two apparently healthy albino male mice, 2-3 months of age, weighing about 20–30 g, were obtained from the National Center for drug control and Research Techniques/AL Nahrain University, and housed in the well-ventilated non-pathogenic environment and with adequate water and food supply with a 12-hour light-dark cycle throughout the study. The animals were acclimatized for a week before the commencement of the experiment.

Drugs and chemicals:

Apremilast, atorvastatin, and cholesterol were obtained as a powder from Hangzhou Hyper Chemicals Limited Company (china).

Induction of hyperlipidemia:

The induction of hyperlipidemia was done by a high-fat diet (2% cholesterol and 1% peanut butter) was added to the usual diet (seeds like sunflower and groundnut, cereals, fruits like grapes and apples, vegetables, and vitamins like A, E, and D3) for 28 days [6].

Preparation of drugs:

Apremilast and atorvastatin solution was prepared freshly by dissolved in 2% ethanol and diluted with distal water to the desired volume.

Experimental design:

An animal experiment uses thirty-two male Albino mice. The mice were divided evenly and randomly into four groups, each with eight mice (n=8), as follows [7]:

Group 1 (normal): standard diet for 28 days without any intervention. Group 2 (induced): High-Fat Diet (HFD) for 28 days, and left without any treatment. Group 3 (treated): HFD for 28 days then atorvastatin 10 mg/kg once a day (p.o.) for another 28 days. Group 4: HFD for 28 days, then apremilast 20 mg/kg once a day (p.o.) for another 28 days.

Blood collection

The animals were fasted for 12 hours while having access to water prior to blood collection. Blood samples were collected by heart puncture under anesthesia and put in test tubes. This was followed by centrifuging at 3000 rpm for 15 minutes at room temperature. The collected serum was stored at 0°C until use. For the estimate of the liver function test and serum lipid profile, serum was employed.

Biochemical analysis

Measurement of lipid profile and liver enzymatic activity:

Standard diagnostic tests were utilized to determine serum total cholesterol (TC), triglyceride (TG), LDL, VLDL, HDL, ALT, AST, ALP, albumin, and total serum bilirubin (TSB) levels in mice with a biochemical auto-analyzer [8].

Measurement of oxidative stress

Animals were slaughtered at the end of the experimental period after blood samples were taken, and the liver organ was removed for examination and split into two pieces for testing[9].

The samples of MDA and glutathione were used to test for oxidative stress were created by first homogenizing a portion of the liver and then centrifuging the homogenates for 15 minutes at 5000 rpm. The MDA and GSH levels in the tissue were measured using the usual diagnostic kits. The competitive inhibition enzyme immunoassay approach is used in this test [10, 11].

Histopathological examination:

All mice were sacrificed and immediately performed an autopsy to obtain livers, then preserved in 10 %Formalin solution[12].

The samples were dehydrated and embedded in paraffin, after routine processing, paraffin sections of each tissue were cut into 5 µm thickness and stained with hematoxylin and eosin (H&E) for histological examination [13].

Statistical analysis

Data entry and analysis were performed using Microsoft Excel 2010 and SPSS version 26. Continuous variables were expressed as mean SD. Categorical variables were presented as frequencies and percentages using the Chi-square test. The Test of Normality (Shapiro-Wilk) showed that the data were non-normally distributed, so a non-parametric test (Mann-Whitney) was used instead of parametric tests (independent t-test and one-way ANOVA). The level of significance was considered when the P value was less than 0.05 [14].

Results

1- Changes in lipid profile among the experimental animal groups:

Analysis of serum levels of (cholesterol, TG, LDL, and VLDL) in the present study revealed a highly significant increase with a significant decrease in HDL among induced (non-treated) animals compared to the control (apparently healthy) group. in contrast, animal groups treated with atorvastatin and apremilast showed a statistically significant reduction in (cholesterol, TG, LDL, and VLDL) with a highly significant increase in HDL compared to the induced (non-treated) group. A comparison of the efficacy of atorvastatin and apremilast revealed that apremilast was able to reduce serum levels of (cholesterol, TG, and VLDL) with a statistical significance ($P \leq 0.05$) while showing almost a comparable result to atorvastatin regarding LDL with statistical significance ($P \geq 0.05$).

Table (1): serum lipid profile analysis of the animal groups

Animal Groups	Serum Lipid Profile Analysis (mg/dl)				
	Cholesterol	TG	LDL	VLDL	HDL
Control (Apparently Healthy)	128.75 ± 17.16	173.28 ± 19.29	56.12 ± 17.85	34.66 ± 3.86	37.98 ± 1.19
Induced (non-treated)	280.36 ± 17.58 a**	238.67 ± 11.05 a*	209.17 ± 16.88 a*	47.73 ± 2.21 a*	23.46 ± 2.32 a*
Atorvastatin (10mg/kg/day)	171.6 ± 3.56 b**	189.88 ± 14.86 b**	99.68 ± 6.25 b**	38.35 ± 3.32 b**	29.82 ± 2.27 b**
apremilast (20mg/kg/day)	156.37 ± 1.74 b,c **	144.59 ± 7.03 b,c**	99.53 ± 4.6 b**c^{NS}	28.92 ± 1.41 b,c**	27.93 ± 3.09 b*,c^{NS}

Results are expressed as mean ± SD, (n=8), and the level of significance was set at (P≤0.05). * Represents significance (P≤0.05 & ≤0.01), ** represents high significance (P≤0.001). (a) represent a comparison between the normal (apparently healthy) group and induced (non-treated) group, (b) represents a comparison between the induction group and other treated groups, (c) represents a comparison between the induced treated with atorvastatin group and apremilast.

TG= triglyceride, **VLDL**= very low-density lipoprotein, **LDL**= low-density lipoprotein, **HDL**= high-density lipoprotein, **SD**= standard deviation

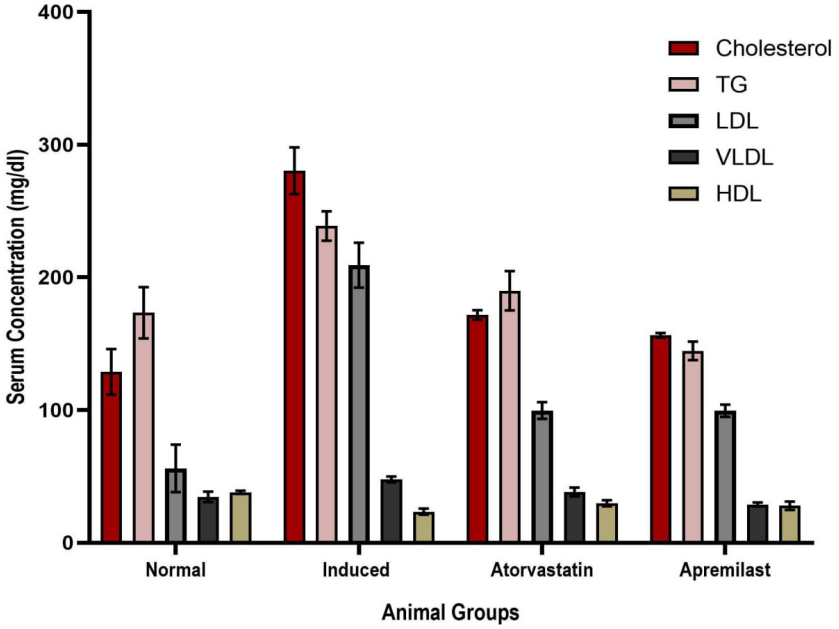

Figure (1): Lipid profile analysis of the animal groups.
Results are expressed as mean ± SD, (n=8), and the level of significance was set at (P≤0.05).
TG= triglyceride, **VLDL**= very low-density lipoprotein, **LDL**= low-density lipoprotein, **HDL**= high-density lipoprotein, **SD**= standard deviation

2- Changes in liver function parameters among experimental animal groups:

The result of the study shows a highly significant increase ($p<0.001$) in serum level of ALP with a significant increase ($p<0.05$) in ALT, AST, albumin, and TSB among the induced (non-treated) group in comparison with the apparently healthy control group, On the other hands, both groups treated with atorvastatin and apremilast showed statistically highly significant ($p<0.001$) decrease in the serum level of ALP, ALT, AST, albumin, and TSB levels when compared with the induced (non-treated) group. In comparison with the atorvastatin-treated group, the apremilast-treated group shows a statistically highly significant($p<0.001$) decrease in AST and ALB, and significant decrease ($p<0.05$) in ALT and TSB, with non-significant differences in level of ALP Table 2.

Table (2): Changes in liver function parameters among animal groups.

Animal Groups	Liver Function Test				
	ALT (u/L)	AST (u/L)	ALP (u/L)	Albumin (u/L)	TSB (mg/dl)
Control (Apparently Healthy)	37.62 ± 2.22	38.03 ± 1.23	21.52 ± 3.01	5.4 ± 0.23	0.68 ± 0.03
Induced (non-treated)	45.18 ± 2.85 a*	44.94 ± 2.07 a*	50.87 ± 1.95 a**	6.61 ± 0.29 a*	1.69 ± 0.12 a*
Atorvastatin (10mg/kg/day)	37.02 ± 2.06 b**	38.25 ± 1.72 b**	31.34 ± 5.26 b**	5.51 ± 0.23 b**	0.8 ± 0.07 b**
Apremilast (20mg/kg/day)	32.77 ± 3.41 b**,c*	35.13 ± 1.42 b,c**	29.16 ± 7.83 b**,c^{NS}	4.91 ± 0.32 b,c**	0.7 ± 0.1 a**, b,c*

Results are expressed as mean ± SD, (n=8), and the level of significance was set at (P≤0.05). * Represents significance (P≤0.05 & ≤0.01), ** represents high significance (P≤0.001). (a) represent a comparison between the normal (apparently healthy) group and induced (non-treated) group, (b) represents a comparison between the induction group and other treated groups, (c) represents a comparison between the induced treated with atorvastatin group and apremilast.

AST= Aspartate Aminotransferase, **ALT**= Alanine Transaminase, **TSB**= total serum bilirubin, **ALP**= Alkaline Phosphatase, **SD**= standard deviation

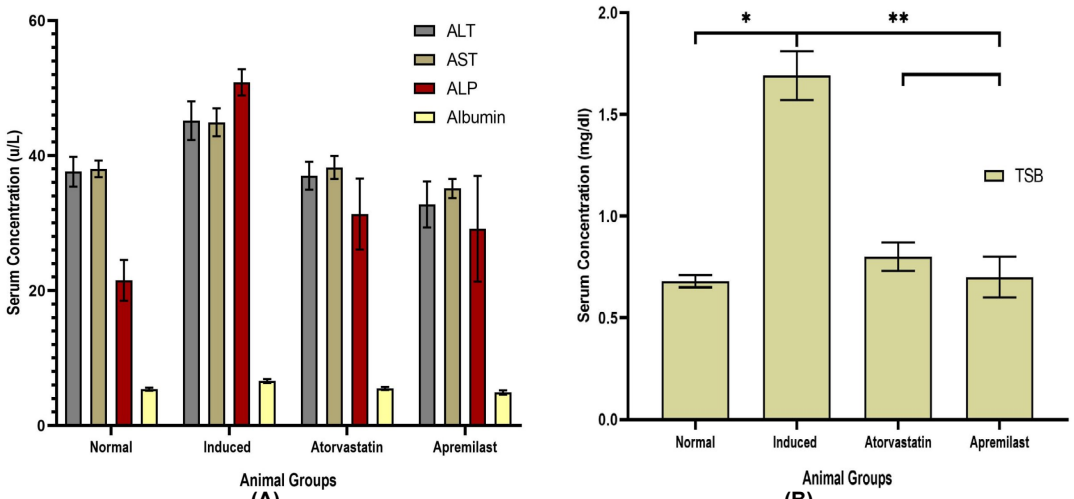

Figure (2): Changes in liver function parameters including (ALT, AST, ALP, and Albumin) (A), Changes in total serum bilirubin (TSB) levels (B) Results are expressed as mean + SD, (n=8), and the level of significance was set at (P≤0.05).
AST= Aspartate Aminotransferase, **ALT**= Alanine Transaminase, **ALP**= Alkaline Phosphatase, **TSB**=total serum bilirubin, SD= standard deviation

3- Changes in oxidative stress parameters among experimental animal groups:

In Table 3, The MDA shows a significantly increased with a highly significant decrease in GSH in the induced (non-treated) group when compared with the normal (apparently healthy) group. While atorvastatin and apremilast-treated groups show a highly significant decrease in MDA with a highly significant increase in GSH when compared with the induced (non-treated) group.

And show a highly significant decrease in MDA with a significant increase in GSH in the apremilast-treated group when compared with the atorvastatin-treated group.

Table (3): Changes in oxidative stress markers among animal groups

Animal Groups	Oxidative Stress Analysis	
	MDA (nmol/ml)	GSH (u/ml)
Control (Apparently Healthy)	229.95 ± 9.61	48.62 ± 5.11
Induced (non-treated)	475.98 ± 44.02 a*	11.65 ± 0.78 a**
Atorvastatin (10mg/kg/day)	334.41 ± 11.15 b**	26.51 ± 2.85 b**
Apremilast (20mg/kg/day)	228.76 ± 7.53 b,c**	23.39 ± 1.2 b**, c*

Results are expressed as mean ± SD, (n=8), and the level of significance was set at (P≤0.05). * Represents significance (P≤0.05 & ≤0.01), ** represents high significance (P≤0.001). (a) represent a comparison between the normal (apparently healthy) group and induced (non-treated) group, (b) represents a comparison between the induction group and other treated groups, (c) represents a comparison between the induced treated with atorvastatin group and apremilast.
MDA= Malondialdehyde, **GSH**= Glutathione peroxidase, **SD**= standard deviation

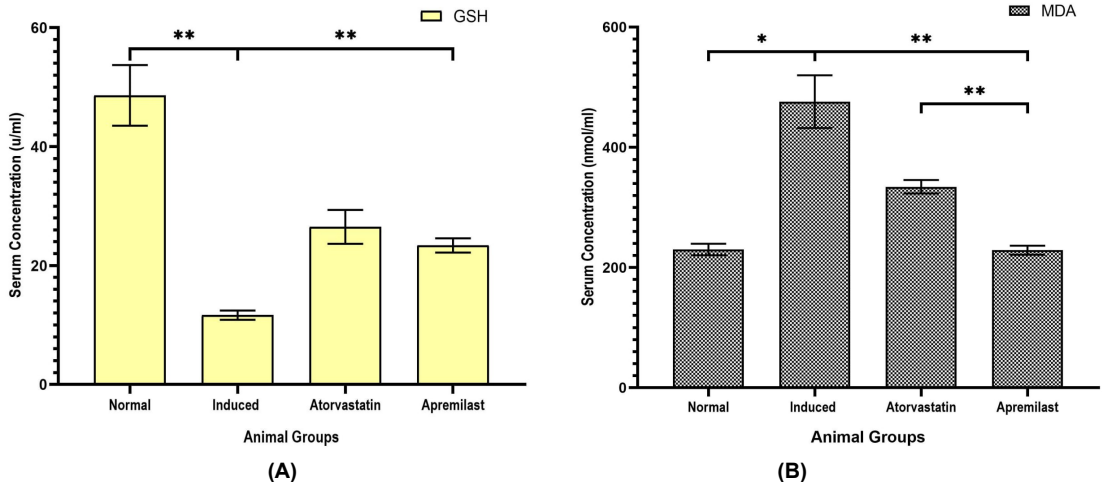

Figure (3): Changes in oxidative stress markers among animal groups. (A) changes in the level of serum glutathione (GSH), (B) changes in the level of malondialdehyde (MDA)
Results are expressed as mean + SD, (n=8), and the level of significance was set at (P≤0.05).
* Represents significance (P≤0.05 & ≤0.01), ** represents high significance (P≤0.001).

4- Histopathological examination of the liver:

In the current study, in induced (non-treated) group showed significant changes in the liver section, such as moderate steatosis and inflammation in the when compared with the control (apparently healthy) group, as shown in image (1).

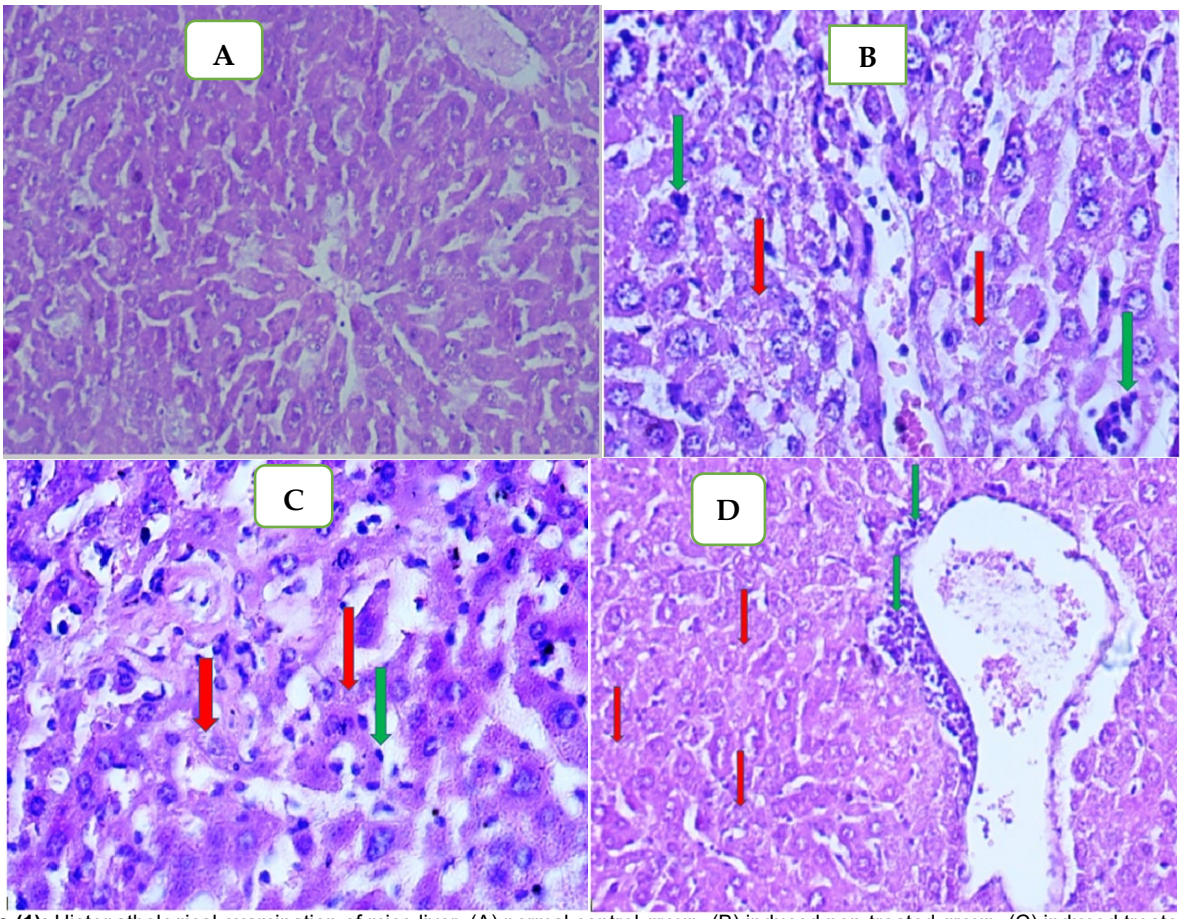

Image (1): Histopathological examination of mice liver. (A) normal control group, (B) induced non-treated group, (C) induced treated with atorvastatin, (D)induced treated with apremilast.
Red arrow= level of steatosis, **Green arrow**= level of inflammation (H&E stain, 400X & 200X)

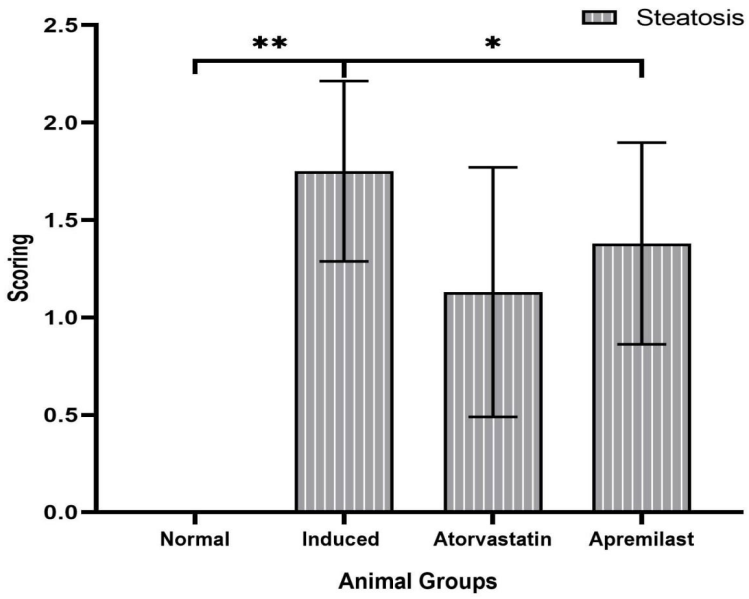

Figure (4): steatosis scoring of the liver among animal groups observed by histopathological examination
Results are expressed as mean + SD, (n=8), and the level of significance was set at (P≤0.05).
* Represents significance (P≤0.05 & ≤0.01), ** represents high significance (P≤0.001).

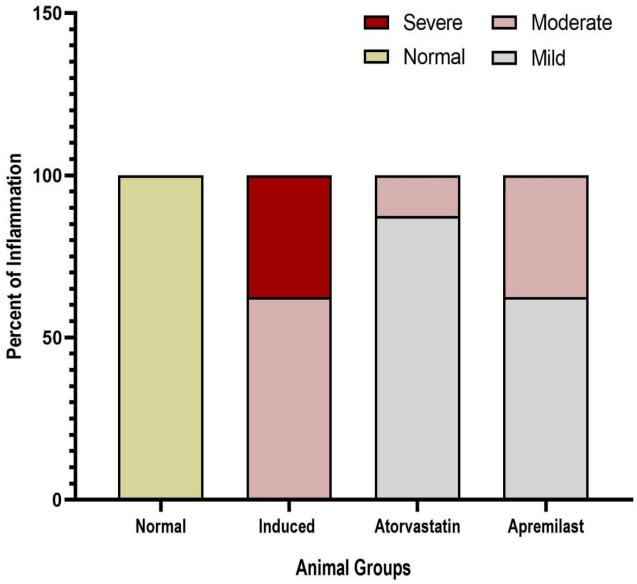

Figure (5): Percentage of inflammation in the histopathological sections of the liver among animal groups
Data are present as percentages (%), (n=8), and the level of significance was set at (P≤0.05)

Discussion

Hyperlipidemia is a chronic condition defined by unusually high amounts of lipids and lipoproteins in the blood, including total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). It is a major cause of arteriosclerosis, coronary heart disease, myocardial infarction, cerebral stroke, and renal failure [15]. The chronic elevation of cholesterol results in atherosclerosis development and negatively impacts the cardiac muscle through increasing oxidative stress, apoptosis, and mitochondrial dysfunction [16]. In addition, hypercholesterolemia induces microvascular dysfunction through inflammation, and nitro-oxidative stress mechanisms may increase the susceptibility to cardiac infarction [17]. Furthermore, the development of nonalcoholic fatty liver disease is mostly caused by the negative interference of HFD with the process of lipid metabolism in the liver. The first line of defense against hyperlipidemia is lifestyle changes, including the consumption of a healthy diet and exercise. When it comes to drug treatment for reducing lipid production and gastrointestinal absorption by using synthetic therapeutic agents like statins, fibrates, and bile acid sequestrants [18]. A number of adverse effects [19], including myopathy, rhabdomyolysis, and an increased risk of gallstone development, have been linked to the use of these drugs. Therefore, it is imperative to find new and strong anti-hyperlipidemic therapies with few side effects [20]. The liver is central to lipoprotein homeostasis and whole-body lipid metabolism because has a crucial role in regulating plasma lipid levels all the way through LDL clearance and HDL cholesterol recruitment [21, 22]. fat accumulation, especially TG within the liver increased through excess intake of a high-fat diet, increase delivery of free fatty acids to the liver[23, 24], inadequate fatty acid oxidation, increased de novo lipogenesis, Consequently elevation in the liver enzymes (ALT, ALP, AST, ALB, and TSB) [25]. Moreover, the elevation in liver enzymes may also be due to excess reactive oxygen species (ROS) production in the mitochondria as a result of lipid overload. The ROS causes hepatic inflammation by activating cytokines. Consequently, ROS and inflammatory cytokines with the excess lipid infiltration, resulted in a condition of liver toxicity [26]. Hence, the liver function markers AST, ALT, and ALB as well as total serum bilirubin TSB MDA level are widely utilized as a marker of lipid peroxidation, and their measurement gives direct evidence for LDL oxidation and is important in predicting free radical-induced injury. Hence, the observed increase in MDA may be attributed to hyperlipidemia, which enhances the process of lipid peroxidation. There was a highly significant increase in the induced (non-treated) group due to the stimulation of polymorph-nuclear leukocytes (PMNLs) and dysfunction of endothelial cells as a result of an increase in the level of ROS [27]. An intracellular hydrophilic antioxidant is glutathione [28]. It is the body's most effective natural defense against oxidative stress and is crucial for maintaining the reduced form of membrane protein SH groups, whose oxidation can change cellular structure and function [29]. showed leakage in the serum and indicated liver damage to the hepatic cells [30]. Atorvastatin as a standard cholesterol-lowering drug, works by inhibiting HMG-CoA reductase, the key enzyme in cholesterol synthesis in the body. is used in the current study which has been associated with a highly significant reduction in lipid profile TC, TG, LDL, and VLDL with a significant increase in HDL along with apremilast (20mg/kg; p.o) treated group, by acting apremilast on the liver and fat tissues lead to increase intracellular cyclic adenosine

3′,5′-monophosphate cAMP levels through activation of protein kinase A (PKA). in the liver, inhibit the accumulation of lipids and reduce TG synthesis by regulating lipid metabolism-related factors such as sterol regulatory element-binding protein 1 (SREBP1) due to an increase in cAMP levels that promote AMP kinase (AMPK) activity.

In fat tissue, increased lipolysis due to the activation of hormone-sensitive lipase (HSL) due to the activation of PKA by increased levels of cAMP leads to the conversion of stored TG to the free fatty acids FFA Chapter Four Discussion 58 and glycerol [31]. Also, apremilast increases cholesterol efflux capacity mediated by apolipoprotein A-1 due to increased expression of ATP cassette binding protein 1 (ABCA1) through the inhibition of PDE4 Consequently, it leads to increased cholesterol removal from macrophage foam cells by HDL. Both atorvastatin and apremilast-treated groups it has been associated with hepatoprotective effects through a significant reduction in liver enzymes ALT, AST, ALP, ALB, and TSB. Other animal studies have linked treatment with atorvastatin to a wide range of unfavorable liver effects. The most common is an asymptomatic and usually transient elevation of serum aminotransferase levels, which is more commonly observed with higher doses of atorvastatin. The most frequent is often a brief rise in serum aminotransferase levels. Although the exact process is yet unknown, it may be brought on by changes in the lipids that make up the hepatocyte membrane, increasing its permeability and causing leakage of liver enzymes as a result. Apremilast treated group showed improvement in liver function parameters due to the anti-inflammatory effects of this PDE4 inhibitor agent. Besides, significant reduction of MDA and increased defense mechanism through increased glutathione level were shown with apremilast (20mg/kg; p.o.) treated groups in comparison with the induced (non-treated) group due to decrease superoxide anion production and ROS generation. This increased the defense mechanism through increased glutathione levels. Histopathological examination of the liver showed improvement in the apremilast-treated group when compared to the induced(non-treated) group. apremilast is reported in the present study to decrease steatosis and inflammation in the liver, and this supports the anti-hyperlipidemic of apremilast.

References

1. W. H. Fang, V. Bonavida, D. K. Agrawal, and F. G. Thankam, "Hyperlipidemia in tendon injury: chronicles of low-density lipoproteins," *Cell and Tissue Research*, pp. 1-12, 2023.
2. B. G. Katzung, M. Kruidering-Hall, and A. J. Trevor, *Katzung & Trevor's Pharmacology Examination & Board Review*. McGraw-Hill Education, 2019.
3. S. Surya, R. A. Kumar, B. Carla, and C. Sunil, "Antihyperlipidemic effect of Ficus dalhousiae miq. stem bark on Triton WR-1339 and high fat diet-induced hyperlipidemic rats," *Bulletin of faculty of pharmacy, Cairo university*, vol. 55, no. 1, pp. 73-77, 2017.
4. D. R. Saxon and R. H. Eckel, "Statin intolerance: a literature review and management strategies," *Progress in cardiovascular diseases*, vol. 59, no. 2, pp. 153-164, 2016.
5. I. S. Padda, R. Bhatt, and M. Parmar, "Apremilast," 2023.
6. L.-X. Wang, K. Liu, D.-W. Gao, and J.-K. Hao, "Protective effects of two Lactobacillus plantarum strains in hyperlipidemic mice," *World Journal of Gastroenterology: WJG*, vol. 19, no. 20, p. 3150, 2013.
7. K. Pandit, S. Karmarkar, and A. Bhagwat, "Evaluation of antihyperlipidemic activity of Ficus hispida Linn leaves in triton wr-1339 (Tyloxapol) induced hyperlipidemia in mice," *Int J Pharm Pharm Sci*, vol. 3, pp. 188-91, 2011.
8. W. A. Coakly, "Handbook of Automated Analysis, Mercel Dekker,," p. 61, 1981.
9. M. BILECENOGLU and T. ÇELIK, "Easternmost occurrence of Didogobius schlieweni Miller, 1993 (Gobiidae) in the Mediterranean Sea," *FishTaxa*, vol. 19, pp. 1-4, 2021.
10. A. M. Hamarash, "Study the phnotypic and productive characters of seven selected genotypes of chickpea (Cicer arietinum L.) cv.(Kabuli and Desi) under rainfall conditions in Sulaimani Province, Iraq," *Kufa Journal for Agricultural Sciences*, vol. 12, no. 2, pp. 43-53, 2020.
11. M. Hameed and K. H. Abdul, "Effect of Trigonella foenum-graecum and Artemisia vulgarisextracts and their combination in streptozotocin induced hyperglycemic mice.A thesis for the degree of Master of Science in Pharmacology," *AL-Nahrain College of Medicine*.
12. D. A. Yela, M. Faber, A. Dantas, C. L. Benetti-pinto, and R. Jales, "Difficulty in Diagnosing of Renal Choriocarcinoma: Case Report," *Jornal Brasileiro de Patologia e Medicina Laboratorial*, vol. 58, p. e4122022, 2022.
13. A. Harborne, *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media, 1998.
14. T. D. V. Swinscow and M. J. Campbell, *Statistics at square one*. Bmj London, 2002.
15. E. Mercado-Palomino, A. Millán-Sánchez, M. Parra-Royón, J. Benítez, and A. Ureña Espa, "SETTER'S ACTION RANGE AS A PERFORMANCE INDICATOR IN MALE VOLLEYBALL," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte*, vol. 22, no. 85, 2022.
16. M. Chena Sinovas, J. Morcillo Losa, M. Rodríguez Hernández, and J. Zapardiel, "MULTIVARIATE TRAINING PLANNING MODEL IN PROFESSIONAL SOCCER," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte*, vol. 22, no. 85, 2022.
17. I. Andreadou *et al.*, "Hyperlipidaemia and cardioprotection: Animal models for translational studies," *British journal of pharmacology*, vol. 177, no. 23, pp. 5287-5311, 2020.
18. A. Koontanatechanon *et al.*, "The effects of increasing dietary fat on serum lipid profile and modification of gut microbiome in C57BL/6N mice," *Journal of Oleo Science*, vol. 71, no. 7, pp. 1039-1049, 2022.
19. C. Demarchi, M. d. Souza, L. S. d. Souza, F. L. Galli, and S. M. Pilati, "Lung histopathological changes in Swiss mice exposed to narghile smoke," *Jornal Brasileiro de Patologia e Medicina Laboratorial*, vol. 58, p. e4442022, 2022.
20. N. NISHIMURA, J. ZHANG, M. ABO, A. OKUBO, and S. YAMAZAKI, "Simultaneous determination of betaines and free amino acids in higher plants by capillary electrophoresis," *Analytical Sciences/Supplements*, vol. 17, no. 0, pp. i929-i932, 2002.
21. K. B. Pandey and S. I. Rizvi, "Markers of oxidative stress in erythrocytes and plasma during aging in humans," *Oxidative medicine and cellular longevity*, vol. 3, no. 1, pp. 2-12, 2010.

22. D. Preiss and N. Sattar, "Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations," *Clinical science,* vol. 115, no. 5, pp. 141-150, 2008.
23. B. Yong, L. Owen, and J. Hoseana, "Mathematical Analysis of an Epidemic Model for COVID-19: How Important Is the People's Cautiousness Level for Eradication?," *Letters in Biomathematics,* 2021.
24. F. Yu, X. Zhang, L. Gao, and J. Lv, "Association between tongue muscle quality and swallowing disorders in older nursing home residents," *Archives of Clinical Psychiatry,* vol. 49, no. 3, 2022.
25. M. Yovera-Aldana, S. Pérez-Cavero, I. Pinedo-Torres, and C. Zubiate-López, "Prevalence of Foot At-Risk and its Associated Characteristics among Outpatients with Diabetes Mellitus in a Peruvian Public Hospital," *Review of Diabetic Studies,* vol. 18, no. 1, pp. 1-9, 2022.
26. E. T. J. Mason *et al.*, "Decoding comparable morphologies: Pigmentation validated for identifying southern California Paralabrax larvae," *FishTaxa,* 2022.
27. P. Gisondi, A. C. Fostini, I. Fossà, G. Girolomoni, and G. Targher, "Psoriasis and the metabolic syndrome," *Clinics in dermatology,* vol. 36, no. 1, pp. 21-28, 2018.
28. B. Salehi *et al.*, "Therapeutic potential of α-and β-pinene: A miracle gift of nature," *Biomolecules,* vol. 9, no. 11, p. 738, 2019.
29. P. Santoso, K. A. Adrianta, and I. G. Wiranatha, "Phytochemical screening and in vivo test of dewandaru (Eugenia uniflora L) fruit extract on mice exposed to cigarette smoke," *International Journal of Health and Medical Sciences,* vol. 4, no. 2, pp. 246-252, 2021.
30. A. R. Abu-Raghif, H. B. Sahib, and S. N. Abbas, "Anti-hyperlipidemic effect of Vitex agnus castus Extracts in Mice," *Int. J. Pharm. Sci. Rev. Res,* vol. 35, pp. 120-125, 2015.
31. R. Gualtierotti and O. De Lucia, "Efficacy and metabolic effect on serum lipids of apremilast in psoriatic arthritis: a case report," *Journal of Clinical Medicine,* vol. 8, no. 3, p. 398, 2019.