Original Article

Atorvastatin and Flaxseed Effects on Biochemical Indices and Hepatic Fat of NAFLD Model in Rats

Zahra Eslami[1], Abdorreza E. Moghanlou[2], Yahya M.N. P. Kandi[3], Mehdi S. Arabi[4], Alireza Norouzi[5], Hamidreza Joshaghani[6]

[1]Metabolic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Golestan Province, Iran, [2]Department of Coaching Education, Istanbul eEsenyurt University, Istanbul, Turkey, [3]Department of Exercise Physiology, Faculty of Physical Education, Islamic Azad University, Islamshahr Branch, Islamshahr, Iran, [4]Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran, [5]Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran, [6]Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver disease that affects about 25% of the general population. No definitive treatment for NAFLD has been identified yet. The aim was to determine the effect of atorvastatin (ATO) and flaxseed on related indicators of NAFLD-induced fat/fructose-enriched diet (FFD).

Materials and Methods: Forty male Wistar rats were divided into five groups. NAFLD groups received FFD and carbon tetrachloride (CCl4) to induce NAFLD. After intervention with ATO (10 mg/kg/day) and/or flaxseed (7.5 g/kg/day), liver enzymes and lipid profiles in serum were determined at eight week of interventions.

Results: Triglycerides (TG) and cholesterol (CHO) in FFD + ATO, FFD + flaxseed, and FFD + ATO + flaxseed had a significant decrease and low-density lipoprotein (LDL) level and LDL/high-density lipoprotein (HDL) ratio showed a significant increase in the FFD + flaxseed compared to the FFD. The levels of aspartate transaminase (AST), alanine transaminase (ALT), and gamma-glutamyltransferase (GGT) were significantly reduced in the FFD + ATO, FFD + flaxseed, and the FFD + ATO + flaxseed. In addition, Alkaline phosphatase (ALP) levels were significantly different between normal and FFD. Fasting blood sugar (FBS) levels were significantly different in the FFD + flaxseed and the FFD + ATO + flaxseed compared to the FFD.

Conclusion: ATO therapy along with flaxseed controls NAFLD-related indices and FBS. Therefore, it can be stated with caution that ATO and flaxseed can be used to improve lipid profile and reduce the complications of NAFLD.

Keywords: Atorvastatin, carbon tetrachloride, flaxseed, fructose, lipids, non-alcoholic fatty liver disease, rats

Address for correspondence: Prof. Hamidreza Joshaghani, Golestan University of Medical Science, Gorgan, Iran.
E-mail: hr.joshaghani99@gmail.com
Submitted: 14-Jan-2022; **Revised:** 22-Apr-2022; **Accepted:** 24-Apr-2022; **Published:** 25-Apr-2023

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the deposition of fat, especially triglycerides (TG), in liver.[1] This disease has a wide spectrum of functional liver disorders and tissue damage similar to alcoholic fatty liver disease (AFLD). However, it does happen in patients who either do not drink alcohol or consume only small amounts of alcohol that indicates the non-alcoholic source of the disease.[2] NAFLD is a relatively silent disease that sometimes manifests itself with an increase in liver enzymes.[3] Inactivity, obesity, and lack of antioxidants in the diet are the main causes of this disease.[4] In disorders such as obesity and fatty liver, insulin resistance leads to increase glucose, fat accumulation, and impaired lipid metabolism in the liver.[5] The prevalence of NAFLD is estimated to be 25% of global population[6] and it is one of the leading causes of death associated with liver disease, and because of the potential

Access this article online

Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_21_22

How to cite this article: Eslami Z, Moghanlou AE, Kandi YM, Arabi MS, Norouzi A, Joshaghani H. Atorvastatin and flaxseed effects on biochemical indices and hepatic fat of NAFLD model in rats. Adv Biomed Res 2023;12:98.

progression of this disease to cirrhosis and liver failure, it has attracted the attention of many researchers to detect this disease and the factors involved in it.[7] NAFLD often has no specific symptoms and remains asymptomatic until it progresses to advanced stages.[8] In the pathogenesis of non-alcoholic steatohepatitis, a two-hit theory was proposed, which stated that in addition to steatosis, other factors, including oxidants, are essential in the development of this disease.[9] In NAFLD, the liver's capacity for energy sources is reduced, which is associated with the accumulation of toxic lipid species.[10]

There are several treatments for this complication. One of the methods is the drug that is prescribed to treat this disease, atorvastatin (ATO). This drug is a subset of statin drugs that long-term use and high doses can cause liver complications, mortality, and increase apoptosis in the cell. It also lowers cholesterol (CHO), TG, low-density Lipoprotein-C (LDL-C), and increases high-density Lipoprotein-C (HDL-C) levels in the blood. ATO has antioxidant, anti-apoptotic, and anti-inflammatory properties.[11] Although ATO has fewer side effects than other statins, it is still questionable, especially at high doses. Important side effects of statins include increased serum levels of transaminase and muscle inflammation.[12] ATO, in addition to reducing liver weight, reduces TG, CHO, and insulin levels. In fact, ATO prevents the accumulation of fat in the hepatocytes through AMP-activated protein kinase (AMPK)-dependent signaling pathway.[13]

Scientific evidence shows consumption of foods rich in omega-3 fatty acids significantly reduces the risk of inflammation, metabolic syndrome, and death from cardiovascular disease, NAFLD, and obesity. One of the most important sources of omega-3 essential fatty acids is flaxseed, which lowers CHO and helps with weight loss.[14] Studies on the anti-inflammatory effects of flaxseed have shown that the receptors for unsaturated fatty acids are mainly the G-protein 120 receptor in the hypothalamus and are activated in response to omega-3 or omega-9 fatty acids. G protein receptor is known as an important anti-inflammatory mediator.[15]

Previous studies have shown that a variety of diets, drugs, and plant-based substances have been used to treat NAFLD, but no proven treatment (with fewer side effects) can be given. Statins, lipid- lowering drugs, are used to improve diseases, such as metabolic syndrome, NAFLD, etc., by ameliorating the lipid profile. Flaxseed also has a potential role in the risk factors of metabolic disorders with its anti-inflammatory and antioxidant effects. Therefore, this study aims to determine the effect of fat/fructose-enriched diet (FFD) along with ATO and flaxseed as well as comparing their parallel effect on biochemical indices and hepatic fat in NAFLD.

Materials and Methods

Animals

In this experimental research, samples randomly allocated by a coin flip. All study staff and analysts were blinded. For each sample, a specific code was assigned and the lists were destroyed to hide the information. Forty male Wistar rats were purchased from Shahid Mirghani Research Institute (200–250 g) and they were randomized into five groups: 1) normal control, 2) FFD control, 3) FFD + ATO, 4) FFD + flaxseed, and 5) FFD + ATO + flaxseed. The animals were left 7 days for acclimatization before the start of the experiment. They were maintained in individual cages under a 12-h dark–light schedule and fed a pelletized commercial chow diet for 1 week after arrival. Water and food were provided ad libitum. Induction of NAFLD was done for 15 weeks.[16] ATO 10 mg/kg (diluted in 6% dimethyl sulfoxide) was gavaged and flaxseed 7.5 g/kg consumed orally for 8 weeks.[17-19] Flaxseed was purchased from Abkar Golestan Agro-industry Company (*VERJEN*). At the end of the experimental period, the animals of groups were anesthetized by injection of a combination of 50 mg/kg ketamine and 5 mg/kg xylazine (Merck, Germany) and then sacrificed after the food was withheld for 12 h.[20]

Biochemical analysis

The concentrations of CHO, HDL, LDL, TG, aspartate transaminase (AST), alanine transaminase (ALT), ALP, gamma-glutamyltransferase (GGT), and FBS were measured by enzymatic colorimetric assays using commercially available detection kits (Sigma).

Statistical analysis

Data are expressed as the mean ± SE. The statistical analyses were carried using the Statistical Package for Social Science (SPSS) software program. Significant differences among the groups were determined by a one-way ANOVA. A probability value of 0.05 was determined to be statistically significant.

Results

Rats were measured at baseline (week 0), after NAFLD induction (week 15), and during the 8 weeks of intervention. The weight of rats at the first week of the study was not significantly different but after inducing of NAFLD, weight of rats was significant in all groups ($P \leq 0.05$) [Table 1]. During the intervention period, normal and FFD control groups had significant weight gain, whereas no significant changes are observed in ATO and/or flaxseed groups ($P \leq 0.05$) [Table 2]. Evaluation of biochemical

Table 1: Mean of rat weight in groups

Group	Week 0 M±SD (g)	Week 15 M±SD (g)
Normal control	238.29±11.52	390.43±14.32
FFD control	243.35±7.05	380.44±16.74
FFD+ATO	229.66±19.44	343.16±12.21
FFD+flaxseed	240.51±12.24	251.48±11.25
FFD+ATO + flaxseed	223.14±15.23	230.12±14.89
P	0.096	0.000**

**$P<0.001$

parameters showed that the interventions led to significant changes in serum levels of TG, CHO, LDL/HDL ratio, and FBS. FFD + ATO group showed reduction in levels of TG (M = 61.60, SE = 6.02), CHO (M = 50.40, SE = 1.02), AST (M = 146.52, SE = 6.96), ALT (M = 66.10, SE = 3.75), and GGT (M = 1.004, SE = 0.054) compared to FFD control ($P \leq 0.05$). Also, the levels of TG (M = 59.00, SE = 5.29), CHO (M = 53.60, SE = 4.51), AST (M = 126.48, SE = 4.36), ALT (M = 62.66, SE = 2.18), GGT (M = 1.08, SE = 0.188), and FBS (M = 126.40, SE = 1.052) were decreased but LDL (M = 6.82, SE = 0.69) level was increased in FFD + flaxseed ($P \leq 0.05$). To evaluate the concurrent effect of ATO and flaxseed, the levels of TG (M = 83.50, SE = 6.83), CHO (M = 54.50, SE = 0.64), AST (M = 139.67, SE = 1.35), ALT (M = 38.35, SE = 2.15), GGT (M = 1.025, SE = 0.047), and FBS (M = 123.50, SE = 0.28) reduced in FFD + ATO + flaxseed compared to FFD control. There are no significant differences in ALP levels in intervention groups compared to FFD control ($P \leq 0.05$) [Figure 1]. The results of ANOVA indicated that liver enzymes and hepatic fat were significantly different in the intervention groups but no differences were observed in liver weight ($P \leq 0.05$) [Figure 2].

Discussion

Histological and biochemical analysis showed the optimal effects of ATO and flaxseed supplementation in the NAFLD groups. In addition to a significant reduction in the activity of liver enzymes, which indicates the improvement of tissue lesions, histological results also confirm these findings.

Because of the significant increase in the prevalence of NAFLD around the world and especially in Iran, the study on its prevention and treatment has received more attention. NAFLD is directly related to lifestyle, especially diet. Diet in developing countries, FFD, and a sedentary lifestyle have increased its prevalence. Today, laboratory animals are used to study diseases. In the case of NAFLD, animal models have been used because of the limitations of obtaining human liver tissue samples for study.

Induction of NAFLD caused an increase in liver enzymes. The level of liver enzymes was significantly reduced by administrating ATO and consuming flaxseed supplementation in FFD groups. NAFLD is associated with cell damage, so the serum levels of AST and ALT increase. Elevated levels of these enzymes in NAFLD are greater than in metabolic syndrome.

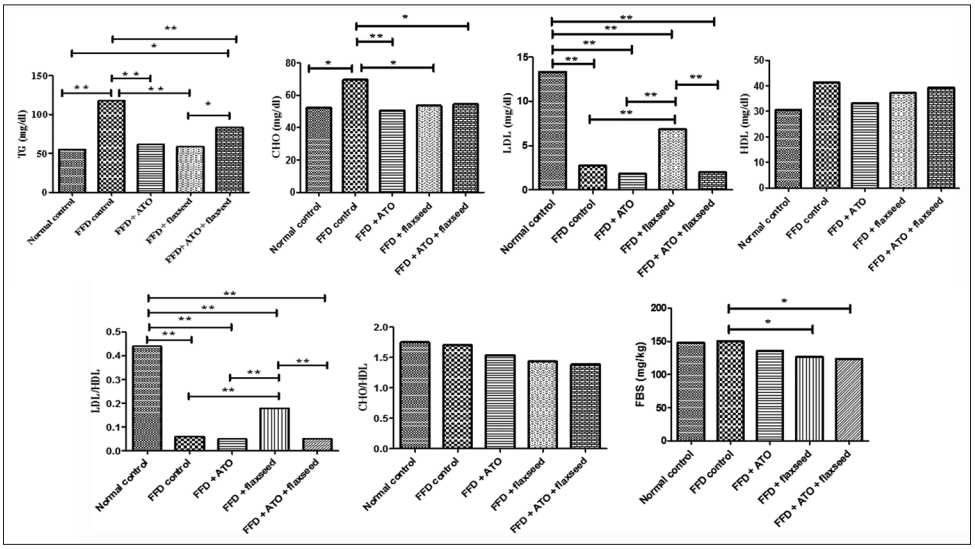

Figure 1: Mean values of biochemical parameters of rats fed a diet supplemented with atorvastatin (ATO), flaxseed, and ATO + flaxseed. Results are means ± SEM, analyzed by one-way ANOVA with the Tukey post hoc test. *$P < 0.05$, **$P < 0.01$

Table 2: Mean of rat weight in intervention period

Group	First week (g)	Second week (g)	Third week (g)	Fourth week (g)	Fifth week (g)	Sixth week (g)	Seventh week (g)	Eighth week (g)
Normal control	390.77	420.84	443.53	418.18	427.63	450.41	452.01	454.99
FFD control	384.96	378.19	392.36	439.59	427.79	441.47	449.91	461.36
FFD + ATO	334.58	348.67	352.04	353.93	353.59	362.12	360.59	363.83
FFD + flaxseed	337.33	333.09	336.79	337.70	333.31	342.57	344.17	357.46
FFD + ATO + flaxseed	381.40	366.88	377.73	384.13	386.20	387.14	388.63	394.25
P	0.034*	0.012*	0.005*	0.000**	0.000**	0.000**	0.000**	0.000**

*P<0.05, **P<0.001

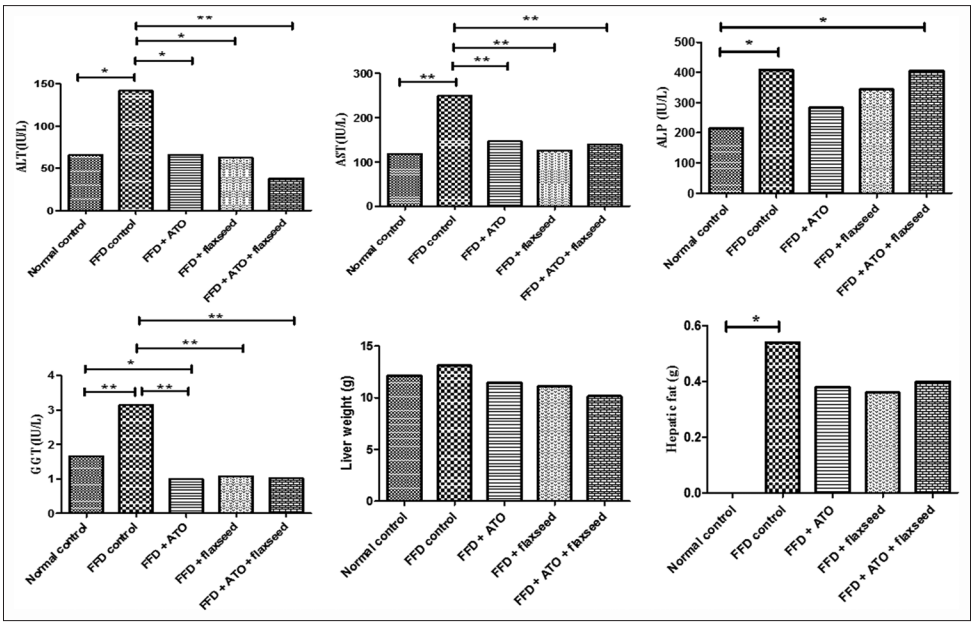

Figure 2: Mean values of liver weight, hepatic fat, and enzymes of rats fed a diet supplemented with atorvastatin and/or flaxseed. Results are means ± SEM, analyzed by one-way ANOVA with the Tukey post hoc test. *$P < 0.05$, **$P < 0.01$

Most drugs used to treat various diseases are metabolized in the liver. The result of this change can be dangerous metabolites that, if the enzymatic systems cause acute and chronic damage on the liver tissue could directly lead to liver toxicity.[21] The results of the study by Ji *et al.* proved the effect of ATO on reducing lipid profile, liver enzymes, inflammation, and improving liver function.[22]

Halalkhor studied the effect of flaxseed and combined exercises on total plasma antioxidant capacity and lipid peroxidation index in overweight women and concluded that consuming flaxseed as a positive antioxidant supplement on lipid peroxidation of cell membranes and prevention of destructive effects of free radicals.[23] Shavandi *et al*.[24] examined the effect of 10 weeks of aerobic exercise with flaxseed on lipids and C-Reactive Protein (CRP) in obese women. They concluded that flaxseed consumption significantly reduced CHO, TG, and HDL levels. In addition to liver enzymes, other biochemical indicators such as lipid profiles, which are usually increased in NAFLD, were also examined. Almost all indicators significantly confirmed the induction of NAFLD. The groups receiving ATO and flaxseed had improved lipid profile, and TG and CHO levels were significantly lower than the control group. ATO is one of the treatment drugs for hyperlipidemia. In fact, this drug reduces the level of lipid and, therefore, reduces the risk of some diseases, including cardiovascular disease. Although the main effect of ATO is lowering CHO, it also reduces TG.[25]

Flaxseed mainly contains omega-3 (ω-3) fatty acids that they reduce the serum concentration of TG and CHO. Omega-3 fatty acids and flaxseed lignan are effective in improving lipid pattern through various mechanisms.[26,27] Some of the effects of ω-3 fatty acids are exerted by the activation of adenosine monophosphate protein kinases. This enzyme acts as a metabolic sensor and balances cellular metabolism, including the oxidation and biosynthesis of fatty acids.[28,29] Omega-3 fatty acids also increase hepatic uptake and oxidation of free fatty acids (FFA) by increasing receptor peroxisome. As a result, the availability of FFA to TG decreases.[30] The results showed a significant decrease in FBS levels in FFD + flaxseed and FFD + ATO + flaxseed compared to the FFD control. The phytoestrogens in flaxseed appear to play an important role in controlling blood glucose and treating diabetes and obesity. Studies in humans and animals predict that lignans in flaxseed control blood glucose levels and insulin sensitivity.[31] It seems that the positive effect of lignans in secoisolariciresinol diglucoside on hypoglycemia can be because of the reduction in insulin resistance and oxidative reactions.[32] Lignan compounds can reduce peroxidation of membrane lipids by trapping hydroxyl radicals and keeping cell membranes intact, thus, increasing glucose uptake.[33] Another effect of flaxseed on lowering blood glucose can be attributed to the presence of ω-3 fatty acid alpha linoleic acid that can affect lipid metabolism and cellular insulin resistance.[34]

Conclusion

Appropriate therapeutic effect of ATO along with flaxseed supplementation can be used as a potentially suitable combination in the treatment of NAFLD. Although the effect of ATO alone and ATO in combination with flaxseed on lipid profile and liver enzymes are almost similar, but Due to the reducing effect of ATO + flaxseed on FBS, their simultaneous use is recommended.

However, further studies are needed to determine the exact mechanism of action and its effect on other complications of NAFLD.

Declarations

Ethics approval

The study was done according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996), and the present protocol was authorized by the local ethics committee (IR.GOUMS.REC.1397.274). All attempts were made to downgrade animal suffering and diminish the number of animals used.

Acknowledgements

The authors thank the staff of Shahid Mirghani Research Institute and Kavosh Laboratory in Golestan province.

Financial support and sponsorship

This study was supported by the Golestan University of Medical Sciences, Iran. (IR.GOUMS.REC.1397.274).

Conflicts of interest

There are no conflicts of interest.

References

1. El-Kader SMA, Ashmawy EMSE-D. Non-alcoholic fatty liver disease: The diagnosis and management. World J Hepatol 2015;7:846-58.
2. Osna NA, Donohue TM, Jr., Kharbanda KK. Alcoholic liver disease: Pathogenesis and current management. Alcohol Res 2017;38:147-61.
3. Adibi A, Maleki S, Adibi P, Etminani R, Hovsepian S. Prevalence of nonalcoholic fatty liver disease and its related metabolic risk factors in Isfahan, Iran. Adv Biomed Res 2017;6:47.
4. Eslami L, Isazadehfar K, Karami M, Eslami L, Rahmani-nia F, Nakhostinroohi B. The effect of 12 weeks regular physical activity and vitamin E in the treatment of non-alcoholic steatohepatitis: A pilot study. Govaresh 2015;20:57-65.
5. Qureshi K, Abrams GA. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol 2007;13:3540-53.
6. de Vries M, Westerink J, Kaasjager KHAH, de Valk HW. Prevalence of nonalcoholic fatty liver disease (NAFLD) in patients with type 1 diabetes mellitus: A systematic review and meta-analysis. J Clin Endocrinol Metab 2020;105:3842-53.
7. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018;24:908-22.
8. Spengler EK, Loomba R. Recommendations for diagnosis, referral for liver biopsy, and treatment of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Mayo Clin Proc 2015;90:1233-46.
9. Alonso C, Fernández-Ramos D, Varela-Rey M, Martínez-Arranz I, Navasa N, Van Liempd SM, *et al*. Metabolomic identification of subtypes of nonalcoholic steatohepatitis. Gastroenterology 2017;152:1449-61.e7.
10. Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: The central role of nontriglyceride fatty acid metabolites. Hepatology 2010;52:774-88.
11. Vargas JI, Arrese M, Shah VH, Arab JP. Use of statins in patients with chronic liver disease and cirrhosis: Current views and prospects. CurrGastroenterol Rep 2017;19:43.
12. Hu M, Cheung BMY, Tomlinson B. Safety of statins: An update. TherAdv Drug Saf 2012;3:133-44.
13. Zhang B, Zhang C, Zhang X, Li N, Dong Z, Sun G, *et al*. Atorvastatin promotes AMPK signaling to protect against high fat diet-induced non-alcoholic fatty liver in golden hamsters. Exp Ther Med 2020;19:2133-42.
14. Albracht-Schulte K, Kalupahana NS, Ramalingam L, Wang S, Rahman SM, Robert-McComb J, *et al*. Omega-3 fatty acids in obesity and metabolic syndrome: A mechanistic update. J Nutr Biochem 2018;58:1-16.
15. Gaspar RC, Veiga CB, Bessi MP, Dátilo MN, Sant'Ana MR, Rodrigues PB, *et al*. Unsaturated fatty acids from flaxseed oil and exercise modulate GPR120 but not GPR40 in the liver of obese mice: A new anti-inflammatory approach. J Nutr Biochem 2019;66:52-62.
16. Eslami Z, Mirghani SJ, Moghanlou AE, Norouzi A, Naseh H, Joshaghani H, *et al*. An efficient model of non-alcoholic fatty liver disease (NAFLD) versus current experimental models: Effects of fructose, fat, and carbon tetrachloride on NAFLD. Hepat Mon 2021;21.
17. Naik HS, Srilatha C, Sujatha K, Sreedevi B, Prasad T. Supplementation of whole grain flaxseeds (Linumusitatissimum) along with high cholesterol diet and its effect on hyperlipidemia and initiated atherosclerosis in Wistar albino male rats. Vet World 2018;11:1433-9.
18. Han KH, Rha SW, Kang HJ, Bae JW, Choi BJ, Choi SY, *et al*. Evaluation of short-term safety and efficacy of HMG-CoA reductase inhibitors in hypercholesterolemic patients with elevated serum alanine transaminase concentrations: PITCH study (PITavastatin versus atorvastatin to evaluate the effect on patients with hypercholesterolemia and mild to moderate hepatic damage). J Clin Lipidol 2012;6:340-51.
19. Ahsan F, Oliveri F, Goud HK, Mehkari Z, Mohammed L, Javed M, *et al*. Pleiotropic effects of statins in the light of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Cureus 2020;12:e10446.
20. Xu Q, Ming Z, Dart AM, Du XJ. Optimizing dosage of ketamine and xylazine in murine echocardiography. Clin Exp Pharmacol Physiol 2007;34:499-507.
21. David S, Hamilton JP. Drug-induced Liver Injury. US Gastroenterol Hepatol Rev 2010;6:73-80.
22. Ji G, Zhao X, Leng L, Liu P, Jiang Z. Comparison of dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. Lipids Health Dis 2011;10:23.
23. Halalkhor F. Effect of flaxseed supplementation and concurrent physical activity on total antioxidant capacity of the plasma and the lipid peroxidation index of overweight women. J Med Plants 2019;18:144-53.
24. Shavandi N, Saremi A, Sh S, Pooryamanesh L. Effect of ten-week aerobic training with flax seed supplementation on blood lipids profile and C-reactive protein in obese women. J Gorgan Univ Med Sci 2016;18:19-26.
25. Feingold KR. Cholesterol Lowering Drugs. In: Feingold KR, Anawalt B, Boyce A, *et al*., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK395573/. [Updated 2021 Mar 30].
26. Parikh M, Netticadan T, Pierce GN. Flaxseed: Its bioactive components and their cardiovascular benefits. Am J Physiol Heart Circ Physiol 2018;314:H146-59.
27. Prasad K. Flaxseed and cardiovascular health. J Cardiovasc Pharmacol 2009;54:369-77.
28. Srivastava RAK, Pinkosky SL, Filippov S, Hanselman JC, Cramer CT, Newton RS. AMP-activated protein kinase: An emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. J Lipid Res 2012;53:2490-514.
29. Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. J Clin Invest 2006;116:1776-83.
30. Hardwick JP, Osei-Hyiaman D, Wiland H, Abdelmegeed MA, Song B-J. PPAR/RXR regulation of fatty acid metabolism and fatty Acid ω-hydroxylase (CYP4) isozymes: Implications for prevention of lipotoxicity in fatty liver disease. PPAR Res 2009;2009.
31. Ebrahimi B, Nazmara Z, Hassanzadeh N, Yarahmadi A, Ghaffari N, Hassani F, *et al*. Biomedical features of flaxseed against different pathologic situations: A narrative review. Iran J Basic Med Sci 2021;24:551-60.
32. Adolphe JL, Whiting SJ, Juurlink BH, Thorpe LU, Alcorn J. Health effects with consumption of the flax lignansecoisolariciresinoldiglucoside. Br J Nutr 2010;103:929-38.
33. Lopez S, Bermudez B, Montserrat-de la Paz S, Jaramillo S, Varela LM, Ortega-Gomez A, *et al*. Membrane composition and dynamics: A target of bioactive virgin olive oil constituents. Biochim Biophys Acta 2014;1838:1638-56.
34. Jamilian M, Tabassi Z, Reiner Ž, Panahandeh I, Naderi F, Aghadavod E, *et al*. The effects of n-3 fatty acids from flaxseed oil on genetic and metabolic profiles in patients with gestational diabetes mellitus: Arandomised, double-blind, placebo-controlled trial. Br J Nutr 2020;123:792-9.