Original Article

Access this article online
Quick Response Code:

Website:
www.carcinogenesis.com
DOI:
10.4103/jcar.jcar_22_02_09

Effect of Phytosterols Fraction of Iraqi Rhus Coriaria in Experimentally Induced Hyperlipidemic Mice

Sarah Falah Hassan[1*], Ahmed R Abu Raghif [1], Enas Jawad Kadhim[2]

Abstract

The aim of this study is to investigate the antihyperlipidemic effect of Phytosterols fraction of Iraqi Rhus coriaria in high-fat diet (HFD)-fed mice. Animals were divided into four groups (n =8). The total duration of the study was 56 days split into two intervals. During the first 28-days interval, mice were administered with HFD, whereas during the second 28-days interval they were administered HFD plus phytosterol fraction (500mg/kg:p.o.) or the standard drug Atorvastatin (10mg/kg:p.o.). Phytosterols treatment to HFD-induced hyperlipidemic mice caused a high significant decrement in the levels of total cholesterol, triglycerides, LDL-C and VLDL-C. Moreover, phytosterols resulted in significant increase in the levels of HDL-C, whereas it caused remarkable decreases in ALT, AST and ALP enzymatic activities also in total serum bilirubin and albumin levels among hyperlipidemic mice. Besides that, Phytosterols treatment showed significant improvement in levels of tissue MDA and GSH in hyperlipidemic mice. Histopathological examination of hyperlipidemic mice showed a disorganized hepatic tissue, marked and diffused cytoplasmic fatty infiltration which was all ameliorated by Phytosterols administration. The results revealed that Phytosterols (500mg/kg;p.o.) possess potential ameliorating benefits against hyperlipidemia induced by HFD on lipid profile, liver function enzymes, oxidative stress parameters and hepatic histo-architecture. Further investigations are recommended and clinical trials are warranted to assess the efficacy and to fully dissect the mode of action underpinning the observed anti-hyperlipidemic effect of phytosterols.

Keywords:

Hyperlipidemic, Rhus coriaria, Phytosterols, lipid profile, Oxidative stress parameters.

Introduction

Hyperlipidemia A rise in one or more components of the lipid profile and/or lipoprotein levels in the blood characterizes the medical condition known as hyperlipidemia, which includes a number of inherited and acquired disorders. As an alternative, a more objective definition of hyperlipidemia includes levels of low-density lipoprotein (LDL), triglycerides (TG), total cholesterol, or lipoproteins that are more than 90 percent over the population average, or that are less than 10 percent below the average for HDL. A wide range of trials and studies have consistently shown that people with elevated levels of LDL cholesterol are more likely to develop atherosclerotic plaques and subsequent vascular disease.

On the other hand, high-density lipoprotein (HDL) cholesterol helps in regulating cholesterol levels to prevent imbalances that could lead to atherosclerotic vascular disease.

The LDL cholesterol goal of each patient is determined by their overall cardiovascular risk, and each patient's medical treatment should be individualized. Managing risk factors, such as hyperlipidemia, to prevent the risk for atherosclerotic cardiovascular disease is referred to as "primary prevention [1, 2].

The choice of pharmaceutical treatment is based on the type of lipid abnormality. The drugs That Primarily Lower Atherogenic Cholesterol (LDL) are statins (the HMG-CoA reductase inhibitors), ezetimibe, resins, PCSK9 inhibitors, and niacin. The fibric acid derivatives (Fibrates), Niacin (nicotinic acid) and Omega - 3 Polyunsaturated Fatty Acids

[1]Department of pharmacology, collage of medicine, Al-Nahrain University, Baghdad, Iraq.
[2]Department of Pharmacognacy and Medicinal Plants, Collage of pharmacy, University of Baghdad, Baghdad, Iraq.

Address for correspondence:
Sarah Falah Hassan, Department of pharmacology, collage of medicine, Al-Nahrain University, Baghdad, Iraq.
E-mail: sue.alshammari@gmail.com

Submitted: 16-Dec-2022
Revised: 08-Aug-2023
Accepted: 23-Sep-2023
Published: 11-Oct-2023

How to cite this article: Hassan S F, Raghif A R A, Kadhim E J. Effect of Phytosterols Fraction of Iraqi Rhus Coriaria in Experimentally Induced Hyperlipidemic Mice. J Carcinog 2023;22(2):73-80

(PUFA) are most effective at lowering TG and VLDL concentrations and increasing HDL cholesterol concentrations [3, 4].

Most of these drugs are linked with a number of adverse side effects including muscle-related complaints such as (myalgia, cramp, myopathy and rhabdomyolysis), flushing, dry skin, diarrhea, abnormal liver function, gall stone, gastric irritation and may provoke renal failure. Therefore, the development of promising new lipid-lowering agents alternatives to allopathic drugs is needed [5]. Rhus coriaria (Sumac) is a commonly used as a spice in the Middle East [6].

Sumac is the common name for the spice product of the Rhus coriaria plant, which belongs to the family Anacardiaceae and the genus Rhus [7]. It is grown in north of Iraq, like Sulaimani, Kirkuk. Erbil, Duhok, and Ninawa. The plants have many pharmacological properties, including antimicrobial, wound healing, antidepressant, diabetic, antirheumatic, antiseptic, anti-inflammatory, antifungal, and antiulcer properties [8, 9].

Phytosterols: Also known as plant sterols and stanols, are natural steroids that can be found in a wide variety of plant parts, such as the roots, stems, leaves, flowers, fruits, and whole grasses [10]. They play a significant role in the cell membranes of plant cells. Phytosterols have ability to lower plasma levels of cholesterol and low-density lipoprotein cholesterol (LDL-C) and may have clinical application for the prevention of cardiovascular diseases (CVDs) and non-alcoholic fatty liver disease (NAFLD) [11]. The present study was planned to investigate the antihyperlipidemic effect of Phytosterols fraction of Iraqi Rhus coriaria in mice [12].

Material and Methods

Plant material

The whole plant of *Rhus coriaria* fruit was collected from the north of Erbil in rawendos in April 2020. The collected plant cleaned, dried at room temperature in a shade area, then pulverized by mechanical mills and weighted[6, 13].

Plant experiment work

It was done in the phototherapy laboratory of pharmacological Department/ Pharmacy College of Baghdad University. The plant *of Rhus coriaria* were collected and treated as follows:

Extraction and fractionation of different active constituents

A (400gm) of shade-dried coarsely powdered plant materials were defatted with hexane for 24 hours then allowed to dry at room temperature (25 °C). The defatted plant materials were extracted with (2 L) of 85% ethanol in soxhlet apparatus until complete exhaustion. The alcoholic extract was evaporated to dryness, under reduced pressure at a temperature not exceeding 40 °C to give a dark greenish-yellow residue designated as a crude fraction 1 (F-A). Crude fraction was acidified with 300ml of 5% hydrochloric acid to pH 2 and partitioned (three times) with equal volume of ethyl acetate to get two layers (aqueous acidic and ethyl acetate layer), this step is necessary to get rid from any basic com- pound found in the crude extract [14].

The ethyl acetate layer of the original alcoholic extract (crude fraction) was evaporated to dryness under reduced pressure and basify with 300ml of 5% sodium hydroxide to pH 10 and extracted with chloroform in the separatory funnel to get two layers, the aqueous basic layer which was separated, evaporated to dryness and acidify with 5% HCL to pH 2 then extracted with ethyl acetate to get fraction designated as fraction 2 (F-B) phenolics and fraction 3 (F-C) neutral phytosterols [15].

Preliminary qualitative phytochemical analysis

Chemical tests were carried out using the ethanol extract from plant and its methanol fraction; we used standard procedures to identify the active constituents [16]. Tests for steroids: H_2SO_4 test: The development of a greenish color was considered as indication for the presence of steroids, when 2 ml of the extract and fractions were treated with sulphuric and acetic acids.

Identification and characterization of the isolated phytosterol fraction by using HPLC

High performance liquid chromatography or commonly known as HPLC is an analytical technique used to separate, identify or quantify each component in a mixture. The mixture is separated using the basic principle of column chromatography and then identified and quantified by spectroscopy.

Animal experiment work

The study was conducted from September 2022 through July 2023 at the department of pharmacology–College of Medicine /AL Nahrain University. The experiments were approved by the Ethical Committee at the College of Medicine /AL Nahrain University.

Thirty-two apparently healthy, albino male mice 2-3 months' age, weight about 20-30g, were obtained from the Higher Institute for Diagnosis of Infertility and Assisted Reproduction Techniques/AL Nahrain University. The animals were acclimatized in standard environmental conditions and fed with food and water *ad libtum* for a week before commencement of the experiment.

Induction of hyperlipidemia

High Fat Diet (2% cholesterol and 1% peanut butter) was added to the standard diet (Seeds as sunflower and groundnut, Cereals, Fruits as grapes and apple, Vegetables, Vitamin as A, E and D_3) in order to induce hyperlipidemia for 28 days [17].Body weights were

measured weekly for all groups.

Extract administrant

phytosterol fraction of Iraqi *Rhus coriaria* was administered via intragastric tube for 28 days [17].

Preparation of drugs

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

Experimental design

The mice were divided into 4 groups, 8 mice each group [18]:

Group 1 (normal): standard diet for 28 days.
Group 2 (induced): High-Fat Diet HFD for 28 days.
Group 3 (treated): HFD for 28 days then atorvastatin 10 mg/kg for further 28 days.
Group 4: HFD for 28 days then phytosterols fraction of *Rhus coriaria* at dose of 500 mg/kg for further 28 days.

Blood collection

Mice were kept fasting for 24 hrs and blood samples were extracted via heart puncture. This was followed by centrifugation at 3000 rpm for15 min at room temperature. The obtained serum was used for the biochemical analysis of lipid profile indices and liver function enzymes.

Biochemical analysis

The biochemical analysis of lipid profile indices and liver function enzymes

The standard diagnostic kits were used for estimation of serum total cholesterol (TC), triglyceride (TG), LDL, VLDL, HDL, ALT, AST, ALP, and total serum bilirubin (TSB)levels by using auto analyzer [19].

Measurement of oxidative stress

At the end of the experimental period and after blood sampling, animals were sacrificed and the liver organ was removed for analysis and divided into two parts for assay. For oxidative stress measurement, the samples of MDA and glutathione were prepared from homogenization of first part of liver tissue, after that, centrifuged homogenates for 15 minutes 5000 rpm. The standard diagnostic kits were used for assay of tissue MDA and GSH level. This assay utilizes the competitive inhibition enzyme immunoassay technique [20, 21].

Histopathological examination

After the animals were sacrificed, the liver was harvested from the mice and instantly fixed in a 10% formalin solution, then. embedded with 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 [22, 23].

Results

Changes in lipid profile among the experimental animal groups:

According to the findings in Table 1, in the induced(non-treated) group it can be shown that feeding mice a high-fat diet led to hyperlipidemia. Both groups treated with atorvastatin (10mg/kg; p.o.) and phytosterol fraction (500mg/kg; p.o.) showed statistically significant decrease in the serum level of cholesterol, TG, LDL, VLDL with a significant increase in the level of HDL ($p<0.05$) in both groups when compared with the induced (non-treated) group.

In comparison with the atorvastatin-treated group, the phytosterol fraction-treated group showed a statistically highly significant($p<0.001$) decrease in TC, TG and VLDL with non-significant differences in LDL and HDL.

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**	238.67 ± 11.05*	209.17 ± 16.88**	47.73 ± 2.21*	23.46 ± 2.32*
Atorvastatin (10mg/kg/day)	171.6 ± 3.56 a**	189.88 ± 14.86 a**	99.68 ± 6.25 a**	38.35 ± 3.32 a**	29.82 ± 2.27 a*
Phytosterol (500mg/kg/day)	158.76 ± 1.01 a,b**	173.03 ± 8.36 a,b**	94.9 ± 3.22 a**b^{NS}	34.61 ± 1.67 a,b**	29.25 ± 3.34 a*b^{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) represents a comparison between the control (apparently healthy) group and the 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 the Induced treated with phytosterol fraction.

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

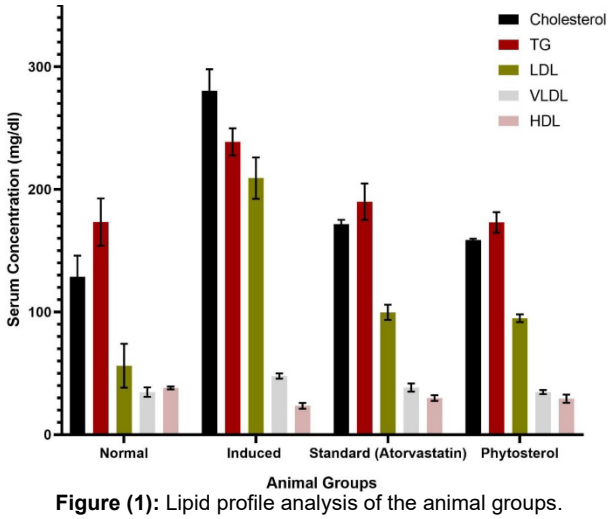

Figure (1): Lipid profile analysis of the animal groups.

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

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 phytosterol fraction showed statistically significant decrease in the serum level of ALP, ALT, AST, albumin, and TSB levels compared to the induced (non-treated) group (p<0.05). In comparison with the atorvastatin-treated group, the phytosterol fraction-treated group shows a statistically highly significant(p<0.001) decrease in ALT and AST with non-significant differences in ALP, TSB, and albumin levels 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*	44.94 ± 2.07*	50.87 ±1.95**	6.61 ± 0.29*	1.69 ± 0.12*
Atorvastatin (10mg/kg/day)	37.02 ± 2.06 a**	38.25 ± 1.72 a**	31.34 ± 5.26 a**	5.51 ± 0.23 a**	0.8 ± 0.07 a**
Phytosterol (500mg/kg/day)	34.29 ± 2.45 a**, b*	35.74 ± 1.35 a,b**	30.71 ± 3.55 a**b^{NS}	5.03 ± 0.38 a**b^{NS}	0.75 ± 0.12 a**b^{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) represents a comparison between the control (apparently healthy) group and the 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 the Induced treated with phytosterol fraction. **AST**= Aspartate Aminotransferase, **ALT**= Alanine Transaminase, **TSB**= total serum bilirubin, **ALP**= Alkaline Phosphatase, **SD**= standard deviation.

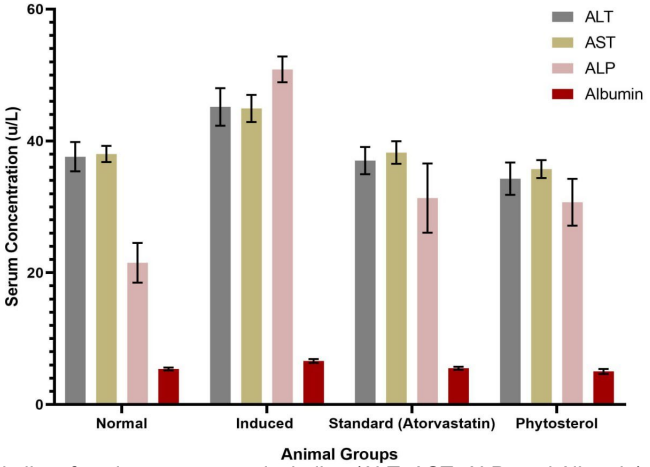

Figure (2): Changes in liver function parameters including (ALT, AST, ALP, and Albumin) among animal groups.

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, **SD**= standard deviation

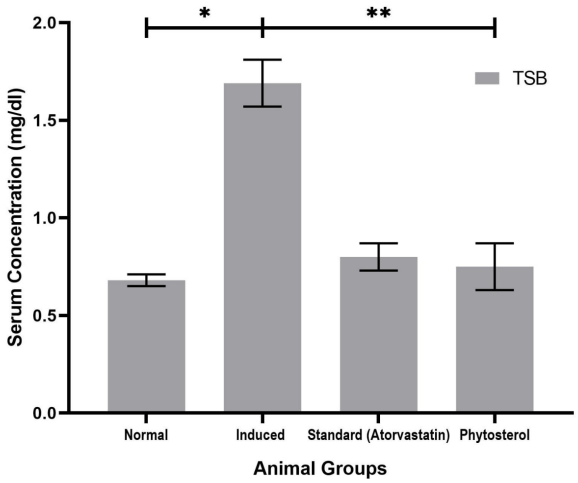

Figure (3.3): Changes in total serum bilirubin (TSB) levels among animal groups as a parameter of liver function.
Results are expressed as mean + SD, (n=8), and the level of significance was set at (P≤0.05). * Represents significance (P≤0.05 & ≤0.01), ** represents high significance (P≤0.001).

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 phytosterol fraction-treated groups show a significant decrease in MDA with a significant increase in GSH when compared with the induced (non-treated) group.

The phytosterol fraction-treated group shows a highly significant decrease in MDA with a highly significant increase in GSH in compared to 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*	11.65 ± 0.78**
Atorvastatin (10mg/kg/day)	334.41 ± 11.15 a**	26.51 ± 2.85 a**
Phytosterol (500mg/kg/day)	227.53 ± 3.45 a,b**	32.85 ± 2.03 a, b**

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) represents a comparison between the control (apparently healthy) group and the 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 the Induced treated with phytosterol fraction. **MDA**= Malondialdehyde, **GSH**= Glutathione peroxidase, **SD**= standard deviation

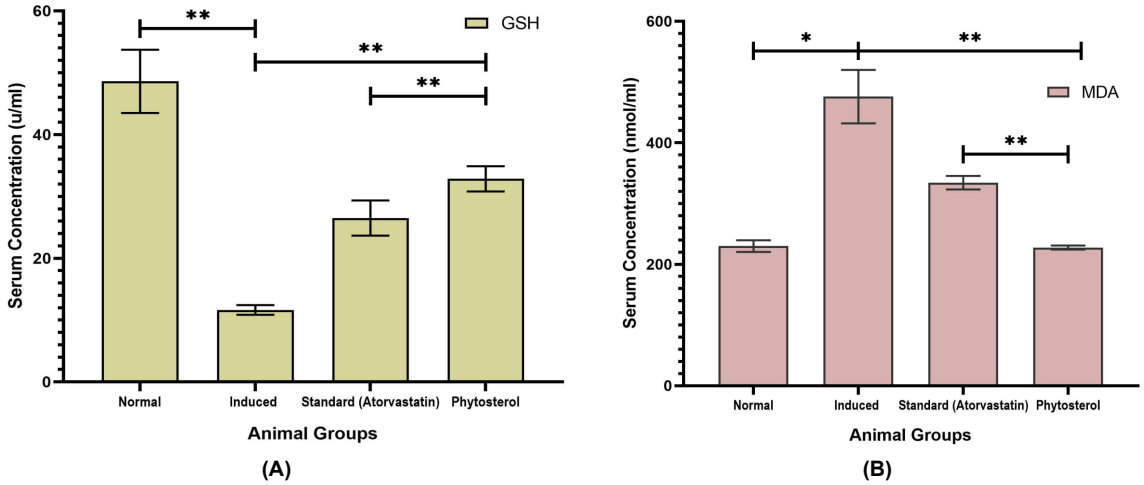

Figure (3.4): Changes in oxidative stress markers among animal groups. (A) changes in the level of serum glutathione (GSH), (B) changes in the level of malondialdehyde (MDA)
Results are expressed as mean + SD, (n=8), and the level of significance was set at (P≤0.05).
* Represents significance (P≤0.05 & ≤0.01), ** represents high significance (P≤0.001).

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 induced non-treated group when compared with the control (apparently healthy) group, as shown in image 4.1

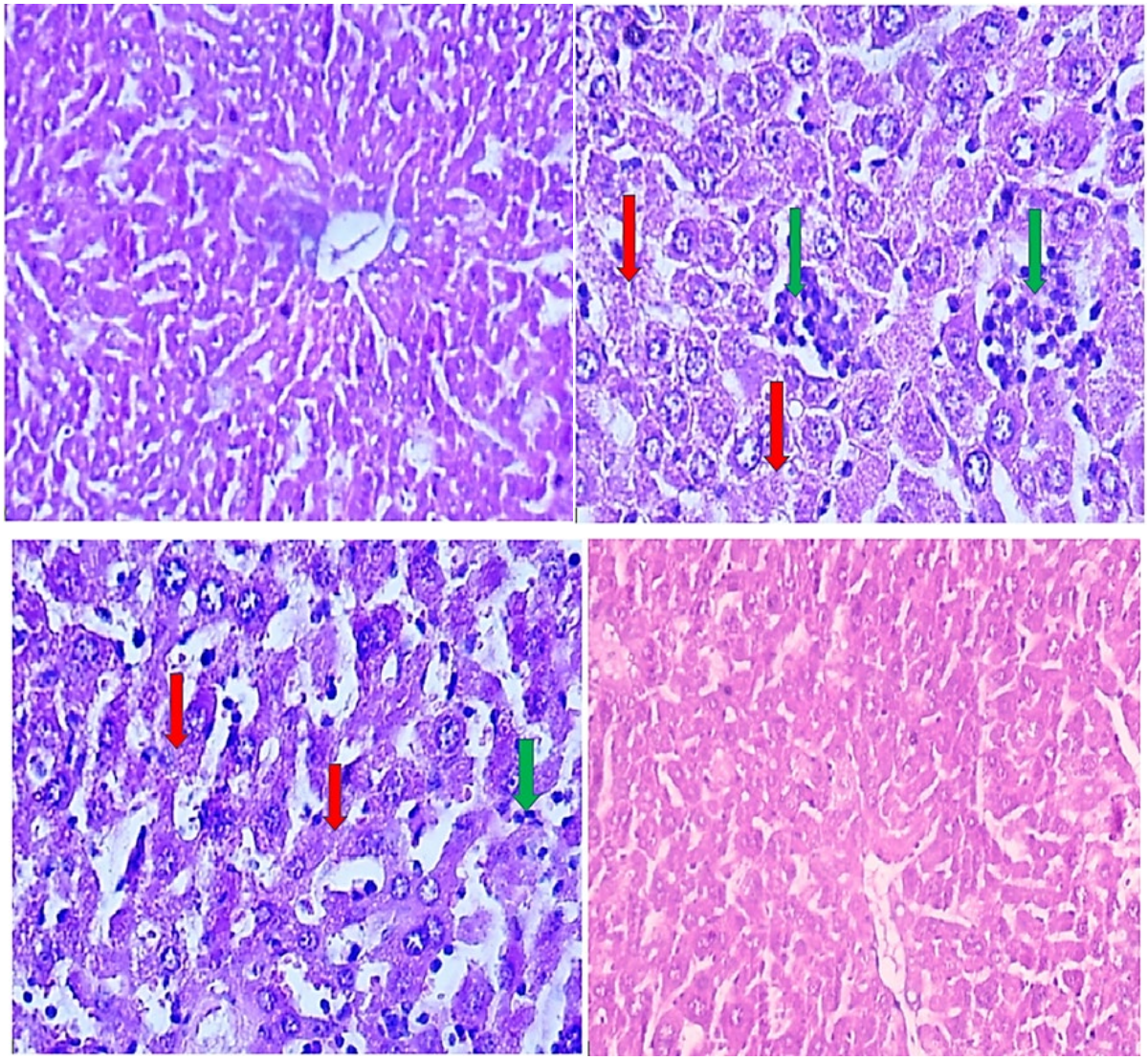

Image (4.1): Histopathological examination of mice liver. (A) normal control group, (B) induced non-treated group, (C) induced treated with atorvastatin, (D) induced treated with phytosterol fraction

Red arrow-level of steatosis, Green arrow-level of inflammation (H&E stain, 400X & 200X)

Discussion

Hyperlipidemia is the pathological state characterized by high levels of serum cholesterol and triglycerides and it is a major risk factor for cardiovascular disease and it present in most patients with myocardial infarction [20].

Currently available practical strategies to control hyperlipidemia include the reduction in both productions of lipids, and their gut absorption using synthetic therapeutic and herbal agents such as statins, bile acid sequestrants and fibrates. However, the use of these agents faced a series of negative and recurrent side effects, most notably elevated risk of gallstone formation, myopathy, and rhabdomyolysis. Hence, developing original and effective antihyperlipidemic therapeutic agents with minimal undesired side effects is urgently required [24]. The opportunity of treating variety of pathological conditions using herbal products and medicinal plants is a rising trend in recent years as they had as safer, well-tolerated therapeutic spectrum with minimal side effects in comparison to synthetic drugs [25].

In this study, the extraction process of the plant done sequentially according to solubility and polarity of the active constituents which has been extracted and isolated to be ready for use in the experiments to assess their hypolipidemic activity [26].

The current study revealed that serum levels of (cholesterol, TG, LDL, and VLDL) showed a marked significance elevation among induced (non-treated)

animals while serum level of HDL show significant decrease in this group. These changes observed in hyperlipidemic group can be explained by the high fat diet induced hyperlipidemia by disturbing lipid metabolism, mainly by decreasing β-oxidation and increasing cholesterol synthesis and oxidative stress by decreasing free radical scavenger enzyme gene expression [27].The dramatic increase in parameters of lipid profile in the current study is attributed to the way in which mice respond to a high cholesterol diet. As the cholesterol intake increase, bile acids reabsorption will also increase which leads to increase its liver uptake [28] Also, increase serum concentrations of total cholesterol, triacylglycerol, and low-density lipoprotein cholesterol in addition to decreased lipoprotein lipase activity, accompanied by a depressed antioxidant defense system [29]. Phytosterol fraction in the current study was significantly able to reduce serum levels of (cholesterol, TG, LDL, and VLDL) in a comparable result to atorvastatin with significant elevation in HDL. The reduction of TC, TG, LDL and VLDL through inhibition of pancreatic lipase which responsible of liberation of triglyceride into fatty acids and glycerol [30]. The inhibition of this enzyme by phytosterols could be a key for controlling hyperlipidemia and obesity through suppression and delay of digestion and absorption of TG [31]. The successful treatment with statin is agreed by [32] when they used HFD-induced hyperlipidemia in one of mice group, a significant rise in a lipid profile marker.

Serum levels of liver enzymes (AST, ALP and ALT) in the current study were significantly increased among the induced (non-treated) group in comparison with the apparently healthy control group due to the disturbance of lipid metabolism because of high fat intake, resulting in accumulation of TG in liver and an increased increment of the liver index, and hepatic steatosis occurred since the liver has a crucial role in regulating plasma lipid level [33]. also due to excess reactive oxygen species (ROS) production in the mitochondria as a result of lipid overload. The ROS cause hepatic inflammation by activation cytokines.

Consequently, ROS and inflammatory cytokines with the excess lipid infiltration, resulted in a condition of liver toxicity. Hence, the liver function markers (AST, ALP and ALT) showed leakage in the serum and indicated liver damage of the hepatic cells. Animal groups treated with atorvastatin, statistically significant reduction in levels of liver enzymes. Statins have strong lipid lowering effect which exerted through inhibition of hepatic (HMG-COA reductase inhibitor) which consider the rate-limiting step of cholesterol synthesis [34]. Phytosterol chemically acts as an antioxidant, a modest radical scavenger [35]. Malondialdehyde (MDA), which is a product of lipid peroxidation or reaction of oxygen with unsaturated lipids [36], were highly significant increase in induced (hyperlipidemic) mice. Glutathione is an intracellular hydrophilic antioxidant and most important endogenous defense mechanism against oxidative stress in body and it plays essential role in maintenance of membrane protein -SH groups in the reduced form, the oxidation of which can cause altered cellular function and structure [37].

phytosterol fraction was able to reduce level of MDA while elevating the level of GSH in comparison to atorvastatin-treated group. This result accords well with Zhao et al study, who had indicated that Phytosterols increased serum GSH hepatic activities of Partridge Shank chickens at 21 days. Furthermore, [38] reported that dietary β-sitosterol increased intestinal GSH and CAT activities, and reduced MDA content in broilers. histopathological examination of mice liver treated with phytosterols showed nearly normal liver tissue. Mirenayat et al, [39] study found that histopathological observations had demonstrated that RC phytosterol extract ameliorated grade-1 hepatic steatosis induced by high fat diet and introducing RC as a future potential therapeutic agent in treating different grades of hepatic steatosis and reducing oxidative damages of a high fat diet on the liver.

References

1. M. F. Hill and B. Bordoni, "Hyperlipidemia," in *StatPearls [Internet]*: StatPearls Publishing, 2022.
2. A. H. J. ABDULLAH, S. A. ABDULLAH, M. S. ZIYADI, and N. A. AL-FAIZ, "Investigation of changes in the fish assemblage building and abundance in the Garmat Ali River, Southern Iraq," *FishTaxa-Journal of Fish Taxonomy,* vol. 25, 2022.
3. P. Kuijpers, "History in medicine: The story of cholesterol, lipids and cardiology," *J. Cradiol. Pract,* vol. 19, pp. 1-5, 2021.
4. Bertram G. Katzung, Marieke Kruidering-Hall, and A. J. Trevor., *Pharmacology Examination & Board Review*. Twelfth Edition Mc Graw Hill 2019.
5. 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.
6. G. Gastélum-Cuadras, "PHYSICAL CAPACITY HERITABILITY FROM PARENTS TO CHILDREN: COMPUTERISED DERMATOGLYPHICS," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte,* vol. 22, no. 85, 2022.
7. K. Sakhr and S. El Khatib, "Physiochemical properties and medicinal, nutritional and industrial applications of Lebanese Sumac (Syrian Sumac-Rhus coriaria): A review," *Heliyon,* vol. 6, no. 1, 2020.
8. E. Silva-Júnior *et al.*, "Phytochemical compounds and pharmacological properties from Schinus molle Linnaeus and Schinus terebinthifolius Raddi (Anacardiaceae)," *Journal of Chemical and Pharmaceutical Research,* vol. 7, no. 12, pp. 389-393, 2015.
9. S. Derese, E. Guantai, Y. Souaibou, and V. Kuete, "Mangifera indica L.(anacardiaceae)," in *Medicinal Spices and Vegetables from Africa*: Elsevier, 2017, pp. 451-483.
10. L. Khrema, A. Saad, A. Alnesser, and C. Capapé, "First record of Baillon's wrasse Symphodus bailloni (Labridae) in the Eastern Mediterranean Sea coast of Syria," *FishTaxa-Journal of Fish Taxonomy,* vol. 25, 2022.
11. X. Li, Y. Xin, Y. Mo, P. Marozik, T. He, and H. Guo, "The bioavailability and biological activities of phytosterols as

modulators of cholesterol metabolism," *Molecules,* vol. 27, no. 2, p. 523, 2022.
12. A. A. Soares Costa *et al.*, "Lung cancer biomarkers. A literature review," *Jornal Brasileiro de Patologia e Medicina Laboratorial,* vol. 58, 2022, doi: 10.1900/JBPML.2022.58.415.
13. R. Pedrero-Tomé, M. Marrodán, and M. Cabañas, "ANTHROPOMETRIC PROFILE OF THE MADRID WOMEN'S SOCCER TEAM U-16 AND U-18," *Revista Internacional de Medicina y Ciencias de la Actividad Física y del Deporte,* vol. 22, no. 85, 2022.
14. J. Mondal, P. Samui, and A. N. Chatterjee, "Effect of SOF/VEL antiviral therapy for HCV treatment," *Letters in Biomathematics,* vol. 8, no. 1, pp. 191–213, 2021, doi: 10.30707/LiB8.1.1682013528.144161.
15. B. Fried and J. Sherma, "Thin layer chromatography, 4th edition revised and expanded," Tylor and France, 2005.
16. H. Wagner and S. Bladt, *Plant drug analysis: a thin layer chromatography atlas*. Springer Science & Business Media, 1996.
17. 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.
18. 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.
19. W. A. Coakly, "Handbook of Automated Analysis," *Mercel Dekker,* p. 61, 1981.
20. 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.
21. J. Richie Jr, L. Skowronski, P. Abraham, and Y. Leutzinger, "Blood glutathione concentrations in a large-scale human study," *Clinical chemistry,* vol. 42, no. 1, pp. 64-70, 1996.
22. T. Swinscow, *Statistics at Square One, 9th Ed (Revised by Campbell MJ)*. BMJ Publishing Group, London, Pages: 69-74 & 96, 1997.
23. A. B. Hauser, L. I. Accordi, M. V. S. Boganha, D. A. Hofelmann, C. G. Meissner, and A. N. d. A. Buchmann, "Comparison between visual inspection of turbidity and quantitative determination of triglycerides in plasma bags," *Jornal Brasileiro de Patologia e Medicina Laboratorial,* vol. 58, 2022, doi: 10.1900/JBPML.2022.58.421.
24. S. H. Aladaileh *et al.*, "Antihyperlipidemic and antioxidant effects of Averrhoa carambola extract in high-fat diet-fed rats," *Biomedicines,* vol. 7, no. 3, p. 72, 2019.
25. M. S. A. Mehraban, O. Tabatabaei-Malazy, R. Rahimi, M. Daniali, P. Khashayar, and B. Larijani, "Targeting dyslipidemia by herbal medicines: A systematic review of meta-analyses," *Journal of ethnopharmacology,* vol. 280, p. 114407, 2021.
26. S. Zhao and D. Zhang, "Supercritical fluid extraction and characterisation of Moringa oleifera leaves oil," *Separation and Purification Technology,* vol. 118, pp. 497-502, 2013.
27. V. S. Nunes, P. M. Cazita, S. Catanozi, E. R. Nakandakare, and E. C. Quintão, "Cholesterol metabolism in mice models of genetic hypercholesterolemia," *Journal of physiology and biochemistry,* vol. 76, pp. 437-443, 2020.
28. 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.
29. A. E. Ashour *et al.*, "Thymoquinone inhibits growth of human medulloblastoma cells by inducing oxidative stress and caspase-dependent apoptosis while suppressing NF-κB signaling and IL-8 expression," *Molecular and cellular biochemistry,* vol. 416, pp. 141-155, 2016.
30. C. De Oliveira *et al.*, "Pancreatic triglyceride lipase mediates lipotoxic systemic inflammation," *The Journal of clinical investigation,* vol. 130, no. 4, pp. 1931-1947, 2020.
31. S. Sut and S. Dall'Acqua, "Food-derived nutraceuticals for hypercholesterolemia management, mode of action and active ingredients," *Food Bioscience,* p. 102866, 2023.
32. S. M. Firdous, S. Hazra, S. C. Gopinath, G. E. El-Desouky, and M. A. Aboul-Soud, "Antihyperlipidemic potential of diosmin in Swiss Albino mice with high-fat diet induced hyperlipidemia," *Saudi journal of biological sciences,* vol. 28, no. 1, pp. 109-115, 2021.
33. C. Fernández-Hernando, Y. Suárez, K. J. Rayner, and K. J. Moore, "MicroRNAs in lipid metabolism," *Current opinion in lipidology,* vol. 22, no. 2, p. 86, 2011.
34. J. Liu, B. K. Gillard, D. Yelamanchili, A. M. Gotto Jr, C. Rosales, and H. J. Pownall, "High Free Cholesterol Bioavailability Drives the Tissue Pathologies in Scarb1−/− Mice," *Arteriosclerosis, Thrombosis, and Vascular Biology,* vol. 41, no. 10, pp. e453-e467, 2021.
35. J. Solivan-Rivera *et al.*, "A neurogenic signature involving monoamine Oxidase-A controls human thermogenic adipose tissue development," *Elife,* vol. 11, p. e78945, 2022.
36. C. J. L. Busch *et al.*, "Malondialdehyde epitopes are sterile mediators of hepatic inflammation in hypercholesterolemic mice," *Hepatology,* vol. 65, no. 4, pp. 1181-1195, 2017.
37. T. S. Nagapan, W. N. Lim, D. F. BaSri, and A. R. Ghazali, "Oral supplementation of L-glutathione prevents ultraviolet B-induced melanogenesis and oxidative stress in BALB/c mice," *Experimental Animals,* vol. 68, no. 4, pp. 541-548, 2019.
38. Q. Cheng, Y. Zhang, Q. Lin, Y. Tian, and Y. Bao, "Study on the antioxidant activity of β-sitosterol and stigmasterol from Sacha Inchi oil and Prinsepia oil added to walnut oil," *Food Science and Technology,* vol. 42, 2022.
39. F. S. Mirenayat, Z. Hajhashemy, M. Siavash, and P. Saneei, "Effects of sumac supplementation on metabolic markers in adults with metabolic syndrome: a triple-blinded randomized placebo-controlled cross-over clinical trial," *Nutrition Journal,* vol. 22, no. 1, p. 25, 2023.