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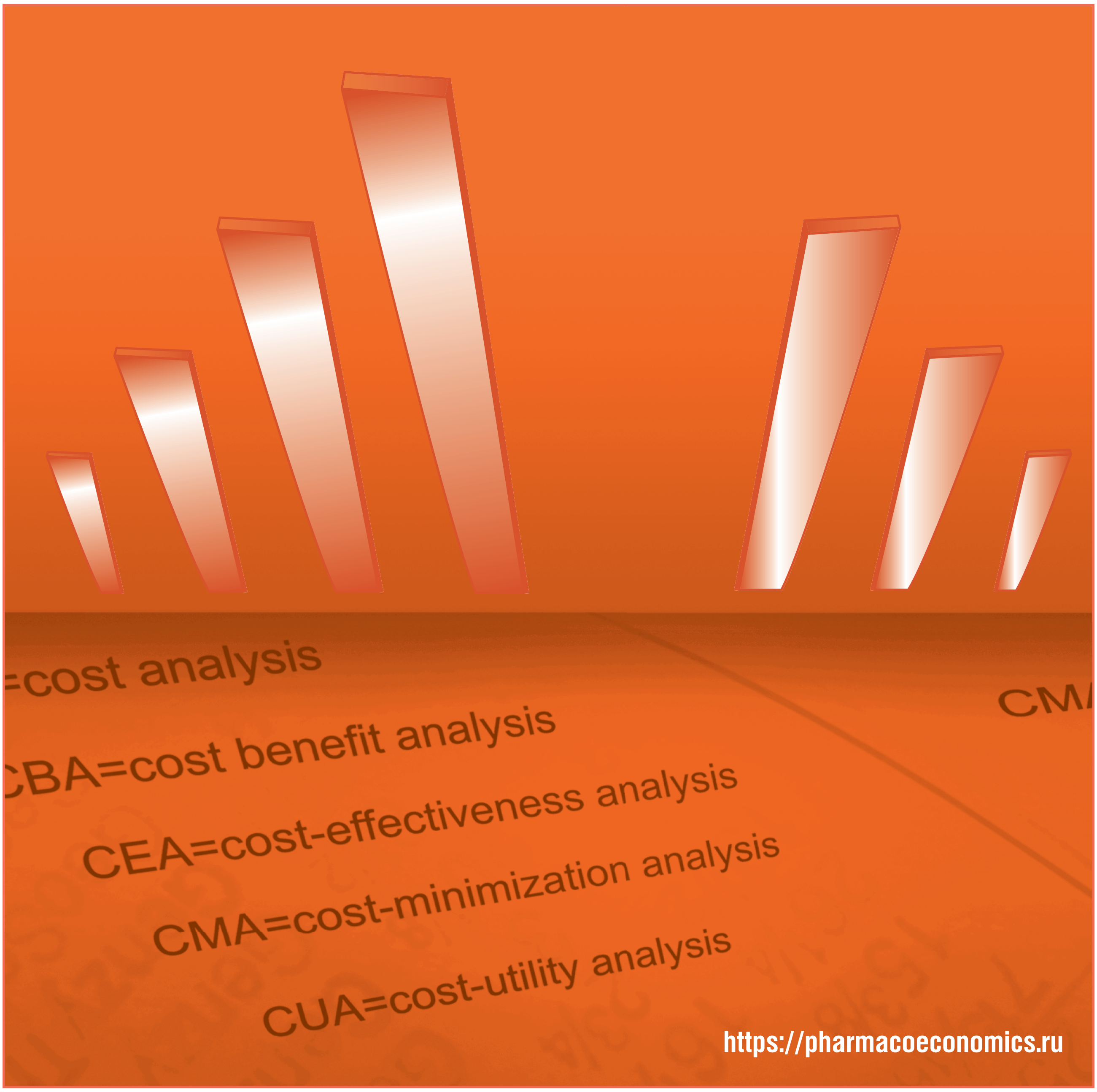

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Amelioration of histopathological damage in mice with induced hyperlipidemia by terpenes and phytosterols extracted from Iraqi chickpea

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ABSTRACT

Objective: To compare the amelioration effects of terpenes and phytosterols extracted from Iraqi chickpea on histopathological damage in mice fed on high-fat diet (HFD).

Material and methods. Whole chickpea plants of the *Fabaceae* family were collected during the flowering period in the northern area of Erbil. The collected plants were cleaned, dried in a shaded area at room temperature, pulverized with mechanical mills, and weighed. Experiments were conducted from October 2021 to April 2022. The research involved 32 healthy albino male mice 2–3 months of age, weighing about 20–30 g. The animals were provided by the Higher Institute for Diagnosis of Infertility and Assisted Reproduction Techniques. One week prior to the onset of the experiment, the animals were acclimatized to standard environmental conditions; food and water were provided *ad libitum*. HFD (2% cholesterol and 1% peanut butter) was added to the standard diet for 28 days to induce hyperlipidemia. In all experimental groups, the body weight was measured weekly. The terpenes and phytosterol fraction extracted from Iraqi chickpea was administered for 28 days via an intragastric tube. Mice were kept fasting for 24 hours and blood samples were extracted via heart puncture. Standard diagnostic kits and an automated analyzer were used for estimation of serum total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), high-density lipoprotein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total serum bilirubin (TSB) levels. The animals were sacrificed, then their liver and heart organs were removed for assay.

Results. Histopathological examination of the hyperlipidemic mice group showed a marked and diffused cytoplasmic fatty infiltration of hepatic and cardiac cells. These effects were successfully ameliorated by administration of terpenes and phytosterols, although treatment with terpenes seemed to be more effective than that with phytosterols. In comparison with phytosterol treatment, terpene treatment in HFD-induced hyperlipidemic mice led to an improvement in lipid profiles, manifested in a highly significant decrease ($p \leq 0.05$) in the serum levels of TC (142.40±14.43 mg/dl), TG (98.89±8.71 mg/dl), LDL (79.43±15.14 mg/dl), and VLDL (19.78±1.74 mg/dl); with a highly significant ($p \leq 0.05$) decrease in the liver enzymatic activities of (ALT (19.19±1.36 U/l), AST (15.98±1.3 U/l), and ALP (20.99±4.43 U/l)) and TSB levels (1.60±0.12 mg/dl) (highly significantly differences with $p \leq 0.05$). Terpenes also led to a statistically significant improvement in the level of tissue malondialdehyde and glutathione in hyperlipidemic mice ($p \leq 0.05$).

Conclusion. In comparison with phytosterols, terpenes exhibit a highly significant ameliorating effect in HFD-induced hyperlipidemia, improving the state of liver and heart tissue, lipid profile, liver function enzymes, and oxidative stress parameters. Further research should assess the efficacy of terpenes and phytosterols and to elucidate the mechanism of their anti-hyperlipidemic action.

KEYWORDS

hyperlipidemia, chickpea, terpenes, phytosterols, lipid profile, oxidative stress

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Уменьшение патогистологических повреждений у мышей с индуцированной гиперлипидемией под воздействием терпенов и фитостеринов из иракского нута

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РЕЗЮМЕ

Цель: сравнить влияние терпенов и фитостеринов, содержащихся в иракском нуте, на уменьшение патогистологических повреждений у мышей, получавших высокожировой рацион питания.

Материал и методы. Цельные растения нута семейства *Fabaceae* были собраны во время цветения на севере провинции Эрбиль (Ирак). Растения очищали, сушили при комнатной температуре в затененном месте, затем измельчали механическими мельницами и взвешивали. Эксперименты проводились в период с октября 2021 г. по апрель 2022 г. на 32 здоровых самцах мышей-альбиносов весом 20–30 г. в возрасте 2–3 мес, предоставленных Институтом диагностики бесплодия и вспомогательных репродуктивных технологий. Животных акклиматизировали к стандартным условиям окружающей среды, обеспечивая кормом и водой *ad libitum* в течение 1 нед до начала эксперимента. Гиперлипидемия была индуцирована добавлением высокожировой пищи (2% холестерина и 1% арахисового масла) к стандартному рациону в течение 28 дней. Измерение массы тела проводилось еженедельно в каждой группе животных. Фракцию терпенов и фитостеринов из иракского нута вводили в течение 28 дней через внутрижелудочный зонд. Затем животные содержались без пищи в течение 24 ч с забором образцов крови через пункцию сердца. Уровни общего холестерина, триглицеридов (ТГ), липопротеинов низкой плотности (ЛПНП), липопротеинов очень низкой плотности (ЛПОНП), липопротеинов высокой плотности, аланинаминотрансферазы (АЛТ), аспартатаминотрансферазы (АСТ), щелочной фосфатазы (ЩФ) и общего билирубина в сыворотке крови определяли с помощью стандартных диагностических наборов и автоматических анализаторов. После вывода животных из эксперимента их печень и сердце были извлечены для анализа.

Результаты. Патогистологическое исследование группы мышей с индуцированной гиперлипидемией показало выраженную диффузную цитоплазматическую жировую инфильтрацию печеночных и сердечных клеток. Состояние животных улучшалось в результате введения как терпенов, так и фитостеринов. При этом лечение терпенами было более эффективным, чем лечение фитостеринами. Наилучшие изменения липидных профилей были отмечены при терапии терпенами, что выражалось в значимом снижении ($p \leq 0{,}05$) сывороточных уровней общего холестерина (142,40±14,43 мг/дл), ТГ (98,89±8,71 мг/дл), ЛПНП (79,43±15,14 мг/дл) и ЛПОНП (19,78±1,74 мг/дл) при значимом снижении ($p \leq 0{,}05$) ферментативной активности печени (АЛТ (19,19±1,36 Ед/л), АСТ (15,98±1,3 Ед/л) и ЩФ (20,99±4,43 Ед/л)) и показателей общего билирубина (1,60±0,12 мг/дл) ($p \leq 0{,}05$). Потенциальное улучшение уровней малондиальдегида и глутатиона у мышей с гиперлипидемией было получено при использовании терпенов с достоверными различиями ($p \leq 0{,}05$).

Заключение. Полученные результаты свидетельствуют о том, что по сравнению с фитостеринами терпены оказывают достоверное влияние на состояние тканей печени и сердца, липидный профиль, функциональные ферменты печени и параметры окислительного стресса при гиперлипидемии, индуцированной высокожировым рационом питания. Рекомендуются дальнейшие исследования для оценки эффективности и установления механизма действия антигиперлипидемических эффектов терпенов и фитостеринов.

КЛЮЧЕВЫЕ СЛОВА

гиперлипидемия, нут, терпены, фитостерины, липидный профиль, оксидативный стресс

Для цитирования

Хвериф Н.Р., Абу Рагхиф А.Р., Кадхим Е.Дж. Уменьшение патогистологических повреждений у мышей с индуцированной гиперлипидемией под воздействием терпенов и фитостеринов из иракского нута. *ФАРМАКОЭКОНОМИКА. Современная фармакоэкономика и фармакоэпидемиология.* 2025; 18 (1): 95–103 (на англ. яз.). https://doi.org/10.17749/2070-4909/farmakoekonomika.2025.275.

INTRODUCTION / ВВЕДЕНИЕ

Hyperlipidemia is manifested in increased levels of cholesterol, both total cholesterol (TC) and low-density lipoproteins (LDL), oxidative stress (OS), and stimulated lipid peroxidation. Overproduction of reactive oxygen species (ROS) leads to severe cellular damage, which is primarily caused by the oxidation of cellular components including DNA and mitochondrial membrane [1]. OS has been documented to play a pivotal role in the pathophysiology and progression of diverse human diseases, including cardiovascular diseases and diabetes mellitus [2].

Hyperlipidemia can be classified as either primary or secondary, depending on the underlying reasons. Changes in lipid levels, including those found in cholesterol, triglycerides, very low-density lipoproteins (VLD), LDL, and intermediate-density lipoproteins (IDL), may lead to negative consequences in humans, such as acute pancreatitis, blood vessel blockage, and gallstone disease [3].

The selection of a suitable pharmaceutical treatment is informed by the type of lipid abnormality. Among the drugs aimed at decreasing LDL are statins, ezetimibe, resins, PCSK9 inhibitors, and niacin. Fabric acid derivatives (fibrates), niacin (nicotinic acid), and omega-3 polyunsaturated fatty acids prove effective in lowering triglyceride (TG) and VLDL concentrations and raising high-density lipoprotein (HDL) cholesterol concentrations [4]. Most of these drugs are associated with a number of side effects, including muscle-related complaints such as (myalgia, cramps, myopathy, and rhabdomyolysis), abnormal liver function, gallstone, gastric irritation. In addition, these drugs may trigger renal failure [4]. Therefore, the development of improved lipid-lowering agents alternatives to allopathic drugs represents a relevant research task [2].

Chickpea is a self-pollinating plant growing better at temperatures ranging within 15–25 °C. This plant, cultivated in more than 50 countries under varied environmental conditions [5], is one of the most consumed pulses worldwide. Chickpea is grown in the north of Iraq, as well as in Sulaimani, Kirkuk, Erbil, Duhok, and Ninawa provinces, ensuring a high quality of spring genotypes [6]. Raw or cooked chickpeas contain dietary bio-active compounds, e.g., sterols, phytic acid tannins, carotenoids, and polyphenols such as isoflavones, whose benefits may extend beyond the basic nutrition requirements of humans [7].

Terpenes are a large and diverse group of natural hydrocarbon secondary metabolites produced by a variety of plants, including tea, thyme, cannabis, conifers, and citrus fruits. Possessing five-carbon isoprene units with a chemical formula of $(C_5H_8)_n$, terpenes are responsible for the fragrance, taste, and pigment of plants [8]. Certain

Highlights

What is already known about the subject?

- Terpenes and phytosterols exhibit anti-hyperlipidemic effects by reducing cholesterol absorption, improving lipid metabolism, and adjusting lipoprotein levels
- Clinical studies showed that phytosterols are capable of reducing low-density lipoprotein levels by 5–15%, with a higher intake resulting in a larger decrease. They may lead to a slightly decrease in triglyceride levels, particularly in hypertriglyceridemia

What are the new findings?

- The anti-inflammatory, antioxidant, and anti-hyperlipidemic properties of terpenes and phytosterols extracted from Iraqi chickpeas were evaluated in animal experiments, revealing their potential as bioactive substances
- Histopathological examination showed significant improvement in the state of hepatocytes and cardio-myocytes in the mice groups receiving terpenes and phytosterols in comparison with the non-treated mice group

How might it impact the clinical practice in the foreseeable future?

- The use of terpenes and phytosterols from Iraqi chickpeas could significantly impact therapeutic practice, potentially leading to new treatment approaches for hyperlipidemia, cardiovascular conditions, and metabolic illnesses
- Terpenes and phytosterols contained in Iraqi chickpea could be introduced in functional meals or supplements to treat hyperlipidemia and associated disorders; to that end, further clinical trials involving humans are required

Основные моменты

Что уже известно об этой теме?

- Терпены и фитостерины оказывают антигиперлипидемическое действие, снижая всасывание холестерина, улучшая липидный обмен и регулируя уровень липопротеинов
- Клинические исследования показали, что фитостерины могут снижать уровень липопротеинов низкой плотности на 5–15%, причем более высокое их потребление приводит к наиболее выраженному результату. Они также могут несколько снизить уровень триглицеридов, особенно при гипертриглицеридемии

Что нового дает статья?

- Противовоспалительные, антиоксидантные и антигиперлипидемические свойства терпенов и фитостеринов из иракского нута выявлены в эксперименте на животных, что демонстрирует их потенциал в качестве биологически активных веществ
- Результаты патогистологического исследования показали значительное улучшение состояния гепатоцитов и кардиомиоцитов в группах мышей, получавших терпены и фитостерины, по сравнению с группой мышей, не получавших лечения

Как это может повлиять на клиническую практику в обозримом будущем?

- Применение терпенов и фитостеринов из иракского нута может существенно повлиять на терапевтическую практику и выработку новых подходов к лечению гиперлипидемии, сердечно-сосудистых заболеваний и болезней обмена веществ
- Терпены и фитостерины, содержащиеся в иракском нуте, могут быть использованы для создания функциональных пищевых продуктов и добавок для лечения гиперлипидемии и связанных с ней расстройств, но требуется проведение клинических испытаний с участием людей

terpenes with a set of antioxidant, anticancer, anti-inflammatory, antidiabetic, antidepressant, antiplasmodial, antimicrobial, astringent, digestive, diuretic, and other activities are widely used in natural folk medicine [8].

Phytosterols are bioactive compounds (steroids) with a chemical structure similar to that of cholesterol and are found naturally in plant species [9]. The most common dietary plant sterols are beta-sitosterol, campesterol, and stigmasterol. Phytosterols are fat-soluble compounds belonging to the triterpene family and comprising a tetracyclic ring with a side chain linked in the C17 position [10]. The health benefits of phytosterols are associated with their ability to reduce TC and LDL levels, thus mitigating the risk of many diseases [9]. Moreover, phytosterols modulate inflammation, exhibit antioxidant, antiulcer, immunomodulatory, antibacterial, and antifungal effects, and participate in wound healing and platelet aggregation inhibition [11].

Objective: To compare the effects of terpenes and phytosterols contained in Iraqi chickpea in mitigating histopathological damage in mice fed on a high-fat diet (HFD).

MATERIAL AND METHODS / МАТЕРИАЛ И МЕТОДЫ

Plant material / Растительный материал

Whole plants of chickpea of the Fabaceae family were collected during the flowering period (November 2021) in the northern area of Erbil. The collected plants were cleaned, dried in a shaded area at room temperature, pulverized with mechanical mills, and weighed.

Plant experiment work / Эксперименты с растениями

Experiments were carried out using the facilities of a phototherapy laboratory, Department of Pharmacognosy and Medicinal Plants (College of Pharmacy, University of Baghdad). The aerial parts of the plants were collected and treated as follows [12]:

– extraction and fractionation of different active constituents;

– preliminary phytochemical screening of various secondary metabolites, such as alkaloids, flavonoids, steroids, tannins, saponins, terpenoids, and cardiac glycosides in the different parts of the plant;

– isolation of sterol and terpene fractions;

– qualitative and quantitative estimation of the isolated sterols and terpenes fractions.

Animal experiments / Эксперименты с животными

The study was conducted during the period from October 2021 to April 2022 at the Department of Pharmacology (College of Medicine, Al Nahrain University). In total, 32 healthy albino male mice, 2–3 months of age and weighing about 20–30 g, were provided by the Higher Institute for Diagnosis of Infertility and Assisted Reproduction Techniques (Al Nahrain University). The animals were acclimatized to standard environmental conditions; food and water were provided ad libitum for a week prior to the commencement of the experiment.

Hyperlipidemia induction and extract administration / Индукция гиперлипидемии и введение экстракта

HFD (2% cholesterol and 1% peanut butter) was added to the standard diet (seeds, i.e., sunflower and groundnuts, cereals, fruits such as grapes and apples, vegetables, vitamins A, E, and D3) for 28 days to induce hyperlipidemia [13]. In all experimental groups, the body weight was measured weekly. Terpenes and phytosterols extracted from Iraqi chickpea were administered for 28 days via an intragastric tube [14].

Experimental design / Дизайн эксперимента

The mice were divided into four groups (8 mice in each group) [15]:

– Group 1 (normal): standard diet for 28 days;

– Group 2 (induced): HFD for 28 days;

– Group 3: HFD for 28 days followed by administration of a terpene fraction of chickpea at a dose of 250 mg/kg/day *per os* for further 28 days;

– Group 4: HFD for 28 days followed by administration of a phytosterol fraction of chickpea at a dose of 250 mg/kg/day *per os* for further 28 days.

Blood collection / Забор крови

Mice were kept fasting for 24 h, following which blood samples were extracted via heart puncture. This was followed by centrifugation at 2,500 rpm for 15 min. The obtained serum was used for the biochemical analysis of lipid profile indices and liver function enzymes [9].

Biochemical analysis / Биохимический анализ

Lipid profile indices and liver function enzymes

Standard diagnostic kits and an automated analyzer were used for estimation of serum TC, TG, LDL, VLDL, HDL, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total serum bilirubin (TSB) levels [13].

Oxidative stress parameters

At the end of the experimental period and following blood sampling, the animals were sacrificed; their liver organs were removed and divided into two parts for assay. For oxidative stress evaluation, one part of the liver tissue was homogenized and centrifuged for 15 min at 5000 rpm to assess the malondialdehyde (MDA) and glutathione (GSH) levels. Standard diagnostic kits were used to carry out an assay of MDA and GSH levels based on the competitive inhibition enzyme immunoassay technique [2].

Histopathological examination / Гистопатологическое исследование

The hepatic and heart tissue samples were first rinsed in phosphate-buffered saline, then fixed in 10% neutral buffered formalin. Standardized dehydration in ascending grades of alcohol and paraffin embedding procedures were conducted. A microtome of paraffin-embedded ultrathin sections (5 mm thickness) was carried out and slides were stained with hematoxylin and eosin. The sections were then analyzed using an Olympus light microscope equipped with a photograph machine [16].

Chickpea extraction / Процесс экстракции нута

Hexane was used for defatted oil from the plant to exclude the surplus fatty components [17] that interfere with the extraction process by adhering to the flask and biological effects inside the body[1]. An alcoholic solution (85% ethanol) was used. After filtration, the ethanol (crude) extract was subjected to evaporation to eliminate alcohol with the purpose of minimizing the harmful effect of ethanol on hepatic indicators. A rotary vacuum evaporator was used to avoid heat degradation of the bioactive constituents during evaporation. Methanol is a well-known solvent possessing the ability to extract semi-polar bioactive compounds [18]; hence, in the current study, it was used to extract terpenes. Petroleum ether, an organic (non-polar) solvent, was used for the extraction of phytosterols (non-polar) [19].

Statistical analysis / Статистический анализ

The Statistical Analysis System (SAS) software (SAS Institute, USA, 2018) was used to investigate the effect of different factors on the study parameters. Unpaired t-test and an analysis of variance (ANOVA) test were used to compare the parameters. The data in tables is presented as a mean value with a standard deviation (M±SD). The differences between the groups were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION / РЕЗУЛЬТАТЫ И ОБСУЖДЕНИЕ

Serum lipid profiles / Липидные профили сыворотки крови

In the present study, the serum levels of lipid profiles of TC, TG, HDL, LDL, and VLDL were monitored, with the corresponding results being depicted in **Table 1**. Feeding the mice with HFD for 28 days led to a highly significant increase in serum TC, TG, LDL, and VLDL with a statistically high significant reduction in HDL in hyperlipidemic mice as compared to the group fed by a normal standard diet. The changes observed in the hyperlipidemic group may be due to a disturbance of lipid metabolism as a result of decreased β-oxidation, increased cholesterol synthesis, and oxidative stress by decreasing enzymatic-free radical scavengers [20].

A highly significant reduction in the serum levels of lipid profile was observed among the terpene- (500 mg/kg/day) treated mice as compared to the hyperlipidemia (non-treated) mice group. These results were further supported by a significant improvement in HDL serum levels as compared to hyperlipidemia mice. According to a high-performance liquid chromatography (HPLC) analysis, the principal active constituents of terpenes are pinene, camphene, limonene, and thujene. The diversity in active constituents in the composition of terpenes impart them various pharmacological properties, such as antihyperlipidemic, anti-inflammatory, antioxidant, antidiabetes, anticancer, and antivirus [2].

A decrease in TC in the mouse group treated with of phytosterols (500 mg/kg/day) compared to the induced (non-treated) mice was noted. These results are further supported by the improved TG, HDL, LDl, and VLDL levels as compared to hyperlipidemia mice. Indeed, plant sterol lowers the absorption of cholesterol and thereby enhances the excretion of fecal steroids resulting in a reduction of the body's lipids [21]. Pancreatic lipase is considered a key enzyme responsible for TG absorption in the small intestine, and the inhibition of this enzyme by phytosterols [22] could be a key approach to controlling hyperlipidemia and obesity through suppression and delay of digestion and absorption of TG [23].

The greatest results were obtained for the terpene (500 mg/kg/day) treated mice as compared to the phytosterol (500 mg/kg/day) treated mice group in terms of serum lipid profiles.

Liver enzyme activity / Активность ферментов печени

The serum levels of ALT, AST, and ALP were significantly increased among the induced (non-treated) group, compared to the normal group. Hence, the liver function markers (ALT, AST, and ALP) as well as TSB showed a noteworthy decrease in the serum, indicating a membrane damage of hepatocytes. These consequences may be related to disturbed lipid metabolism under the action of a high fat intake, which resulted in accumulation of TG in the liver and an increase in the liver index and hepatic steatosis [24]. It is known that the liver plays a crucial role in regulating blood lipid levels through LDL clearance and HDL recruitment (see Table 1) [25].

[1] Al-Hakeemi A. Isolation of Trigonelline from Iraqi Fenugreek seed and studying its effect on blood glucose level and lipid profile in normal and alloxan-diabetic rabbits. M. Sc. Thesis; 2002.

Table 1. Comparison of the groups treated with terpenes (500 mg/kg/day) and phytosterols (500 mg/kg/day) with the non-treated group by ANOVA test

Таблица 1. Сравнение групп, получавших терпены (500 мг/кг/сут) и фитостерины (500 мг/кг/сут), с группой индуцированной гиперлипидемии, не получавшей лечения, по различным параметрам с помощью теста ANOVA

Parameter / Параметр	Group / Группа			
	Normal / Контрольная (норма)	Induced (non-treated) / С индуцированной гиперлипидемией без лечения	Terpenes (500 mg/kg/day) // Лечение терпенами (500 мг/кг/сут)	Phytosterols (500 mg/kg/day) // Лечение фитостеринами (500 мг/кг/сут)
TC, mg/dl // Общий холестерин, мг/дл	135.80±17.20	241.98±5.5 [a*]	142.40±14.43 [b*]	158.89±10.96 [b*]
TG, mg/dl // ТГ, мг/дл	85.92±14.04	183.87±13.75 [a*]	98.89±8.71 [b*]	107.88±15.12 [b*]
VLDL, mg/dl // ЛПОНП, мг/дл	17.18±2.81	36.79±2.75 [a*]	19.78±1.74 [b*]	21.57±3.02 [b*]
LDL, mg/dl // ЛПНП, мг/дл	67.58±19.83	185.03±10.03 [a*]	79.43±15.14 [b*]	91.85±11.75 [b*]
HDL, mg/dl // ЛПВП, мг/дл	49.37±6.10	16.59±5.63 [a*]	43.21±3.46 [b*]	45.46±7.87 [b*]
ALT, U/l // АЛТ, Ед/л	17.73±3.69	95.77±26.0 [a*]	19.19±1.36 [b*]	34.41±5.87 [b*]
AST, U/l // АСТ, Ед/л	15.33±3.83	196.39±53.4 [a*]	15.98±1.3 [b*]	20.16±2.91 [b*]
ALP, U/l // ЩФ, Ед/л	22.52±3.02	52.87±1.96 [a*]	20.99±4.43 [b*]	21.71±3.06 [b*]
TSB, mg/dl // Общий билирубин, мг/дл	1.29±0.19	4.87±1.10 [a**]	1.60±0.12 [b*]	1.74±0.31 [b*]
MDA, nmol/ml // МДА, нмоль/мл	0.53±0.23	4.10±1.31 [a*]	0.59±0.08 [b*]	0.76±0.17 [b*]
GSH, U/ml // Глутатион, ед/мл	315.77±66.97	57.24±19.85 [a*]	298.35±38.19 [b*]	265.36±10.38 [b*]

Note. *TC – total cholesterol; TG – triglycerides; VLDL – very low-density lipoproteins; LDL – low-density lipoproteins; HDL – high-density lipoproteins; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; TSB – total serum bilirubin; MDA – malondialdehyde; GSH – glutathione; a – comparison of the induced (non-treated) group versus the normal group; b – comparison of the terpene (500 mg/kg/day) and phytosterol (500 mg/kg/day) treated mice groups versus the induced (non-treated) group. * Statistically significant differences (p≤0.05). ** Highly statistically significant differences (p≤0.001). The differences between the phytosterol (500 mg/kg/day) treated mice group versus the terpene (500 mg/kg/day) treated mice group are not significant.*

Примечание. *ТГ – триглицериды; ЛПОНП – липопротеины очень низкой плотности; ЛПНП – липопротеины низкой плотности; ЛПВП – липопротеины высокой плотности; АЛТ – аланинаминотрансфераза; АСТ – аспартатаминотрансфераза; ЩФ – щелочная фосфатаза; МДА – малоновый диальдегид; a – сравнение группы мышей с индуцированной гиперлипидемией без лечения с контрольной группой; b – сравнение групп мышей, получавших терпены (500 мг/кг/сут) и фитостерины (500 мг/кг/сут) с группой с индуцированной гиперлипидемией без лечения. * Статистически значимые различия (p≤0,05). ** Высокодостоверные различия (p≤0,001). Различия между группой мышей, получавших фитостерины (500 мг/кг/сут), и группой мышей, получавших терпены (500 мг/кг/сут), не являются значимыми.*

A highly significant reduction in the serum levels of liver enzymes (ALT, AST, and ALP) followed the administration of terpenes (500 mg/kg/day), compared to the group of hyperlipidemia mice (see Table 1). Terpenes protect cells from the damage induced by oxidative stress, which is generally considered to be a cause of degenerative diseases [26]. In addition, a highly significant reduction in TSB levels indicates the liver ameliorating effects of terpenes (500 mg/kg/day).

A highly significant improvement in ALT, AST, ALP, and TSB was observed with the administration of phytosterols at a dose of 500 mg/kg/day. The underlying reason may be related to the diversity of phytochemical compounds contained in phytosterols, which possess a radical scavenging activity and hepatoprotective properties [11].

Concerning the liver enzyme activity, the terpene (500 mg/kg/day) treated mice group should more pronounced than the phytosterol (500 mg/kg/day) treated mice group.

Antioxidant activity / Антиоксидантная активность

MDA, which is a product of lipid peroxidation or oxygen reaction with unsaturated lipids, showed a highly significant increase in the group of induced (non-treated) mice [27]. The elevated levels of MDA in this group suggest an increased lipid peroxidation in fat deposits, which may have a detrimental effect on hepatic cells and other hepatocytes. In addition, the results showed a statistically highly significant reduction in tissue GSH in the induced (non-treated) group in comparison with the healthy group (see Table 1).

GSH is the most important endogenous defense mechanism against oxidative stress in the body. It plays an essential role in the maintenance of membrane protein -SH groups in the reduced form, the oxidation of which can alter cellular functions and structures [28].

A highly significant reduction of tissue MDA and an intensified defense mechanism through a highly significant increased glutathione level were noted in the group receiving terpenes (500 mg/kg) in comparison with the induced (non-treated) group (see Table 1). The antioxidant effects of terpenes are manifested through elevated catalase, superoxide dismutase, and peroxidase activities as well as through elevated levels of GSH, restoring the mitochondrial membrane [29].

A highly significant reduction of tissue MDA was also registered in the phytosterol-treated group in comparison with the induced (non-treated) group. Phytosterols act as free radical scavengers, cell membrane stabilizers, and antioxidant enzyme boosters [11]. These factors intensify the natural defense mechanism through a highly significant increase in GSH levels.

Therefore, with regard to antioxidant activity, terpene treatment (500 mg/kg/day) outperformed phytosterol treatment (500 mg/kg/day).

Histopathological examination / Гистопатологическое исследование

In the present study, the induced (non-treated) group showed a marked and diffuse cytoplasmic fatty infiltration and a granular degeneration of both hepatic (**Fig. 1**) and cardiac cells (**Fig. 2**) in comparison with the healthy (normal) mice group. This agrees well with the published data [2, 30].

The results obtained showed a significant improvement in the state of hepatocytes and cardiomyocytes among the terpene (500 mg/kg/day)-treated mice group in comparison with the induced (non-treated) mice group (**Fig. 3**). These findings indicate the antihyperlipidemic and anti-inflammatory effects of terpenes, which may be exerted through inhibited pro-inflammatory cytokines similar to those

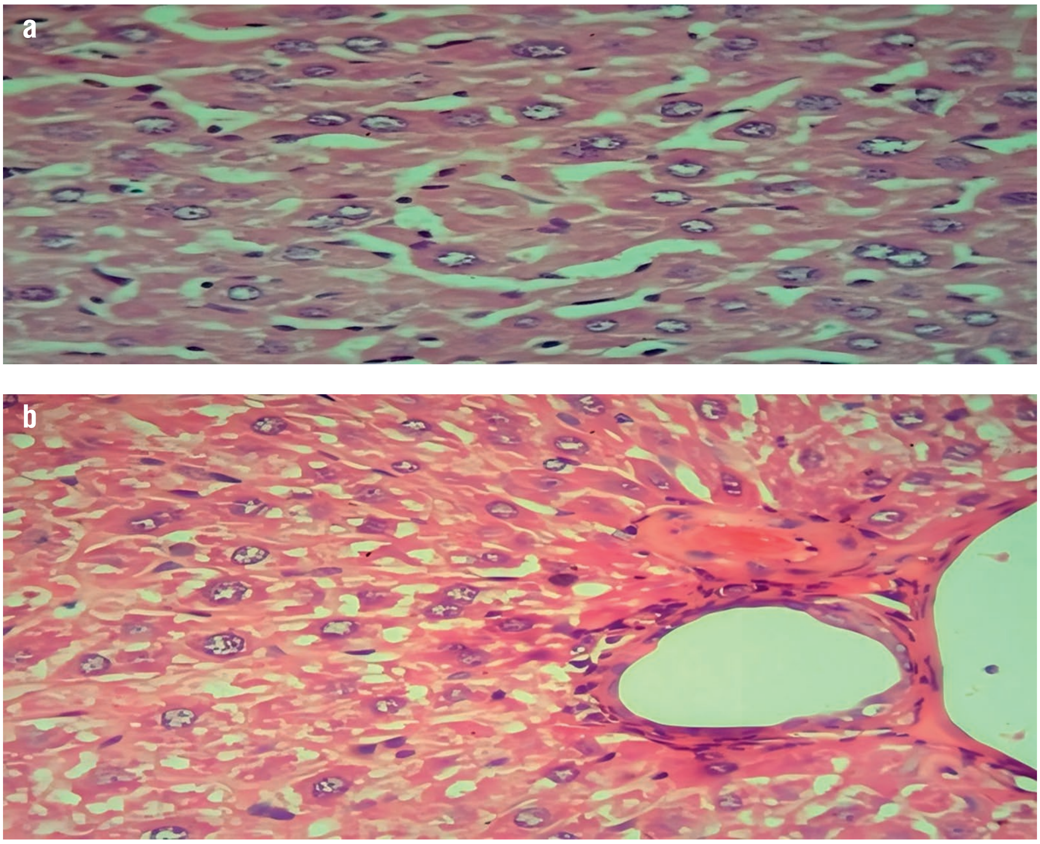

Figure 1. Histopathological examination of the mouse liver (hematoxylin and eosin staining, magnification ×40):
a – normal hepatocytes in the normal control group; **b** – marked and diffuse fatty steatosis with chronic inflammation of hepatocytes in the induced (non-treated) group

Рисунок 1. Патогистологическое исследование тканей печени мышей (окрашивание гематоксилином и эозином, увеличение ×40):
a – нормальные гепатоциты в контрольной группе;
b – выраженный диффузный жировой стеатоз с хроническим воспалением гепатоцитов в группе индуцированной гиперлипидемии без лечения

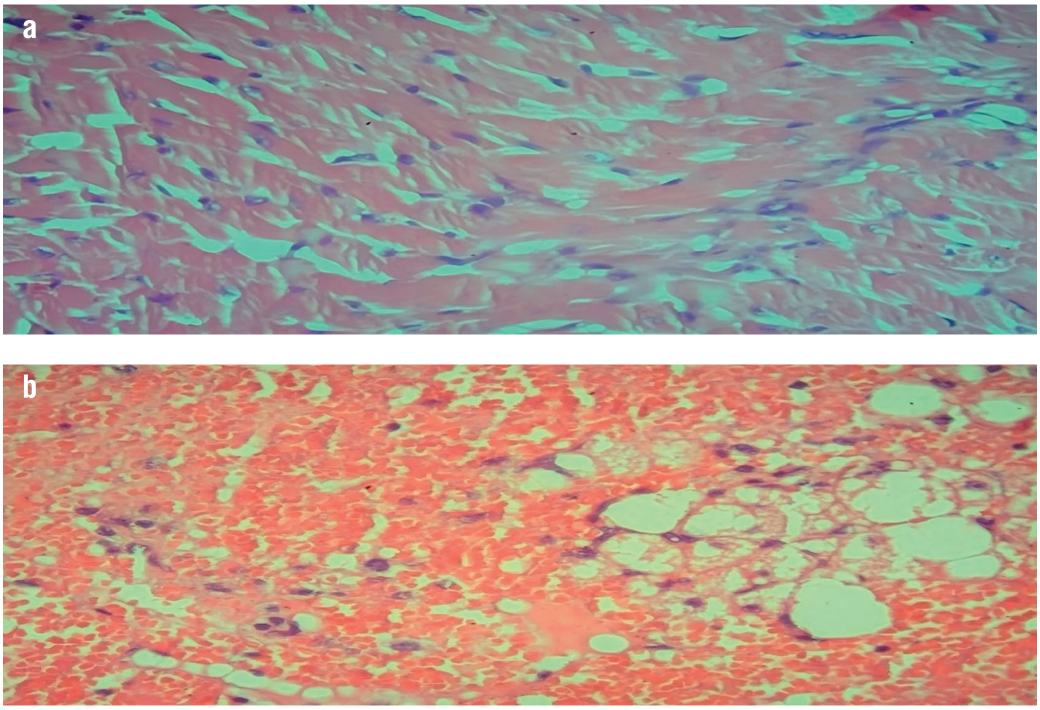

Figure 2. Histopathological examination of the mouse heart (hematoxylin and eosin staining, magnification ×40):
a – normal cardiac muscle with centrally arranged nuclei in the normal control group; **b** – marked fat deposition, neutrophilic cell infiltration, and vascular congestion in the induced (non-treated) group

Рисунок 2. Патогистологическое исследование тканей сердца мышей (окрашивание гематоксилином и эозином, увеличение ×40):
a – нормальная сердечная мышца с центрально расположенными ядрами в контрольной группе;
b – выраженное жироотложение, инфильтрация нейтрофильными клетками и закупорка сосудов в группе индуцированной гиперлипидемии без лечения

occurring with limonene pre-treatment [31]. In addition, the effect of lipid metabolism may be due to the presence of camphene, which was previously reported to prevent hepatic steatosis and to exhibit a hypolipidemic effect in high-fat diet mice [32].

In addition, our study revealed an improvement in the histopathological pictures of the liver and heart tissue in the phytosterol (500 mg/kg/day) treated mice group (**Fig. 4**). This indicates the antihyperlipidemic and anti-inflammatory effects of phytosterols, which may be due to reduced macrophage and neutrophil-mediated inflammatory processes and pro-inflammatory cytokine concentrations [33].

The mice treated with phytosterols (500 mg/kg/day) showed an improvement in the cellular histoarchitectural morphology, while the terpene (500 mg/kg/day) treated mice showed a distinct quasi-normal histopathological profile.

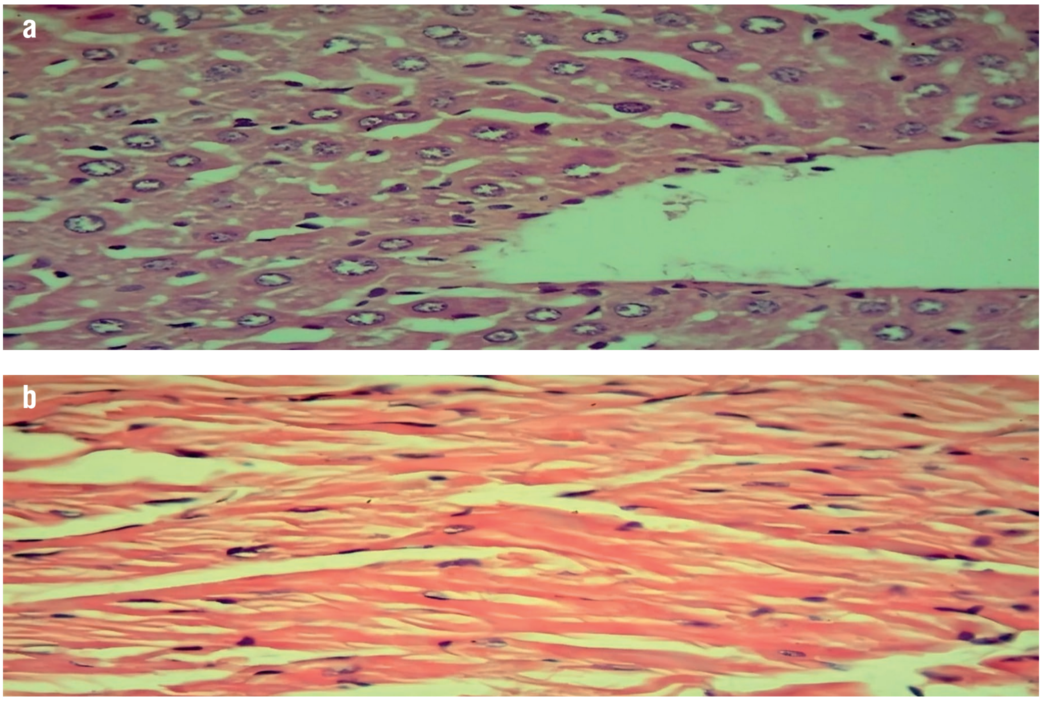

Figure 3. Histopathological examinations of mice treated with terpenes (500 mg/kg/day) (hematoxylin and eosin staining, magnification ×40):
a – liver examination showing normal hepatocytes with scattered fatty steatosis; **b** – heart examination showing normal cardiac muscle

Рисунок 3. Патогистологическое исследование тканей мышей, получавших терпены (500 мг/кг/сут) (окрашивание гематоксилином и эозином, увеличение ×40):
a – исследование печени показало нормальные гепатоциты с рассеянным жировым стеатозом;
b – исследование сердца выявило нормальную сердечную мышцу

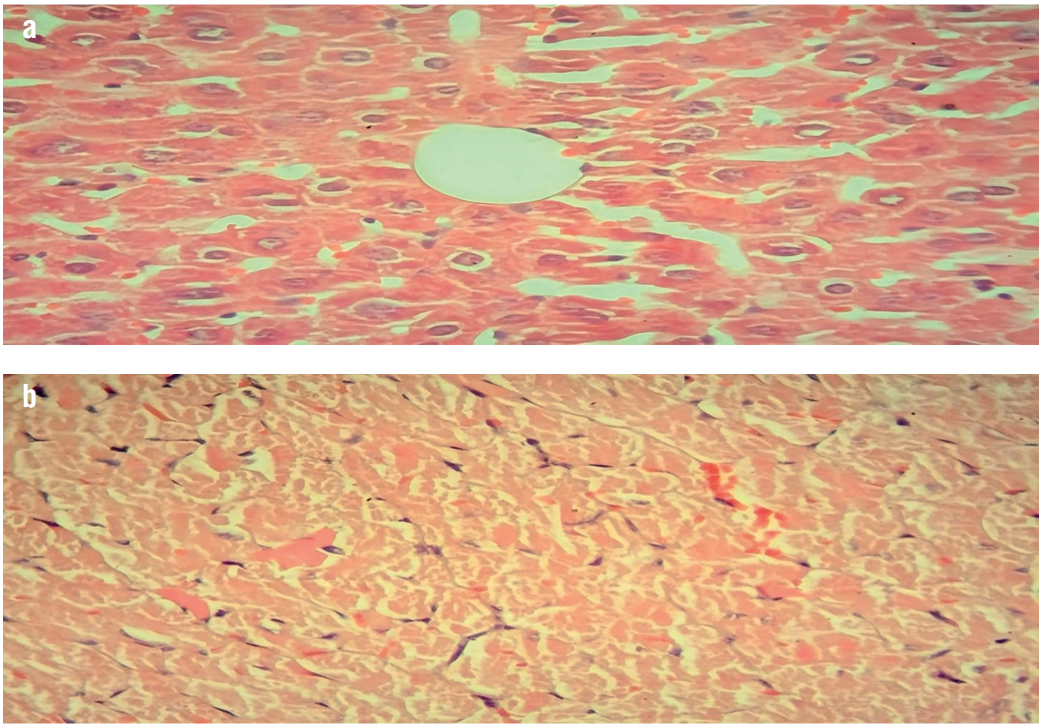

Figure 4. Histopathological examination of mice treated with phytosterols (500 mg/kg/day) (hematoxylin and eosin staining, magnification ×40):
a – liver examination showing mild fatty steatosis and scattered mild degenerative changes; **b** – heart examination showing normal cardiac muscle and mild fat deposition with vascular congestion

Рисунок 4. Патогистологическое исследование тканей мышей, получавших фитостерины (500 мг/кг/сут) (окрашивание гематоксилином и эозином, увеличение ×40):
a – исследование печени выявило умеренный жировой стеатоз и рассеянные незначительные дегенеративные изменения;
b – исследование сердца показало нормальную сердечную мышцу и небольшое отложение жира с закупоркой сосудов

Research prospects / Перспективы исследований

The potential challenges in translating the results obtained in mouse models to human studies include the bioavailability, pharmacokinetics, and side effects of the studied substances. Their further research in terms of efficacy and safety is required.

The investigated absorption-related fractions alter stability and solubility; as a result, such variables as enzymes, transporters, and natural flora influence absorption and metabolism. Therefore, absorption is a critical aspect in determining the efficacy of the studied substances in people. Their solubility should be improved using modern approaches. The metabolism of phytochemicals can differ dramatically between species, resulting in distinct bioactive metabolite profiles. As a result, these substances have an effect on metabolism, which is important for determining the rate of terpene and phytosterol metabolizing enzymes. Phytosterols and terpenes may impact the first-pass hepatic metabolism (bioavailability).

The available information shows that phytosterol supplements are reasonably safe and well-tolerated. The reported side effects are, as a rule, modest and include constipation, nausea, upset stomach, heartburn, gas, and stool discoloration. Chickpea-derived compounds may cause an allergic response. Hormonal alterations should be observed due to the similarity of phytosterol molecules.

CONCLUSION / ЗАКЛЮЧЕНИЕ

The terpene fraction (500 mg/kg/day *per os*) of Iraqi chickpea demonstrated highly significant antihyperlipidemic, anti-inflammatory, and antioxidant effects. The diversity in the presence of phytochemical compounds, such as pinene, camphene, limonene, and thujene, may be responsible for the inhibition of lipid peroxidation.

The phytosterol fraction (500 mg/kg/day *per os*) of Iraqi chickpea demonstrated highly significant antioxidant, antihyperlipidemic, and anti-inflammatory effects. The diversity in the presence of phytochemical compounds, such as beta-sitosterol, campesterol, and stigmasterol may be responsible for the inhibition of lipid peroxidation.

The effects of terpenes (500 mg/kg/day) and phytosterols (500 mg/kg/day) extracted from Iraqi chickpea were comparable in different parameters. Terpenes (500 mg/kg/day) showed the highest results concerning histopathology outcomes and led to a significant improvement of serum lipid profiles, liver enzyme activity, and antioxidant activities. Although no significant differences were observed between terpenes (500 mg/kg/day) and phytosterols (500 mg/kg/day) in increasing the levels of HDL, phytosterols demonstrated better results.

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Data sharing	**Раскрытие данных**
Raw data could be provided upon reasonable request to the corresponding author	Первичные данные могут быть предоставлены по обоснованному запросу автору, отвечающему за корреспонденцию
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