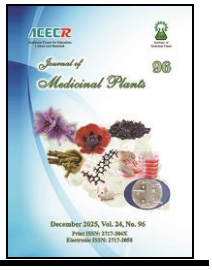
J. Med. Plants 2025; 24(96): 53-67

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Hepatoprotective and hypolipidemic effects of *Hibiscus gossypifolius*, *Trachyspermum copticum*, *Taraxacum officinale*, and *Rosmarinus officinalis* extracts in a rat model of diet-induced hypercholesterolemia

Ali Zarei[1], Naser Hosseini[2], Majid Ramezani[3], Abbas Alimoradian[4], Saeed Changizi-Ashtiyani[5,*]

[1]*Department of Physiology, Estahban School of Paramedical Sciences, School of Nursing Hazrat Zahra (P.B.U.H) Abadeh, Shiraz University of Medical Sciences, Shiraz, Iran*
[2]*Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran*
[3]*Department of Internal Medicine, School of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran; Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Department of Pharmacology, School of Medicine, Arak University of Medical Sciences, Arak, Iran*
[5]*Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Keywords:
Hibiscus gossypifolius
Rosmarinus officinalis
Taraxacum officinale
Trachyspermum copticum
Hypercholesterolemia
Medicinal Plants

ABSTRACT

Background: Hypercholesterolemia is a key risk factor for cardiovascular and metabolic diseases, prompting the search for natural alternatives with fewer side effects than conventional drugs such as orlistat. **Objective:** This study aimed to comparatively evaluate the hepatoprotective and hypolipidemic efficacy of hydroalcoholic extracts from *H. gossypifolius*, *Trachyspermum copticum*, *Taraxacum officinale*, and *Rosmarinus officinalis* in a rat model of diet-induced hypercholesterolemia. **Methods:** Fifty-six male Wistar rats were allocated into seven groups (n= 8). Groups received either a standard diet, a high-cholesterol diet (HCD; 2 % cholesterol) with saline, or the HCD supplemented via daily gavage with orlistat (10 mg/kg) or one of the plant extracts (500, 800, 200, 300 mg/kg, respectively) for 48 days. Biochemical parameters, including lipid profile, liver enzymes, and renal markers, were analyzed. **Results:** The extracts demonstrated distinct therapeutic specializations. *T. officinale* was the most effective in reducing body weight gain. *T.copticum* and *R. officinalis* showed superior hepatoprotective effects, significantly lowering AST, and ALT/ALP levels, respectively. Contrary to the initial hypothesis, none of the plant extracts reduced LDL or total cholesterol more effectively than the HCD-control; orlistat was the most effective hypolipidemic agent. *R. officinalis* showed a non-significant trend towards reducing creatinine levels. **Conclusion:** This study shows the investigated plant extracts have specific, complementary effects. *T. officinale* potently reduces weight gain, whereas *T. copticum* and *R. officinalis* provide significant hepatoprotection. However, these extracts did not show superior hypolipidemic efficacy compared to the control, a finding that requires further study. The potential of these plants for weight management and liver protection merits future research with standardized extracts.

Abbreviations: WHO, World Health Organization; FBS, fasting blood sugar; TG, triglyceride; BUN, Blood Urea Nitrogen; LDL, Low-Density Lipoprotein; VLDL, Very-Low-Density Lipoprotein; HDL, High-Density Lipoproteins; AST, Aspartate Transaminase; ALT, alanine aminotransferase; ALP, Alkaline Phosphatase; TSH, Thyroid-Stimulating Hormone; T3, Triiodothyronine; T4, Thyroxine; NF-κB, Nuclear Factor Kappa B; TNF-α, Tumor Necrosis Factor; IL-1β, *Interleukin-1 beta;* COX-2, *Cyclooxygenase-2;* IL-6, *Interleukin 6;* ROS, Reactive Oxygen Species

*Corresponding author: ashtiyani.s@iums.ac.ir

doi:

Received 29 June 2025; Received in revised form 14 December 2025; Accepted 22 December 2025

1. Introduction

Obesity is one of the most important problems of public health. According to the WHO in 2022, about 2.5 billion adults (18 years and older) worldwide are overweight, of whom approximately 890 million are obese. This corresponds to about 43 % of adults being overweight and 16 % being obese. Additionally, the World Obesity Atlas 2025 predicts that the number of adults living with obesity will increase from 524 million in 2010 to more than 1.13 billion by 2030, representing an increase of over 115 % [1]. This problem is increasing due to industrialization, fast food consumption, and decreasing physical activity [2]. Indeed, obesity is the consequence of an intake of caloric surplus via dietary consumption and the subsequent accumulation of adipose tissue relative to standard physiological processes [3].

The increase of lipid absorption can cause increased accumulation of fat, the increase of lipogenesis, the decrease of lipolysis, and a combination of these three mechanisms [4]. The epidemiologic evidence shows that a high-fat diet is one of the effective factors in the incidence of metabolic syndrome, which includes different disorders of the endocrine system, such as obesity, hyperlipidemia, dysglycemia, dyslipidemia, and hypertension. Also, it can increase the risk of developing atherosclerosis, cardiovascular diseases, and finally, type 2 diabetes [5, 6]. Nowadays, multiple drugs, such as orlistat, are used for treating obesity [7]. Orlistat, a pharmacological agent utilized in the management of obesity, is a pancreatic lipase inhibitor, but it has multiple side effects such as cholestasis, liver failure, hepatic necrosis, [8], hypertension, headache, dry mouth, insomnia, and constipation [7, 9]. Additionally, various pharmacological agents, including statins, are employed in the management of hypercholesterolemia; however, this class of medications is associated with several adverse effects, including hepatic and renal complications. Therefore, researchers are looking for alternative therapeutic methods like using medicinal plants and their derivatives, as safer and more effective ways for treating obesity and reducing the side effects of drugs [4, 10, 11]. The *Hibiscus gossypifolius* Mill**,** *Trachyspermum copticum***,** *Taraxacum officinale*, and *Rosmarinus officinalis* have been used long-standing in folk and traditional medicine across various cultures, as well as their documented therapeutic properties.

H. gossypifolius (Sour Tea) has been extensively studied for its potent antihyperlipidemic effects. Its therapeutic properties are primarily attributed to a rich profile of bioactive compounds, including anthocyanins, which inhibit LDL oxidation, and protocatechuic acid (PCA), which has been shown to reduce serum cholesterol and atherogenic index [12, 13]. These properties make *H. gossypifolius* a prime candidate for investigating natural cholesterol-lowering interventions. Similarly, *T. copticum* (Ajwain), a staple in traditional medicine, contains key volatile oils like thymol and carvacrol. These constituents have demonstrated significant lipid-lowering capabilities in multiple studies, with proposed mechanisms including the inhibition of HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis [14-16]. This strong biochemical basis makes *T. copticum* a compelling subject for this investigation. Another plant with significant therapeutic potential is *T. officinale*. (Dandelion). With a long history in folk medicine for liver and kidney support, its selection is scientifically justified by a complex phytochemical profile. It is a notable source of phenolic acids (caffeic

and chlorogenic acid), flavonoids (luteolin), and polysaccharides, which are recognized for their potent antioxidant and anti-inflammatory properties—crucial for addressing the metabolic imbalances in hypercholesterolemia [17-19]. Finally, *R. officinalis* (Rosemary) was included due to its recognized hypolipidemic properties. Its effects are largely attributed to phenolic diterpenes like carnosic acid and carnosol, alongside rosmarinic acid. These compounds are known to suppress adipogenesis, reduce liver triglycerides, and provide strong antioxidant activity, positioning rosemary as a strong candidate for mitigating diet-induced metabolic disorders [4, 20, 21]. The present investigation assessed the impact of hydroalcoholic extracts derived from the aforementioned botanical species on various metabolic indicators and potential hepatic and renal impairment in rats subjected to a hypercholesterolemic diet, while also making comparisons with orlistat.

2. Materials and methods

2.1. Plant material collection and extraction

The aerial parts of *H. gossypifolius*, *T. copticum*, *T. officinale*, and *R. officinalis* were procured from the Arak University Herb Farm. The botanical identity of each plant was authenticated by a plant systematics expert at the Shahid Beheshti University Herbarium (HSBU). Voucher specimens for each species have been deposited at the herbarium under the following accession numbers: *H. gossypifolius* (HSBU2025962), *T. copticum* (HSBU2025963), *T. officinale* (HSBU2025964), and *R. officinalis* (HSBU2025965)."

Following collection, the plant materials were shade-dried at ambient temperature and subsequently pulverized into a coarse powder using a mechanical grinder. The powders were stored in airtight containers to prevent degradation. The hydroalcoholic extracts were prepared using the percolation method. Briefly, the powder of each plant was macerated in 80 % (v/v) aqueous methanol for 72 hours at room temperature, with periodic agitation. The resulting mixture was filtered through Whatman No. 1 filter paper to separate the marc from the liquid extract. The filtrate was then concentrated under reduced pressure at 40 °C using a rotary evaporator (model RV10 Control, IKA-Werke, Germany). The final paste-like residue was completely dried in a desiccator over anhydrous silica gel to yield a solid extract. For daily administration, a fresh solution was prepared by dissolving the required amount of the dried extract in distilled water [22, 23].

2.2. Phytochemical rationale and extraction method

The selection of the four medicinal plants for this study was based on extensive evidence from traditional use and modern pharmacological research, which has identified their rich phytochemical profiles as responsible for their metabolic benefits. While this study did not involve quantitative standardization a recognized limitation the extraction protocol was specifically designed to maximize the yield of relevant bioactive compounds. The use of 80 % aqueous methanol as the solvent is a well-established method for efficiently extracting a broad spectrum of polar and semi-polar secondary metabolites, including the phenolic acids, flavonoids, and terpenes that are abundant in these species. It is important to note that this study utilized non-standardized extracts. While the extraction protocol was optimized to capture a broad spectrum of bioactive compounds, the specific concentrations of key active principles (e.g., anthocyanins, thymol, carnosic acid) were

not quantitatively determined. This is a recognized limitation that affects the precision of dose-response interpretations and the reproducibility of the findings [24].

As detailed in Table 1, each plant possesses a unique arsenal of active compounds with known hypolipidemic and antioxidant properties. For instance, *H. gossypifolius* is rich in anthocyanins and protocatechuic acid, while *T. copticum* is a potent source of thymol and carvacrol. Similarly, the therapeutic effects of *T. officinale* are linked to its flavonoids and phenolic acids, and *R. officinalis* is characterized by its high content of phenolic diterpenes such as carnosic acid. The preparation of these extracts was therefore optimized to ensure the presence of these key bioactive classes, providing a robust basis for assessing and comparing their collective pharmacological effects in the context of hypercholesterolemia.

Table 1. Key bioactive compounds of the investigated plants with potential Hypolipidemic effects

Plant	Part Used	Key Compounds Hepatoprotective/ Hypolipidemic Effects	Supporting References
H. gossypifolius	Calyces	Anthocyanins, Protocatechuic acid (PCA), Flavonoids (e.g., quercetin)	[13, 25]
T. copticum	Seeds	Phenolic Monoterpenes (Thymol, Carvacrol)	[14, 26]
T. officinale	Aerial Parts	Flavonoids (e.g., luteolin), Phenolic Acids (e.g., chlorogenic acid)	[17, 18, 19]
R. officinalis	Aerial Parts	Phenolic Diterpenes (Carnosic acid, Carnosol), Rosmarinic Acid	[20, 21, 22]

Table content has been slightly simplified to enhance readability and focus on the most cited compounds in the main text.

2.3. High-cholesterol diet (HCD) preparation

The HCD (2 % cholesterol) was prepared by dissolving 20 g of pure cholesterol (Merck, Germany) and 5 g of cholic acid (Fluka Chemika) in 10 ml of warm olive oil. This mixture was then thoroughly blended with 1 kg of standard rat chow. The diet was prepared fresh every two days and stored at 4 °C [27].

2.4. Animals and Ethical Approval

The study was conducted on 56 male Wistar rats, each weighing between 200 and 220 g, obtained from the laboratory animal breeding center of Baqiyatallah University of Medical Sciences. The animals were housed under standard environmental conditions, including a controlled temperature of 22 °C a 12-hour light/dark cycle at 40 – 50 % humidity, and ad libitum access to food and water. All procedures were performed in strict accordance with the guidelines for the care and use of laboratory animals as stipulated by the Ministry of Health and Medical Education of Iran, and the study protocol received ethical approval (approval number: 1393-03-98-1842).

2.5. Experimental design and treatment protocol

The rats were randomly allocated into seven groups, with eight animals (n= 8) per group. The experimental period lasted for 48 consecutive days. Group I (Negative Control) received a standard chow diet, while all other groups were fed a HCD containing 2 % cholesterol. The specific daily treatments, administered via oral gavage, for each group are detailed in Table 2. The hydroalcoholic extracts used in this study were derived from the aerial parts of the respective medicinal plants.

Table 2. Experimental design and treatment protocol

Group	Group Name	Diet	Daily Treatment (via oral gavage)
I	Negative Control	Standard Rat Chow	0.2 mL Distilled Water
II	HCD	High-Cholesterol Diet (2 % Cholesterol)	0.2 mL Normal Saline
III	*H. gossypifolius*	High-Cholesterol Diet (2 % Cholesterol)	500 mg/kg *H. gossypifolius* extract [25]
IV	*T. copticum*	High-Cholesterol Diet (2 % Cholesterol)	800 mg/kg *T. copticum* extract [26]
V	*T. officinale*	High-Cholesterol Diet (2 % Cholesterol)	200 mg/kg *T. officinale* extract [27]
VI	*R. officinalis*	High-Cholesterol Diet (2 % Cholesterol)	300 mg/kg *R. officinalis* extract [28]
VII	Orlistat	High-Cholesterol Diet (2 % Cholesterol)	10 mg/kg Orlistat [29]

2.6. Data and sample collection

Throughout the study, daily food consumption was recorded, and the change in appetite was calculated as the difference between the average food intake on the final and first days of the experiment. Body weight was measured at the beginning and end of the 48-day period. On the final day, animals were fasted overnight, and blood samples were collected for biochemical analysis. The following parameters were assessed: serum levels of creatinine, urea, fasting blood sugar (FBS), the lipid profile (including total cholesterol, triglycerides (TG), LDL, VLDL, and HDL), liver enzymes (AST, ALT, and ALP), and thyroid hormones (TSH, T3, and T4).

2.7. Biochemical measurement

The Pars Azmoon Company obtained the assay kit for TG, cholesterol, LDL, HDL, VLDL, FBS, AST, ALT, and ALP. Pishtaz Teb provided an assay kit for thyroid hormones. Measurements were made using the auto-analyzer device (Selectra, XL, Netherlands). An auto-analyzer device (Technicon, RA-1000, USA) was used to measure creatinine and urea.

2.8. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS version 16.0. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test. A p-value of $P \leq 0.05$ was considered statistically significant. The normality of data distribution was confirmed using the Shapiro-Wilk test. Homogeneity of variances was verified using Levene's test. Data that met these assumptions were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test.

3. Results

3.1. Weight

All plant extracts showed a significant weight reduction when compared to the HCD group ($P < 0.05$). However, *T. officinale* proved to be the most effective for weight loss. It

created the most substantial difference in weight relative to the HCD group ($P < 0.01$) (Table 3).

3.2. Appetite

T. copticum and *T. officinale* resulted in the smallest increase in appetite. The increase in appetite (g) was calculated as the difference between the initial and final average food consumption (Table 3).

3.3. Creatinine

The levels of creatinine were notably reduced in the orlistat and *R. officinalis* groups compared to the HCD, *H. gossypifolius* ($P < 0.05$), and the *T. officinale* groups ($P < 0.01$). The orlistat group showed the most significant reduction in creatinine levels (Table 4).

3.4. FBS and BUN

The administration of *R. officinalis* led to the greatest reduction in FBS and BUN. (Tables 5, 6).

3.5. Serum lipid profile

Among the plant extracts investigated in this research, *H. gossypifolius* showed the most significant reduction in LDL and total serum cholesterol, with a notable difference when compared to the negative control group ($P < 0.05$) (Table 7). Additionally, it resulted in the greatest decrease in TG and VLDL, although this difference was not significant compared to the negative control group. (data not shown). Analysis of the serum lipid profile revealed that none of the plant extracts significantly lowered total cholesterol or LDL levels compared to the HCD-control group. Contrary to our hypothesis and some literature, the groups receiving *H. gossypifolius*, *T. copticum*, *T. officinale*, and *R. officinalis* extracts showed mean values for LDL and total cholesterol that were not lower than the HCD-control. In contrast, the orlistat treatment group showed a clear and significant reduction in these parameters.

3.6. Liver enzymes

All herbal extracts reduced the liver enzymes compared to the HCD group. However, *R. officinalis* effectively reduced ALP and ALT, while the administration of *T. copticum* resulted in the lowest level of AST compared to other herbal extracts (Table 8).

3.7. Thyroid Hormones

In the results section, regarding Table 9, the serum levels of thyroid-stimulating hormone (TSH) and thyroxine (T4) were evaluated. The group treated with *H. gossypifolius* extract showed a significant reduction in T4 levels compared to the high-cholesterol diet (HCD) control group, while no significant changes in TSH were observed among the other treatment groups (Table 9).

Table 3. Mean weight (g) ± SEM, the average weight and appetite difference increased at the end of the experiments for each group

Groups	Mean weight	The difference in the average weight	The mean appetite increased
Negative control	255.48 ± 8.9	26.29	2.4
HCD	281.77 ± 6.5	0.77	3
H. gossypifolius (500 mg/kg)	249.23 ± 2.1	-32.54**	1.9
T. copticum (800 mg/kg)	262.6 ± 45.31	-19.32*	0.2
T. officinale (200 mg/kg)	247.80 ± 3.9	-33.97***	1.2
R. officinalis (300 mg/kg)	258.80 ± 3.7	-22.97**	1.7
Orlistat (10mg/kg)	260.57 ± 4.2	-21.2	2.2

*: Significant difference between *T. copticum* and HCD groups (P < 0.05).
**: Significant difference between *H. gossypifolius*, *T.officinale, and R. officinalis* compared to HCD groups (P < 0.01).
***: The most effective in weight loss compared to the HCD group (P < 0.01).
Difference of the average weight (g): The difference between the average weight of the group and the average weight in the HCD group.
Appetite increased (g): the difference between the first and the last average consumed food.
SEM: standard error of the mean.

Table 4. Mean of serum creatinine (mg/dl) ± SEM at the end of experiments in each group.

Groups	Creatinine
Negative control	0.62 ± 0.015
HCD	0.72 ± 0.025*
H. gossypifolius (500 mg/kg)	0.74 ± 0.032†
T. copticum (800 mg/kg)	0.75 ± 0.053**
T. officinale (200 mg/kg)	0.69 ± 0.047
R. officinalis (300 mg/kg)	0.67 ± 0.042++
Orlistat (10mg/kg)	0.59 ± 0.024

*: Significant difference between orlistat and HCD groups (P < 0.05).
†: Significant difference between orlistat and *H. gossypifolius* groups (P <0.05).
**: Significant difference between orlistat and *T. copticum* groups (P<0.01).
++: The most effective in decreasing creatinine level compared to the HCD group (P < 0.01).
SEM: standard error of the mean.

Table 5. Mean of FBS (mg/dl) ± SEM at the end of experiments in each group

Groups	FBS
Negative control	131.12 ± 8.7
HCD	147.17 ± 13.3
H. gossypifolius (500 mg/kg)	159.28 ± 13.2
T. copticum (800 mg/kg)	189.62 ± 28.4
T. officinale (200 mg/kg)	199.67 ± 23.7
R. officinalis (300 mg/kg)	121.60 ± 8.8
Orlistat (10mg/kg)	157.42 ± 11.4

FBS: fasting blood sugar
SEM: standard error of the mean.

Table 6. Mean of serum BUN (mg/dl) ± SEM at the end of experiments in each group

Groups	BUN
Negative control	47.26 ± 1.6
HCD	46.10 ± 3.6
H. gossypifolius (500 mg/kg)	40.17 ± 3
T. copticum (800 mg/kg)	45.30 ± 1.7
T. officinale (200 mg/kg)	40.1 ± 1.8
R. officinalis (300 mg/kg)	38.78 ± 2.2
Orlistat (10mg/kg)	44.38 ± 2.5

SEM: standard error of the mean.
BUN: Blood Urea Nitrogen

Table 7. Mean serum LDL and total cholesterol (mg/dl) ± SEM at the end of experiments for each group

Groups	LDL	Cholesterol
Negative control	31.8 ± 2.9	73 ± 5.7
HCD	85 ± 24.28	144.5 ± 35.3
H. gossypifolius (500 mg/kg)	100.2 8± 14.29*	163.71 ± 20.79
T. copticum (800 mg/kg)	112.5 ± 22.3*	191.12 ± 33.8†
T. officinale (200 mg/kg)	104.83 ± 16.73*	192.67 ± 27.2†
R. officinalis (300 mg/kg)	113 ± 19.63*	198.75 ± 30.9†
Orlistat (10 mg/kg)	53.43 ± 6.4	92.85 ± 11.1

†: Significant difference between *T. copticum, T. officinale, and R. officinalis* compared to the negative control group ($P < 0.05$).
*: Significant difference between *T. copticum, T. officinale, R. officinalis, and H. gossypifolius* compared to the negative control group ($P < 0.05$).
SEM: standard error of the mean.
LDL: Low-Density Lipoprotein.

Table 8. Mean values of enzyme profiles of the liver (U/L) ± SEM at the end of experiments in each group

Groups	ALT	ALP	AST
Negative control	16.88 ± 1.4	44.08 ± 3.9	29.84 ± 2.5
HCD	29.4 ± 1.5	110.97 ± 10.9**	61.83 ± 4.4*
H. gossypifolius (500mg/kg)	13.03 ± 1.4	84.66 ± 8.6	42.89 ± 3.8
T. copticum (800 mg/kg)	29.32 ± 6.3	83.05 ± 10.6	34.72 ± 5
T. officinale (200 mg/kg)	23.3 ± 6.07	84.87 ± 7.1	39.7 ± 4.5
R. officinalis (300 mg/kg)	13.72 ± 4.2	81.97 ± 6.5	55.4 ± 7.1
Orlistat (10mg/kg)	24.77 ± 6.1	86.54 ± 13.1	75.75 ± 15*

*: Significant difference between HCD and orlistat compared to negative control groups ($P < 0.05$).
**: Significant difference between positive and negative control groups ($P < 0.01$).
ALT: Alanine aminotransferase
ALP: Alkaline phosphatase
AST: Aspartate transaminase
SEM: standard error of the mean.

Table 9. Mean serum TSH and T_4 (mmol/L) ± SEM at the end of experiments for each group

Groups	TSH	T4
Negative control	0.52 ± 0.07	78.42 ± 2.5
HCD	1.17 ± 0.13†	89.43 ± 2.9
H. gossypifolius (500 mg/kg)	0.93 ± 0.04†	60.22 ± 3.4*
T. copticum (800 mg/kg)	1.1 ± 0.11	61.39 ± 5.1
T.officinale (200 mg/kg)	1.03 ± 0.07	65.2 ± 3.9
R. officinalis (300 mg/kg)	1.04 ± 0.05	65.36 ± 4.2
Orlistat (10mg/kg)	0.91 ± 0.07	70.37 ± 3.3

†: Significant difference between *H. gossypifolius* and HCD groups ($P < 0.05$).
*: Significant difference between *H. gossypifolius* and HCD groups ($P < 0.05$).
TSH: Thyroid-stimulating Hormone
T4: Thyroxine
SEM: standard error of the mean.

4. Discussion

This study provides a valuable, comparative preclinical investigation into the therapeutic potential of four medicinal plants against diet-induced hypercholesterolemia. The primary strength of the work lies in its head-to-head comparison under identical experimental conditions, which allows for a clear differentiation of each plant's unique pharmacological profile. The discussion below critically examines the findings for each plant, highlighting their distinct primary effects, proposed mechanisms, and the implications of the results.

1. H. gossypifolius: The Potent Hypolipidemic Agent

A central and unexpected finding of this study was that the *H. gossypifolius* extract did not lower total cholesterol or LDL levels in our model compared to the HCD-control. This contrasts with several previous reports which highlight its potent antihyperlipidemic effects [12, 13, 22]. This discrepancy underscores the context-dependency of phytotherapeutic outcomes. Potential explanations for the lack of efficacy in this model include the specific dose used, which may have been sub-therapeutic for this particular high-cholesterol diet (HCD) model. The composition of the HCD itself, particularly the inclusion of cholic acid to enhance cholesterol absorption, might have overwhelmed or interfered with the extract's mechanism of action, potentially related to the inhibition of dietary cholesterol absorption or modulation of hepatic metabolism. Furthermore, variations in the phytochemical profile of the non-standardized extract could account for differences in efficacy compared to other studies that used standardized preparations or different solvents. This finding highlights the critical importance of model-specific and dose-response evaluations, and suggests that the hypolipidemic benefits of *H. gossypifolius* may not be universal across all experimental conditions of hypercholesterolemia [28, 29].

Notable Secondary Finding & Critical Question: A highly significant finding was that the *H. gossypifolius* group exhibited the lowest levels of thyroxine (T4). This warrants critical attention. While beyond the study's primary scope, this observation raises a crucial safety consideration. Is this a potential adverse endocrine-disrupting effect? This must be a focus of future toxicological studies before any clinical recommendation can be made [30, 31].

Critical Analysis & Proposed Mechanism: This aligns with extensive literature on the plant's rich anthocyanin and flavonoid content. The proposed mechanism of AMPK activation, leading to the inhibition of HMG-CoA reductase (the target of statins) and SREBP-1c, is a robust explanation for its potent cholesterol- and triglyceride-lowering effects. This positions *H. gossypifolius* not merely as a general "healthy" herb, but as a candidate with a specific, potent mechanism for managing dyslipidemia [25].

Notable Secondary Finding & Critical Question: A highly significant and unexpected finding was that the *H. gossypifolius* group exhibited the lowest levels of thyroxine (T4). This warrants critical attention. While beyond the study's primary scope, this observation raises a crucial safety consideration. Is this a therapeutic effect (e.g., reducing metabolic rate in obesity) or an adverse endocrine-disrupting effect? This must be a primary focus of future toxicological and mechanistic studies before any clinical recommendation can be made. The profound lipid-lowering benefit cannot be viewed in isolation from this potential impact on thyroid function [25].

2. T. officinale (Dandelion): The Effective Weight-Loss Promoter

Primary Finding: *T. officinale* was unparalleled in promoting weight loss, showing the most substantial reduction in body weight gain.

Critical Analysis & Proposed Mechanism: The authors' proposed dual mechanism is compelling:

1. Pancreatic Lipase Inhibition: Bioactive compounds like luteolin may inhibit dietary fat absorption, mirroring the pharmacological action of orlistat. This provides a direct, mechanistic explanation for the weight loss effect [32].

2. Prebiotic Effect: Oligofructans in dandelion could modulate gut microbiota towards a profile associated with leanness [32].

Implication: This makes *T. officinale* a particularly interesting candidate for obesity management where weight reduction is the primary goal. Its effects appear to be more targeted towards energy intake and absorption rather than direct, potent lipid-lowering like *H. gossypifolius* [25, 32].

3. T. copticum & R. officinalis: The specialized hepatoprotective duo

Primary Findings: These two plants showed superior hepatoprotective effects. *T. copticum* was most effective at reducing AST, while *R. officinalis* was most effective at reducing ALT and ALP.

Critical Analysis & Proposed Mechanism:

***T. copticum*:** The reduction in AST is convincingly linked to its volatile oils (thymol, γ-terpinene). The proposed mechanism of enhancing antioxidant defenses (CAT, GST) and suppressing pro-inflammatory cytokines (TNF-α) addresses the core pathways of oxidative stress and inflammation in diet-induced liver injury [33].

***R. officinalis*:** The significant reduction in ALT and ALP is strongly attributed to its potent antioxidant diterpenes (carnosic acid, carnosol). The additional observation of reduced creatinine levels suggests a valuable nephroprotective potential, expanding its therapeutic profile beyond the liver. The synergy between its hypolipidemic (via AMPK) and direct antioxidant actions provides a comprehensive protective mechanism [34, 35].

Implication: This specialization suggests that *T. copticum* and *R. officinalis* could be

prioritized in conditions where liver protection is paramount, such as in non-alcoholic fatty liver disease (NAFLD) secondary to hypercholesterolemia. Their mechanisms are complementary, targeting different aspects of hepatocyte injury [33-35] (Table 10).

Table 10. Comparative summary of the therapeutic effects and mechanisms of action of the investigated plant extracts

Plant	Key Finding in This Study	Primary Bioactive Compounds	Proposed Primary Mechanism	References
H. gossypifolius	Greatest reduction in TC & LDL-C	Anthocyanins, Quercetin	AMPK activation → Inhibition of HMG-CoA reductase & SREBP-1c	[25]
T. officinale	Greatest reduction in body weight	Luteolin, Sesquiterpene lactones	Inhibition of pancreatic lipase → Reduced fat absorption	[32]
T. copticum	Greatest reduction in AST	Thymol, γ-terpinene	Enhanced antioxidant enzymes (CAT, GST); Anti-inflammatory (↓TNF-α)	[33]
R. officinalis	Greatest reduction in ALT & ALP	Carnosic acid, Rosmarinic acid	Direct antioxidant action; Enhanced antioxidant enzymes; AMPK activation	(34, 35)

4. Orlistat (Reference Drug): The Benchmark with Limitations

Context: Orlistat served as a HCD and provided a crucial benchmark. It showed effective lipid-lowering and weight control.

Critical Perspective: The study effectively uses orlistat to highlight a key message: while the herbal extracts may not always surpass the drug in efficacy for a single parameter, they offer a multi-targeted and potentially safer profile. For instance, orlistat showed no significant hepatoprotective benefit (AST was high in this group), whereas the plants did. This underscores the potential of the plants to address multiple facets of the metabolic syndrome (dyslipidemia, weight gain, liver injury) simultaneously, unlike the single-mechanism drug [32].

Study Limitations and Future Directions

This study has certain limitations that should be considered. The primary constraint is the use of non-standardized hydroalcoholic extracts, where variations in plant source and extraction methods may affect bioactive compound concentrations, potentially influencing the reproducibility of the results.

To address this, future research should employ quantitative methods such as HPLC to standardize key active compounds (e.g., anthocyanins, thymol, carnosic acid), enabling precise dose-response analyses and consistent replication of findings.

Notably, secondary observations such as reduced T4 levels with *H. gossypifolius* and improved renal markers with *R. officinalis* highlight promising avenues for further investigation into their endocrine and systemic effects. The primary constraint is the use of non-standardized hydroalcoholic extracts. Future research must employ quantitative methods (e.g., HPLC) to standardize key active compounds, enabling precise dose-response analyses and consistent replication.

Ultimately, translation of these findings requires long-term preclinical safety studies followed by rigorous clinical trials to confirm the efficacy and safety of standardized extracts in humans.

5. Conclusion

This preclinical study demonstrates that the hydroalcoholic extracts of *T. officinale*, *T. copticum*, and *R. officinalis* effectively

mitigate specific complications associated with diet-induced hypercholesterolemia in rats, revealing a distinct therapeutic specialization. *T. officinale* excelled in promoting weight loss, while *T. copticum* and *R. officinalis* showed superior hepatoprotective activity. Contrary to our initial hypothesis and some existing literature, the extract of *H. gossypifolius* did not demonstrate a significant hypolipidemic effect in this particular model, a finding that warrants further investigation into the factors influencing its efficacy. Furthermore, its significant impact on thyroxine levels necessitates careful safety evaluation. Collectively, these results suggest that the therapeutic application of these plants should be target-specific. Further research, particularly involving standardized extracts, different dosing regimens, and clinical trials, is warranted to validate these findings and explore their translational potential.

Author contributions

The authors express their sincere gratitude to the Head of the Endocrinology and Metabolism Research Center (Prof. Larijani), the Deputy of Research (Dr. Mehrdad), and the Deputy of Research at Arak University of Medical Sciences, for providing the necessary facilities.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

A.Z & S.CA & M.R: Visualization, Investigation, WritingOriginal draft preparation. A.Z, N.H & A.A: Conceptualization, Methodology, Writing, Reviewing and Editing, Supervision. N.H: Plant diagnosis and identification.

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How to cite this article: Zarei A, Hosseini N, Ramezani M, Alimoradian A, Changizi-Ashtiyani S. Hepatoprotective and hypolipidemic effects of *H. gossypifolius*, *Trachyspermum copticum* (L.), *Taraxacum officinale*, and *Rosmarinus officinalis* extracts in a rat model of diet-induced hypercholesterolemia. ***Journal of Medicinal Plants*** 2025; 24(96): 53-67.
doi: