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Antihyperlipidemic and Antiobesity Effects of Parmotrema tinctorum Ethanolic Extract in Olive Oil-Induced Hyperlipidemic Rats

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ABSTRACT

Hyperlipidemia and obesity represent significant global health challenges associated with heightened risks of angina, atherosclerosis, and stroke due to elevated blood lipid levels. Exploring natural agents as potential therapeutic interventions is promising in addressing these conditions. In this study, the antihyperlipidemic and anti-obesity effects of ethanolic extract from Parmotrema tinctorum were evaluated using an olive oil-induced hyperlipidemic rat model. Rats were divided into distinct treatment groups, including simvastatin, distilled water (control), and two doses of the extract (200 mg/kg and 400 mg/kg). The experiment was conducted over 28 days. Administration of Parmotrema tinctorum extract led to significant reductions in body and liver weight gain. The serum lipid profile exhibited dose-dependent decreases in total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). Additionally, there were notable reductions in the atherogenic index and blood glucose levels. The observed effects were attributed to potential anorectic actions mediated through the central nervous system. The extract did not affect gastric emptying and demonstrated inhibition of de novo cholesterol biosynthesis. This study underscores the potential of ethanolic extract from Parmotrema tinctorum as a dual-action therapeutic agent for addressing hyperlipidemia and obesity. The findings highlight its ability to modulate lipid metabolism, suggesting implications for managing cardiovascular and metabolic disorders. Further research is needed to elucidate the underlying mechanisms of its effects and to explore its potential application in clinical settings.

Key words: *Ethanolic extract from Parmotrema tinctorum (EEPT), Obesity, Hyperlipidemia, Low-density lipoprotein cholesterol (LDL-C), Very low-density lipoprotein cholesterol (VLDL-C)*

INTRODUCTION

Obesity stands as a serious health challenge stemming from difficulties in effectively processing carbohydrates and lipids within the body. Due to this body will start storing more amount of fats in adipose tissue as well in vital organs like the liver, heart, muscles, and pancreas [1]. This concern touches individuals from all walks of life, disregarding factors like gender, age, ethnicity, or race [2]. Hyperlipidemia, characterized by elevated levels of fats in the bloodstream, is a crucial catalyst in the alarming escalation of atherosclerosis. This critical condition

involves the accumulation of plaque within the arteries, posing a severe threat to our overall health. These disorders are frequently connected to conditions including coronary heart disease, stroke, and peripheral vascular problems. A combination of genetic elements, unhealthy eating habits involving high-calorie meals, saturated fats, and cholesterol, coupled with inadequate physical activity, collectively play a role in the emergence of this condition [3, 4]. The escalating rates of obesity and the aging population will undoubtedly amplify the prevalence of these medical concerns in the future. Dyslipidemias, covering conditions like high lipid levels and low levels of beneficial cholesterol HDL-C, amplify the risk of atherosclerosis. This issue stems from a blend of genetic factors and poor dietary choices, such as consuming calorie-rich foods, saturated fats, cholesterol, and not engaging in enough physical exercise. This pattern is prevalent in developed countries worldwide [5].

Parmotrema tinctorum, a particular type of lichen, belongs to the Parmeliaceae family due to its distinctive leaf-like structure. It manifests as a flat and leafy appearance with irregular sections, varying in diameter from a few centimeters to several. The upper surface presents a palette of colors ranging from pale gray to brownish-green or yellow, often showcasing a textured surface. The underside, on the other hand, exhibits a lighter color and comes equipped with rhizomes, specialized structures aiding in securing surfaces like rocks or trees. Up top, one can find reproductive structures called apothecia that resemble discs or cups, within which spores reside. This unique structure equips *Parmotrema tinctorum* to thrive across diverse environments, adding to its ecological significance and potential health benefits attributed to its chemical composition [6]. Prosorediosic acid, protocetraric acid, usnic acid, α-collatolic acid, β-alectoronic acid, atranorin, chloroatranorin, lecanoric acid, methyl orsenillate, orsenillic acid, methyl lecanoric acid, lichenin, isolichenin, and vital vitamins such as vitamin C are among the chemical compounds that distinguish Parmotrema tinctorum [7]. Traditionally, this lichen has been harnessed to enhance food flavors, address skin conditions, offer pain relief, and serve as a remedy within Indian, Chinese, Homeopathic, and Western medicinal practices. It can be used to effectively manage joint discomfort, treat alopecia, relieve obstructions, alleviate pharyngitis, tackle rabies infections, combat worm infestations, ease motion sickness, and potentially contribute to heart health [8-10]. *Parmotrema tinctorum* showcases various pharmacological effects, encompassing antibacterial, antimicrobial, antioxidant, anticancer, and anti-diabetic properties [11-14].

Although the extensive pharmacological potential of *Parmotrema tinctorum* is recognized, its possible roles in addressing obesity and high lipid levels remain relatively uncharted. Hence, we're presently delving into the potential antihyperlipidemic and antiobesity effects of an ethanolic extract of *Parmotrema tinctorum*, especially in a rat model with increased lipid levels brought on by olive oil.

MATERIALS AND METHODS

Plant material

The plant *Parmotrema tinctorum* was collected from Kanhangad, Kerala, India, and authenticated by Botanist, Pilkula Nisargadhama, Mangalore. The lichens are now dried, cleaned, and made into a coarse powder using a mechanical grinder mixer. The powder was stored for future use.

Preparation of plant extract

350 grams of powdered material underwent continuous hot extraction with ethanol using a Soxhlet apparatus. The resulting extract was first filtered through cotton wool, then through Whatman No. 1 filter paper. Finally, it was dried at 40-50°C to yield a 46% w/w (weight by weight) brownish, sticky extract. This extract was then stored in the refrigerator (4-8°C) for future use [15].

Animals

This study followed guidelines set by the Committee for Control and Supervision of Experimental Animals (CCSEA). The specific procedures were also approved by an internal ethics committee for animal research. Male and female Wistar albino rats weighing 180-200 grams were obtained from Geniron Biolabs Pvt. Ltd. in Bangalore, India. These animals were housed in a CCSEA-approved facility at Srinivas College of Pharmacy in Mangalore. Each cage held six rats and provided standard lab conditions: 12 hours of light followed by 12 hours of dark. The rats had unlimited access to both commercial pellet food and water. The animal house maintained a temperature of 25°C with a humidity of 50%. Throughout the experiments, researchers strictly adhered to ethical guidelines.

Study design

Group 1 (Control): These rats received a saline solution (0.9% acacia) to serve as a baseline comparison.
Group 2 (Model control): These rats were given just olive oil (5 ml/kg) to establish a model with potentially elevated blood lipid levels. They also received saline (0.9% acacia) one hour before the olive oil.
Group 3 (Simvastatin + Olive Oil): These rats were given a standard cholesterol-lowering drug (simvastatin, 20 mg/kg) one hour before olive oil (5 mg/kg) treatment.
Group 4 (EEPT 200mg/kg + Olive Oil): These rats were pretreated with a dose of the test substance (EEPT, 200 mg/kg) mixed with saline (0.9% acacia) one hour before olive oil treatment.
Group 5 (EEPT 400mg/kg + Olive Oil): These rats received a higher dose of the test substance (EEPT, 400 mg/kg) with saline (0.9% acacia) one hour before olive oil treatment.

The designated animals received the planned treatments for 28 days. Daily, one hour before their regular medications and *Parmotrema tinctorum* extracts (EEPT), all groups received olive oil [16]. The study next assessed how these therapies affected blood lipids, blood sugar, liver tissue structure (histology), body weight, relative liver weight, food intake, and atherogenic index (AI) for heart disease risk.

Biochemical parameters

Collection of blood samples
After 28 days of treatment, blood samples were collected from the rats on day 29. A thin, glass capillary tube was carefully inserted into the inner corner of the rat's eye (retro-orbital plexus) to collect blood in Eppendorf tubes. The tubes were then left undisturbed for 30 minutes at 37°C (body temperature). Finally, a centrifuge was used to separate the clear serum from the blood cells by spinning at 2500 rpm for 10 minutes [17].

Biochemical analysis
All the blood serum samples were analyzed for several key markers of heart health. These markers included total cholesterol (TC), HDL cholesterol, and triglycerides (TG). Standard enzymatic methods were used to measure total cholesterol and triglycerides, while HDL cholesterol was measured using a precipitation method [18, 19]. An automated analyzer (ERBA Autoanalyser) with commercially available test kits (ERBA Diagnostic Mannheim GmbH, Germany) was used for these analyses. In addition to directly measured values, researchers also calculated levels of LDL cholesterol (bad cholesterol) and VLDL cholesterol (another type of lipoprotein) using a formula by Friedewald [20]. Finally, they calculated atherogenic indexes, which are indicators of heart disease risk based on cholesterol levels.

Estimation of blood glucose by GOD/POD method
In the presence of glucose oxidase, glucose is converted to hydrogen peroxide and gluconic acid. Peroxidase catalyzes the reaction between hydrogen peroxide, phenol, and 4-aminoantipyrine to form a red quinoneimine dye complex. Calculate the amount of glucose in the sample by measuring the absorbance at 505 nm. This immediately correlates with the color's intensity when it forms [21].

Estimation of total protein
When proteins bind to copper ions in the alkaline biuret reagent medium, they form a purple-colored complex whose absorbance is proportionate to the concentration of proteins. This complex can be measured at 546 nm using a spectrophotometer or colorimeter with a yellow-green filter to compare the absorbance of the standard to that of the blank [22].

Histopathology studies
Rat liver tissues were obtained from humanely euthanized, anesthetized animals, and then fixed in formalin for preservation. To prepare for microscopic examination, the samples underwent dehydration with alcohol, followed by embedding in paraffin wax. Ultra-thin sections (5 micrometers) were cut from the embedded tissue, stained with a special dye to differentiate cellular structures, and finally examined under a high-powered optical microscope at 100x magnification [23].

Statistical analysis
Statistical analysis of the experimental data was performed using GraphPad Prism software (version 9.5.1). The analysis involved a two-step process: first, an analysis of variance (ANOVA) test to assess overall differences

between groups, followed by Dunnett's test for multiple comparisons between a control group and individual treatment groups. P-values less than 0.001, 0.01, and 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

This study investigated how various treatments affected multiple aspects of health in rats, with detailed results presented in **Tables 1-5**. Rats fed olive oil (Group II) displayed a significantly increased appetite, consuming considerably more food each day compared to the control group (Group I) ($p<0.001$). Interestingly, all other treatment groups (Groups III, IV, and V) showed a marked decrease in food intake compared to the olive oil group ($p<0.01$, $p<0.001$). Body weight mirrored this trend. Olive oil feeding led to a substantial weight gain in Group II compared to control ($p<0.001$). Conversely, all other treatments caused significant weight loss relative to the olive oil group ($p<0.001$). The EEPT extract, in particular, exhibited a dose-dependent effect, with a higher dose leading to a greater reduction in body weight **(Table 2)**. Olive oil also had a significant impact on blood lipids in Group II. Compared to control, rats fed olive oil had a notable increase in total cholesterol and a decrease in HDL cholesterol, the "good" cholesterol ($p<0.001$ for both). Notably, all other treatments significantly improved this profile. These groups displayed a rise in HDL cholesterol and a decrease in total cholesterol ($p<0.01$, $p<0.001$, $p<0.05$ for Groups III, IV, and V respectively). This positive shift also translated to a decline in the atherogenic index, a risk factor for heart disease, in the treated groups **(Table 4)**. Similar trends were observed for triglyceride levels, with a significant increase in Group II-fed olive oil ($p<0.001$) and significant decreases in all other treatment groups ($p<0.001$, $p<0.01$, $p<0.001$ for Groups III, IV, and V respectively). LDL and VLDL levels mirrored these trends as well. Blood sugar levels followed a similar pattern. Compared to control, rats fed olive oil (Group II) had a significant increase in blood sugar ($p<0.01$). Conversely, all other treatments significantly lowered blood sugar levels ($p<0.001$, $p<0.05$, $p<0.01$ for Groups III, IV, and V respectively). Lastly, total protein levels also varied across groups. Olive oil feeding (Group II) resulted in a significant increase in total protein compared to control ($p<0.01$). In contrast, all other treatments caused significant decreases in protein levels compared to the olive oil group ($p<0.05$, $p<0.01$).

Histopathology

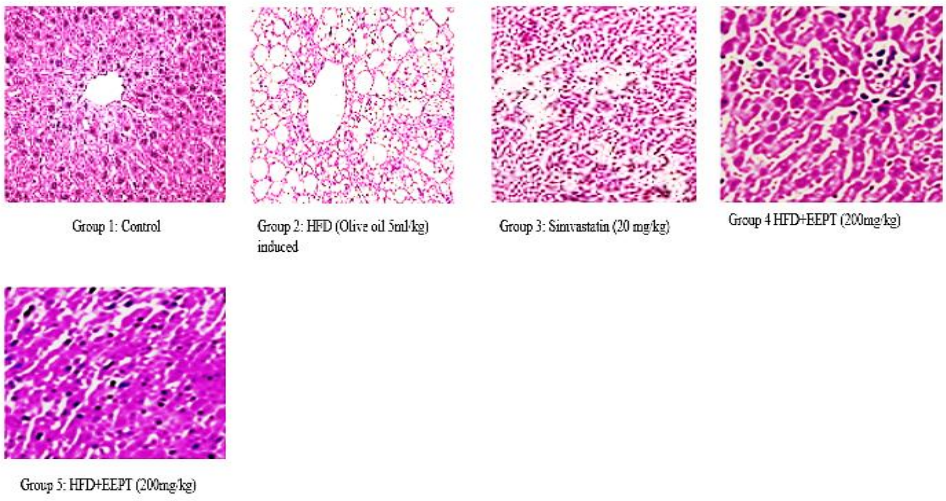

Figure 1. Effects of EEPT on histopathology of liver from HFD-induced hyperlipidemic rats.

Table 1. Effect of EEPT on daily food intake

Sl. No.	Group	Treatment	Food intake			
			1st day	7th day	14th day	28th day
1	I	Control	122.78±0.23	125.45±1.39	127.93±0.60	132.45±0.42
2	II	Olive oilinduced (5ml/kg/p.o)	142.98±0.52***	149.11±0.320***	150.91±0.469***	156.39 ±0.84***
3	III	Simvastatin (20mg/kg/p.o) + HFD	108.85±0.37***	105.85±0.50***	103.85±0.44***	101.85±0.51***

4	IV	EEPT (200mg/kg/p.o) + HFD	125.40±0.35**	123.57±0.42***	118.30±0.57***	116.30±0.57**
5	V	EEPT (400mg/kg/p.o) + HFD	119.50±0.03***	118.11±0.77***	115.94±0.79**	111.35±0.58***

The data in the tables are presented as mean values ± SEM, allowing for comparisons between groups. Statistical significance is denoted by asterisks, with * indicating p<0.05 (most important), ** for p<0.01, *** for p<0.001, and "ns" for non-significant differences.

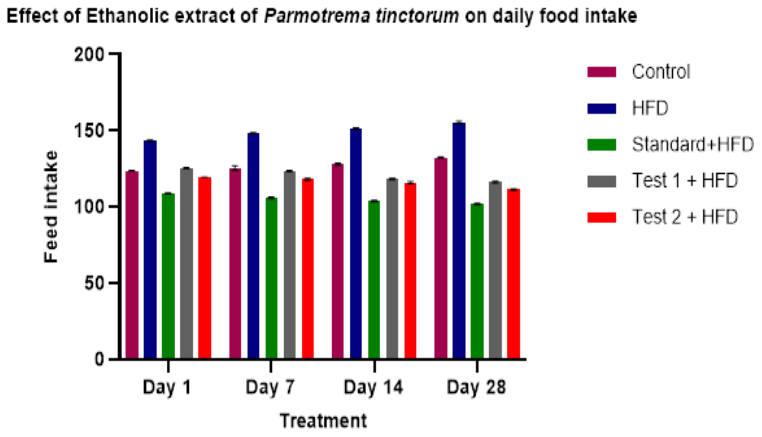

Figure 2. Effect of EEPT on daily food intake.

Table 2. Effect of Ethanolic extract of *Parmotrema tinctorum* on weight gain.

Sl. No	Groups	Treatment	Weight onday1	Weight onday 29	Weight gain
1	I	Control	162.56±0.98	184.12±0.23	19.01±0.25
2	II	Olive oil induced(5ml/kg/p.o)	173.11±0.34	292.01±0.948	118.91±0.54***
3	III	Simvastatin (20mg/kg/p.o)	162.09±0.29	175.32±1.21	9.33±1.12***
4	IV	EEPT (200mg/kg/p.o)	167.91±0.192	188.128±0.71	17.729±0.76***
5	V	EEPT (400mg/kg/p.o)	166.12±0.12	180.19±3.14	13.98±0.412***

The data in the tables are presented as mean values ± SEM, allowing for comparisons between groups. Statistical significance is denoted by asterisks, with * indicating p<0.05 (most important), ** for p<0.01, *** for p<0.001, and "ns" for non-significant differences.

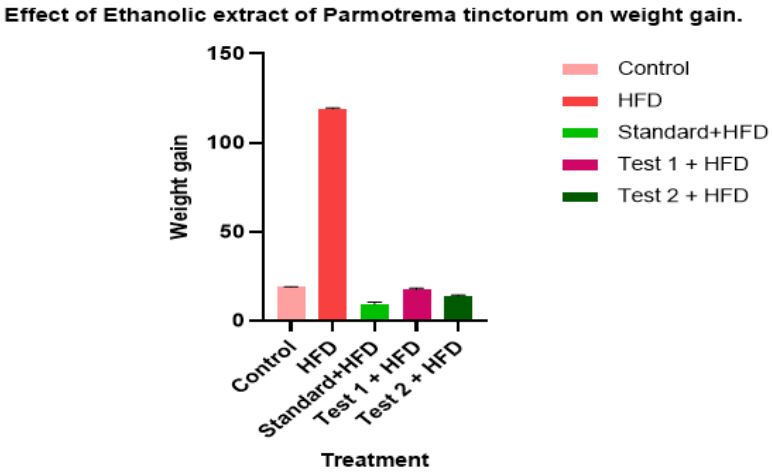

Figure 3. Effect of EEPT on weight gain.

Table 3. Effect of EEPT Lipid Profile

Groups	Treatment	Cholesterol	TG	LDL-C	VLDL-C	HDL-C
I	Control	80.260±1.249	164.290±2.183	23.082±0.291	32.832±0.422	48.928±0.863

II	Olive oil induced (5ml/kg/p.o)	152.817±0.66***	255.783±3.203***	44.710±0.520***	51.157±0.641***	32.235±0.607***
III	Simvastatin (20mg/kg/p.o)	99.595±1.125***	174.523±2.950***	23.040±0.395***	34.905±0.590***	59.325±0.994***
IV	EEPT (200mg/kg/p.o)	116.567±0.475**	189.800±1.911**	28.425±0.546***	38.97±0.245**	50.867±0.481*
V	EEPT (400mg/kg/p.o)	110.12±0.481***	182.48±0.643***	26.100±0.138***	36.72±0.248***	54.200±1.26**

The data in the tables are presented as mean values ± SEM, allowing for comparisons between groups. Statistical significance is denoted by asterisks, with * indicating p<0.05 (most important), ** for p<0.01, *** for p<0.001, and "ns" for non-significant differences.

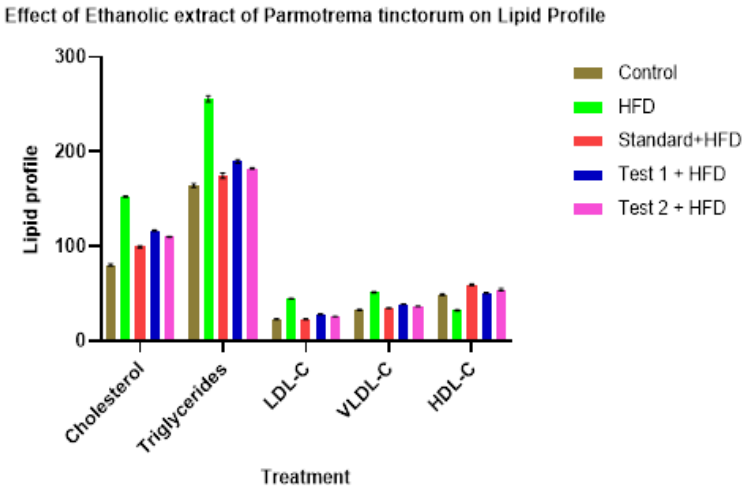

Figure 4. Effect of EEPT on Lipid Profile.

Table 4. Atherogenic index and percentage protection of EEPT.

Sl. No	Group	Treatment	Atherogenic index	% protection
1	I	Control	3.342	-
2	II	Olive oil induced(5ml/kg/p.o)	7.951	-
3	III	Simvastatin (20mg/kg/p.o)	2.929	62.98
4	IV	EEPT (200mg/kg/p.o)	3.882	38.459
5	V	EEPT (400mg/kg/p.o)	3.409	46.158

The table provides information about different groups, their respective treatments, atherogenic indices, and the percentage of protection.

Table 5. Effect of EEPT on blood glucose and total protein levels

Sl. No	Group	Treatment	Blood glucose(mg/dl)	Total protein(gm/dl)
1	I	Control	75.89±0.919	5.435±0.12
2	II	Olive oil induced(5ml/kg/p.o)	133.13±1.547**	11.327±0.12**
3	III	Simvastatin (20mg/kg/p.o)	87.72±1.72***	4.46±0.083**
4	IV	EEPT (200mg/kg/p.o)	103.12±1.34*	6.24±0.047*
5	V	EEPT (400mg/kg/p.o)	93.30±0.849**	5.05±0.0411**

The data in the tables are presented as mean values ± SEM, allowing for comparisons between groups. Statistical significance is denoted by asterisks, with * indicating p<0.05 (most important), ** for p<0.01, *** for p<0.001, and "ns" for non-significant differences.

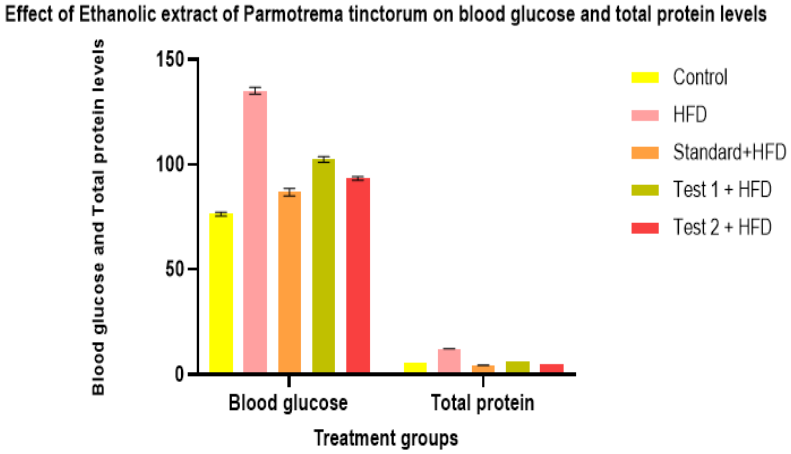

Figure 5. Effect of EEPT on blood glucose and total protein levels.

Atherosclerosis is a medical condition characterized by the accumulation of lipids within the artery walls, and it is closely linked to elevated lipid levels in the bloodstream Diet has a substantial impact on hyperlipidemia and obesity, with high-fat diets being particularly significant contributors [24]. Olive oil, a common dietary component, adds to the complexity by potentially intensifying these conditions through its propensity to enhance intestinal lipid absorption [25]. To address the intricate correlation between diet and health, we will undertake a comprehensive investigation into the healing possibilities offered by the ethanolic extract of *Parmotrema tinctorum* (EEPT).

EEPT stands out due to its rich composition of saponins, compounds that have shown promise in inhibiting intestinal lipid absorption and pancreatic lipase activity [14]. Furthermore, saponins are recognized for their role in enhancing cholesterol excretion through bile acids [26]. The intricate metabolic consequences of various dietary fats cannot be overlooked. Compared to saturated fats found in butter, beef, and palm oil, olive oil stands out with its abundant monounsaturated fatty acids, providing unique metabolic advantages Moreover, it has been found that sugars like sucrose and fructose have an astonishing capability to greatly elevate blood lipid levels, especially triglycerides. Indeed, the impact of these carbohydrates can often exceed that of other carbohydrate types [27].

EEPT, aside from displaying hypolipidemic properties, showcases the intriguing ability to reduce food intake, potentially serving as a regulator of lipid metabolism. Emphasizing the significance of elevated LDL cholesterol levels is vital, as they significantly enhance the chances of developing atherosclerotic cardiovascular diseases. Equally crucial is the association between high levels of HDL cholesterol and triglycerides with an increased cardiovascular risk [28]. EEPT showing anti-hyperlipidemic and anti-hypercholesterolemic effects opens up a promising avenue for scientific inquiry into addressing the intricate web of hyperlipidemia and obesity.

In the realm of scientific investigation, EEPT emerges as a fascinating subject that warrants further exploration and scrutiny as we strive to unravel the complex dynamics of lipid metabolism and its impact on our cardiovascular health.

CONCLUSION

Our research thoroughly investigates the potential therapeutic benefits of EEPT, an ethanolic extract obtained from *Parmotrema tinctorum*. We specifically focus on its ability to tackle two critical health problems hyperlipidemia and obesity. These health issues play a crucial role in the development of atherosclerosis, a condition characterized by the accumulation of lipids in our arteries. What makes EEPT intriguing is its rich content of saponins, compounds that have shown promise in influencing our lipid profiles and even regulating our food intake. This indicates the potential of EEPT as a therapeutic agent for managing lipid metabolism and potentially combating conditions like hyperlipidemia and obesity. Given the intricate relationship between our diet, how our bodies process lipids, and its impact on cardiovascular health, EEPT warrants further investigation. This discovery paves the way for exciting scientific investigations, providing a glimmer of hope in finding innovative solutions that can greatly improve public health.

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CONFLICT OF INTEREST : None

FINANCIAL SUPPORT : None

ETHICS STATEMENT : The experimental protocol and the number of animals used in this research study were approved by the Institutional Animals Ethics Committee (IAEC) in its meeting held on 18th August 2023.

REFERENCES

1. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: The linking mechanism and the complications. Arch Med Sci. 2017;13(4):851-63. doi:10.5114/aoms.2016.58928
2. Flores-Dorantes MT, Díaz-López YE, Gutiérrez-Aguilar R. Environment and gene association with obesity and their impact on neurodegenerative and neurodevelopmental diseases. Front Neurosci. 2020;14:863. doi:10.3389/fnins.2020.00863
3. Nelson R. Hyperlipidemia as a risk factor for cardiovascular disease. Prim Care Clin Office Pract. 2013;40(1):195-211. doi:10.1016/j.pop.2012.11.003
4. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233-41. doi:10.1038/35025203
5. Pepe RB, Lottenberg AM, Fujiwara CT, Beyruti M, Cintra DE, Machado RM, et al. Position statement on nutrition therapy for overweight and obesity: Nutrition department of the Brazilian association for the study of obesity and metabolic syndrome (ABESO—2022). Diabetol Metab Syndr. 2023;15(1):124. doi:10.1186/s13098-023-01037-6
6. Ohmura Y, Takeshita S, Kawachi M. Photobiont diversity within populations of a vegetatively reproducing lichen, Parmotrema tinctorum, can be generated by photobiont switching. Symbiosis. 2018;77(1):59-72. doi:10.1007/s13199-018-0572-1
7. Kanmani P, Rajiv Kannan A. Evaluation of morphological responses in Parmotrema tinctorum lichen using back propagation neural networks collected from Servarayan Hills of Tamilnadu, India. Int Res J Pharmacy. 2017;8(4):95-9. doi:10.7897/2230-8407.080456
8. Crawford S. Lichens used in traditional medicine. In: Lichen Secondary Metabolites. 2014. p. 27-80. doi:10.1007/978-3-319-13374-4_2
9. Malhotra S, Subban R, Singh A. Lichens - role in traditional medicine and drug discovery. Internet J Altern Med. 2008;5(2):1-5. doi:10.5580/3d9
10. Plante J, Eason C, Snyder A, Elston D. Tofacitinib in the treatment of lichen planopilaris: A retrospective review. J Am Acad Dermatol. 2020;83(5):1487-9. doi:10.1016/j.jaad.2020.05.104
11. Raj P, Prathapan A, Sebastian J, Antony A, Riya M, Rani M, et al. Parmotrema tinctorum exhibits antioxidant, antiglycation, and inhibitory activities against aldose reductase and carbohydrate digestive enzymes: An in vitro study. Nat Prod Res. 2014;28(18):1480-4. doi:10.1080/14786419.2014.909420
12. Thadhani V, Karunaratne V. Potential of lichen compounds as antidiabetic agents with antioxidative properties: A review. Oxid Med Cell Longev. 2017;2017(1):2079697. doi:10.1155/2017/2079697
13. Syed Zameer Ahmed K, Ahmed SSZ, Thangakumar A, Krishnaveni R. Therapeutic effect of Parmotrema tinctorum against complete Freund's adjuvant-induced arthritis in rats and identification of novel isophthalic ester derivative. Biomed Pharmacother. 2019;112:108646. doi:10.1016/j.biopha.2019.108646
14. Shiromi PSAI, Hewawasam RP, Jayalal RGU, Rathnayake H, Wijayaratne WMDGB, Wanniarachchi D, et al. Chemical composition, and antimicrobial activity of two Sri Lankan lichens, Parmotrema rampoddense, and Parmotrema tinctorum against methicillin-sensitive and methicillin-resistant Staphylococcus aureus. Evid Based Complement Alternat Med. 2021;2021(1):9985325. doi:10.1155/2021/9985325
15. Shiriki D, Ubwa ST, Yusufu MI, Shambe T. Extraction methods and inhibition studies of ten plant extracts on nine yam rot pathogenic microorganisms. Food Nutr Sci. 2019;10(04):439-58. doi:10.4236/fns.2019.104033
16. Adeneye AA, Adeyemi OO, Agbaje EO. Anti-obesity and antihyperlipidaemic effect of Hunteria umbellata seed extract in experimental hyperlipidemia. J Ethnopharmacol. 2010;130(2):307-14. doi:10.1016/j.jep.2010.05.009
17. Sikarwar M, Patil M. Antihyperlipidemic activity of Salacia chinensis root extracts in triton-induced and atherogenic diet-induced hyperlipidemic rats. Indian J Pharmacol. 2012;44(1):88. doi:10.4103/0253-7613.91875

18. Rautela GS, Liedtke RJ. Automated enzymic measurement of total cholesterol in serum. Clin Chem. 1978;24(1):108-14. doi:10.1093/clinchem/24.1.108
19. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res. 1970;11(6):583-95. doi:10.1016/s0022-2275(20)42943-8
20. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502. doi:10.1093/clinchem/18.6.499
21. Mosae Selvakumar P. Phenol sensing studies by 4-amino antipyrine method-A review. Org Med Chem Int J. 2018;5(2). doi:10.19080/omcij.2018.05.555657
22. Liu Z, Pan J. A practical method for extending the biuret assay to protein determination of corn-based products. Food Chem. 2017;224:289-93. doi:10.1016/j.foodchem.2016.12.084
23. Bano N, Najam R. Histopathological and biochemical assessment of liver damage in albino Wistar rats treated with cytotoxic platinum compounds in combination with 5-fluorouracil. Arch Med Sci. 2019;15(4):1092-103. doi:10.5114/aoms.2019.86064
24. Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, et al. Pathophysiology of atherosclerosis. Int J Mol Sci. 2022;23(6):3346. doi:10.3390/ijms23063346
25. Gaforio JJ, Visioli F, Alarcón-de-la-Lastra C, Castañer O, Delgado-Rodríguez M, Fitó M, et al. Virgin olive oil and health: Summary of the III International conference on virgin olive oil and health consensus report, JAEN (Spain) 2018. Nutrients. 2019;11(9):2039. doi:10.3390/nu11092039
26. Shi Y, Guo R, Wang X, Yuan D, Zhang S, Wang J, et al. The regulation of alfalfa saponin extracts on key genes involved in hepatic cholesterol metabolism in hyperlipidemic rats. PLoS One. 2014;9(2):e88282. doi:10.1371/journal.pone.0088282
27. Hewlings S. Coconuts and health: Different chain lengths of saturated fats require different consideration. J Cardiovasc Dev Dis. 2020;7(4):59. doi:10.3390/jcdd7040059
28. Soliman G. Dietary cholesterol and the lack of evidence in cardiovascular disease. Nutrients. 2018;10(6):780. doi:10.3390/nu10060780