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

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A R T I C L E I N F O

Keywords:
Ethanolic Extract from Parmotrema Tinctorum (EEPT), Obesity, Hyperlipidemia, Low-Density Lipoprotein Cholesterol (LDL-C), and Very Low-Density Lipoprotein Cholesterol (VLDL-C).

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A B S T R A C T

Background: Hyperlipidemia and obesity are prevalent global health concerns linked to increased risks of atherosclerosis, coronary artery diseases, and cerebral vascular diseases due to elevated blood lipid levels. Targeting these conditions via natural agents presents a potential therapeutic avenue.
Methodology: Using an olive oil-induced hyperlipidemic rat model, the study evaluated the antihyperlipidemic and antiobesity effects of ethanolic extract from Parmotrema tinctorum. Rats were divided into distinct treatment groups, receiving simvastatin, distilled water (control), and two doses of the extract (200 mg/kg and 400 mg/kg). The experiment spanned 28 days.
Result: The administration of Parmotrema tinctorum extract resulted in significant reductions in body and liver weight gain. The serum lipid profile demonstrated dose-dependent decreases in total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). Moreover, atherogenic index and blood glucose levels were notably reduced. The effect of extract was attributed to potential anorectic actions mediated through the central nervous system. The extract did not influence gastric emptying and showed the inhibition of de novo cholesterol biosynthesis. Conclusion: The study highlights the potential of ethanolic extract from Parmotrema tinctorum as a dual-action therapeutic agent for addressing hyperlipidemia and obesity. The findings underscore its capacity to modulate lipid metabolism, offering potential implications for managing cardiovascular and metabolic disorders. Further research is warranted to elucidate the mechanisms underlying its observed effects and to explore its translational potential in clinical settings.

1. INTRODUCTION

Obesity stands as a serious health challenge stemming from difficulties in effectively processing carbohydrates and lipids within the body. Due to which body will start storing more amount of fats in adipose tissue as well in vital organs like 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. 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, belonging to 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 to 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]. *Parmotrema tinctorum* is marked by a diverse assortment of chemical compounds, featuring praesorediosic 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 essential vitamins like vitamin C [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.

2. MATERIALS AND METHODS

2.1. Plant material

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

2.2. Preparation of Plant extract

For the preparation of an ethanolic extract, 350g of powder was mixed with then subjected to continuous hot extraction with ethanol in a Soxhlet apparatus. The extract was filtered through a cotton plug, followed by Whatman filter paper (No. 1), and dried at 40-50 °C to get a brownish sticky mass. The % yield of the extract was 46%. The extract was stored in the refrigerator at 4 -8°C for further use [15].

2.3. Animals

Experiments were performed in accordance with the Committee for Control and Supervision of Experimental Animals (CCSEA). The experimental protocol in the study was approved by the Institutional Animals Ethics Committee. Wistar albino rats of either sex weighing 180-200g were procured from the Geniron Biolabs Pvt. Ltd. Bangalore, Karnataka. Animals were housed at CCSEA approved animal house of Srinivas College of Pharmacy, Mangalore. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory condition (12 hr. light and 12 hr. dark cycle) and had free access to commercial pellet diet with water ad libitum. The animal house temperature was maintained at 25±20C with relative humidity at (50±15%). Ethical norms were strictly followed during all the experiments.

2.4. Study design

Group-1: Served as control received 0.9% acacia (5ml/kg/p.o).

Group-2: The animals were pre-treated with 0.9% acacia 1 h before oral treatment with 5ml/kg of

olive oil served as model control.

Group-3: The animals were pre-treated with (20mg/kg/p.o) simvastatin, 1 h before oral treatment with (5mg/kg/p.o.) of olive oil.

Group-4: The animals ware pre-treated with (200mg/kg/p.o) of EEPT in 0.9% acacia, respectively, 1 h before the olive oil treatment.

Group-5: The animals ware pre-treated with (400mg/kg/p.o) of EEPT in 0.9% acacia, respectively, 1h before the olive oil treatment.

For the designated group of animals, the previously mentioned treatment plan was followed for 28 days. Every day, 1 hour before receiving the normal medication and the ethanol extracts of *Parmotrema tinctorum*, all the animals received olive oil [16]. Investigations were conducted into how these drugs affect body weight, relative liver weight, food consumption, serum lipids, atherogenic index (AI), blood glucose, and the histology of the liver.

2.5. Biochemical Parameters

2.5.1. Collection of blood samples

On day 29 of experiment, the animal blood samples were collected in Eppendorf tubes from the retro-orbital plexus of rats by inserting a fine capillary gently in the inner angle of the eye. The tubes containing the blood samples were allowed to stand for 30 min at 37^{o} c and clear serum was separated at 2500 rpm for 10 min using micro centrifuge [17].

2.5.2. Biochemical analysis

All samples were used for following biochemical investigations. The blood serum under this model has been analysed for the marker parameters such as total cholesterol (TC), High density lipoprotein cholesterol (HDL-C) and triglycerides (TG). Serum total cholesterol and triglycerides were estimated by enzymatic methods of CHOD-PAP and GPO-Trinder method respectively [18]. Estimation of HDL-C was done by precipitation method [19]. All parameters were analysed by ERBA Autoanalyser (Spectrophotometric) with standard biochemical kits (ERBA Diagnostic Mannheim GmbH, Germany). Serum concentrations of Very low density lipoprotein-Cholesterol (VLDL-C) and Low density lipoprotein-Cholesterol was calculated using Friedewald's formula [20]. The atherogenic indices were calculated.

2.5.3. Estimation of blood glucose by GOD/POD method:

Glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of glucose present in the sample and measure the absorbance at 505 nm [21].

2.5.4. Estimation of Total protein:

Proteins bind with copper ions in the alkaline medium of biuret reagent and produce a purple-colored complex whose absorbance is proportional to the protein concentration and measured the absorbance of standard and test against and blank on a colorimeter with yellow green filter or on a spectrophotometer at 546 nm [22].

2.6. Histopathology Studies

Rat livers were excised after humane sacrifice of the animals under anesthesia and fixed in 10% formalin. Fixed tissues were completely dehydrated in absolute ethanol and processed routinely for embedding in paraffin wax. From these 5µm sections were prepared and stained with hematoxylin–eosin dye. Stained slides were viewed using an optical photomicroscope at 100× magnification [23].

2.7. Statistical Analysis

Graph Pad Prism software, version 9.5.1 was used in the statistical analysis of experimental data. The statistical Analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's test. p values $p<0.001$, $p<0.01$, $p<0.05$ were considered as significant.

3. EMPIRICAL ANALYSIS

In this research, we examined the impact of various treatments on different aspects of physiological health, as outlined in Tables 1 to 5. Group II animals, which received olive oil in their diet, showed a marked increase in their daily food consumption compared to Group I animals, a difference that was statistically significant ($p<0.001$). Conversely, Group III, IV, and V animals exhibited noteworthy reductions in their daily food intake when compared to Group II, with statistical significance ($p<0.01$, $p<0.001$). Regarding body weight, Group II animals exhibited a substantial increase between day 1 and 29 when compared to Group I animals, with a statistically significant difference ($p<0.001$). In contrast, Group III, IV, and

V animals displayed significant decreases in body weight compared to Group II. The administration of the EEPT extract at two different dose levels resulted in a dose-dependent reduction in body weight (Table 2). Turning our attention to the lipid profiles (Table 3), Group II animals fed with olive oil exhibited significant increases in total cholesterol and a decrease in HDL cholesterol, both with statistical significance ($p<0.001$), when compared to Group I. Conversely, Group III, IV, and V animals demonstrated significant increases in HDL levels and significant decreases in total cholesterol ($p<0.01$, $p<0.001$, $p<0.05$). Triglyceride levels significantly increased in Group II ($p<0.001$) but decreased significantly in Groups III and IV ($p<0.001$, $p<0.01$), and significantly in Group V ($p<0.001$). LDL and VLDL levels showed similar trends, with significant increases in Group II ($p<0.001$) and significant decreases in Groups III, IV, and V ($p<0.001$). The atherogenic index decreased in the treated groups, with percentage protection percentages of 63.14% for Group III, 38.46% for Group IV, and 46.16% for Group V (Table 4). Blood glucose levels in Group II significantly increased ($p<0.01$) compared to Group I, while Groups III, IV, and V exhibited significant decreases ($p<0.001$, $p<0.05$, $p<0.01$) (Table 5). Lastly, total protein levels (Table 5) significantly increased in Group II ($p<0.01$) compared to Group I, whereas Group III, IV, and V animals displayed significant decreases ($p<0.05$, $p<0.01$) compared to Group II. These results indicate complex physiological responses to different treatments, with potential implications for overall health.

3.1. Histopathology

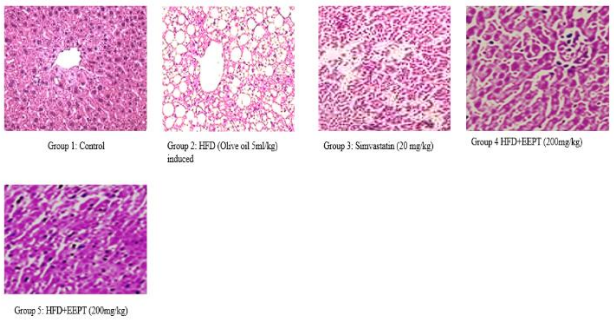

Figure 1. Effects of ethanolic extract of Parmotrema tinctorum on histopathology of liver from HFD-induced hyperlipidemic rats

Table 1: Effect of Ethanolic extract of *Parmotrema tinctorum* on daily food intake

Sl. No.	Group	Treatment	Food intake			
			Day 1	Day 7	Day 14	Day 28
1	I	Control	123.76±0.23	125.45±1.39	127.93±0.60	132.45±0.42
2	II	Olive oil induced (5ml/kg/p.o)	143.4±0.52***	148.4±0.321***	151.4±0.47***	155.45±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 values presented in the table represent the Mean ± SEM of animals. Statistical comparisons were made between different groups as follows: Group I vs. Group II and Group II vs. Group III, IV, and V. The significance levels were denoted as follows: *p<0.05, **p<0.01, ***p<0.001, and "ns" indicated non-significant differences.

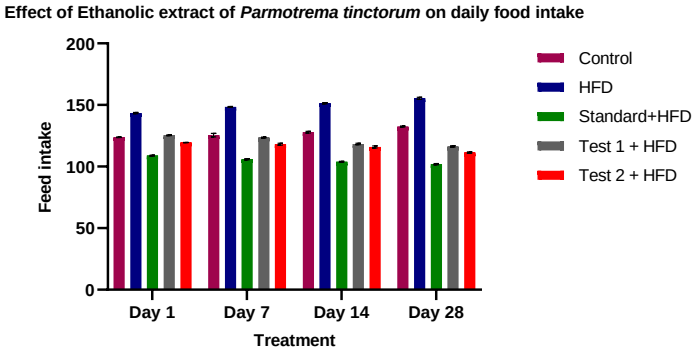

Figure 2. Effect of Ethanolic extract of Parmotrema

tinctorum on daily food intake

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

Sl. No	Groups	Treatment	Weight on day1	Weight on day 29	Weight gain
1	I	Control	163.45±0.82	182.41±o.63	18.96±0.24
2	II	Olive oil induced (5ml/kg/p.o)	172.65±0.43	291.68±0.95	119.03±0.55***
3	III	Simvastatin (20mg/kg/p.o)	165.15±0.3	174.60±1.26	9.45±1.13***
4	IV	EEPT (200mg/kg/p.o)	168.76±0.22	186.16±0.70	17.73±0.75***
5	V	EEPT (400mg/kg/p.o)	167.16±0.24	181.08±3.02	14.08±0.42***

The values presented in the table represent the Mean ± SEM of animals. Statistical comparisons were made between different groups as follows: Group I vs. Group II and Group II vs. Group III, IV, and V. The significance levels were denoted as follows: *p<0.05, **p<0.01, ***p<0.001, and "ns" indicated non-significant differences.

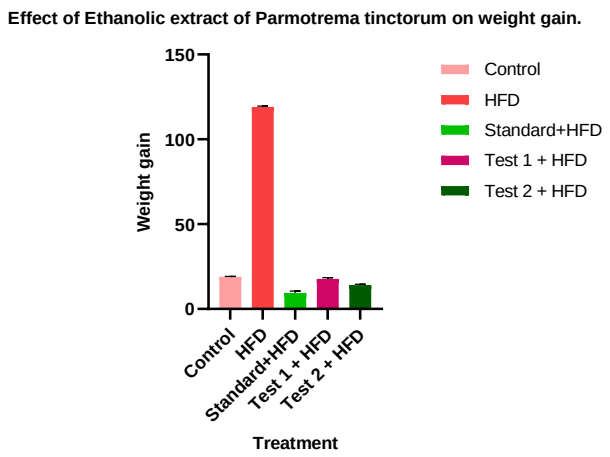

Figure 3. Effect of Ethanolic extract of Parmotrema tinctorum on weight gain.

The values presented in the table represent the Mean ± SEM of animals. Statistical comparisons were made between different groups as follows: Group I vs. Group II and Group II vs. Group III, IV, and V. The significance levels were denoted as follows: *p<0.05, **p<0.01, ***p<0.001, and "ns" indicated non-significant differences.

Table 3**:** Effect of Ethanolic extract of *Parmotrema tinctorum* on 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/k	110.12±0.481***	182.48±0.643***	26.100±0.138***	36.72±0.248***	54.200±1.26**

	g/p.o)					

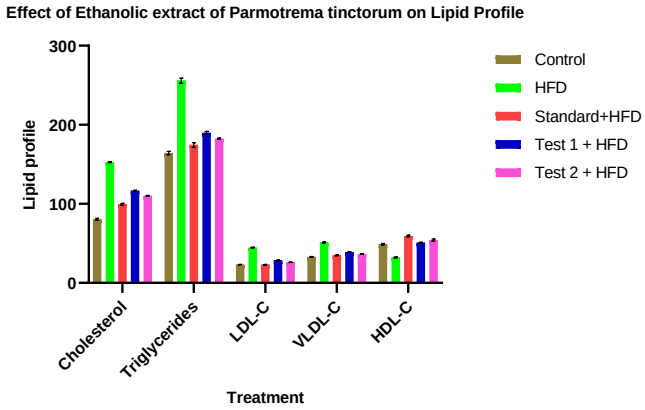

Figure 4. Effect of Ethanolic extract of Parmotrema tinctorum on Lipid Profile

Table 4: Atherogenic index and percentage protection of Ethanolic extract of *Parmotrema tinctorum*.

Sl. No	Group	Treatment	Atherogenic index	% protection
1	I	Control	3.34	-
2	II	Olive oil induced (5ml/kg/p.o)	7.95	-
3	III	Simvastatin (20mg/kg/p.o)	2.93	63.14
4	IV	EEPT (200mg/kg/p.o)	3.89	38.46
5	V	EEPT (400mg/kg/p.o)	3.41	46.16

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

Table 5**:** Effect of Ethanolic extract of *Parmotrema tinctorum* on blood glucose and total protein levels

Sl. No	Group	Treatment	Blood glucose (mg/dl)	Total protein(gm/dl)
1	I	Control	76.41±0.925	5.425±0.008
2	II	Olive oil induced (5ml/kg/p.o)	135.12±1.567**	12.237±0.057**
3	III	Simvastatin (20mg/kg/p.o)	86.77±1.749***	4.36±0.063**
4	IV	EEPT (200mg/kg/p.o)	102.367±1.29*	6.44±0.050*
5	V	EEPT (400mg/kg/p.o)	93.30±0.849**	5.045±0.042**

The values presented in the table represent the Mean ± SEM of animals. Statistical comparisons were made between different groups as follows: Group I vs. Group II and Group II vs. Group III, IV, and V. The significance levels were denoted as follows: *p<0.05, **p<0.01, ***p<0.001, and "ns" indicated non-significant differences.

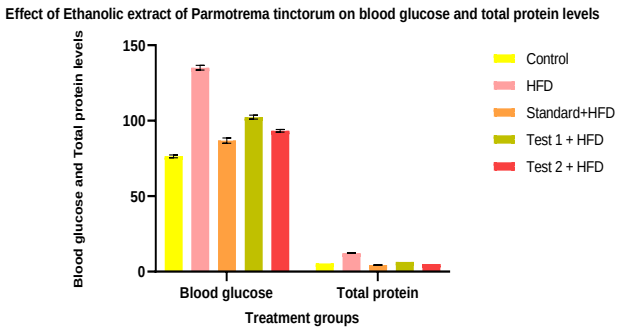

Figure 5**.** Effect of Ethanolic extract of *Parmotrema tinctorum* on blood glucose and total protein levels.

4. DISCUSSION

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 hyperlipidaemia 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 absolutely 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-hyperlipidaemic and anti-hypercholesterolemic effects open up a promising avenue for scientific inquiry into addressing the intricate web of hyperlipidaemia 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.

5. CONCLUSIONS

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 like hyperlipidaemia 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 hyperlipidaemia 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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