RESEARCH ARTICLE

Evaluation of Polyherbal Formulation for Antihyperlipidemic Activity

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Received: 15th September, 2023; Revised: 05th February, 2024; Accepted: 28th February, 2024; Available Online: 25th March, 2024

ABSTRACT

Hyperlipidemia, characterized by elevated plasma concentrations of lipids and lipoproteins, emerged as a decisive menace for cardiovascular disorders. Imminent side effects linked to synthetic drugs; there is a growing interest in exploring herbal remedies for managing hyperlipidemia. This study delves into examining the antihyperlipidemic effects of a polyherbal formulation composed of extracts from *Ailanthus excelsa* Roxb (leaves and stems), *Cymbopogon citratus* (leaves), and *Allium sativum* (bulbs).

The PHF was orally administered at 200 and 400 mg/kg doses. Results revealed a noteworthy reduction in serum and liver biochemical parameters in hyperlipidemic rats treated with PHF. Additionally, the extract demonstrated a noteworthy decrease in the action of HMG-CoA reductase, indicating a potential mechanism for its antihyperlipidemic activity.

To sum up, PHF administered at doses of 200 and 400 mg/kg exhibited effective antihyperlipidemic activity in both animal models. The observed inhibition of HMG-CoA reductase enzyme pathway suggests a plausible mechanism of action for the extract's therapeutic effects. These findings contribute to the expanding body of evidence supporting the clinical relevance of herbal drugs in hyperlipidemia treatment, offering a potential avenue for developing alternative and safer therapeutic interventions for cardiovascular health.

Keywords: Polyherbal, HMG-CoA, Antihyperlipidemic, Cardiovascular, Lipid.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.1.15

How to cite this article: Agrawal A, Dubey SK. Evaluation of Polyherbal Formulation for Antihyperlipidemic Activity. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):101-105.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Hyperlipidemia, a metabolic chaos is a key risk factor for cardiovascular disorders (CVD). Traditional medicinal practices, particularly those rooted in Ayurveda, have identified various herbal remedies for managing hyperlipidemia without the potential side effects associated with synthetic drugs. Here, we explored antihyperlipidemic latent of a polyherbal formulation derived from *Ailanthus excelsa* Roxb (leaves and stems), *Cymbopogon citratus* (leaves), and *Allium sativum* (bulbs) in rat induced with hyperlipidemia using various agents.[1-5]

The choice of these specific botanicals is grounded in their traditional use as antihyperlipidemic agents, as documented in Ayurvedic literature. *A. excelsa* Roxb, *C. citratus*, and *A. sativum* have individually exhibited lipid – lowering properties, making them promising candidates for a synergistic polyherbal formulation. Triton WR-1339 and HFD-induced hyperlipidemia are well-established experimental paradigms for studying lipid metabolism and evaluating the efficacy of potential antihyperlipidemic agents.[6-9]

Here antihyperlipidemic effects of hydroalcoholic extract of polyherbal formulation at varying dose are explored. Evaluation will encompass the impact on serum and liver cholesterol and triglyceride levels, as well as modulation of HMG-CoA reductase activity.[10]

MATERIALS AND METHODS

Collection and Authentication

Locally sourced leaves and stems of *A. excelsa* Roxb, leaves of *C. citratus*, and bulbs of *A. sativum* were purchased. Collected specimens were authenticated and voucher specimens were secured and deposited for prospect reference. The leaves of *C. citratus* were meticulously washed, chopped, and air–dried at temperatures ranging from 35 to 40°C. Following drying, the leaves were subjected to multiple milling processes using an electric grinder.

In the case of *A. excelsa* Roxb leaves and stem, as well as *A. sativum* bulbs, they were shed-dried and subsequently powdered. These powdered materials underwent an exhaustive uninterrupted searing extraction method in a soxhlet's

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apparatus with 70:30 ratio of water: alcohol. Qualitative analysis was engaged to pigeonhole chemical constituents present in all extracts.[11] Resulting masses were dried and vigilantly stored in airtight containers in a cold environment for future use.

Formulation of Polyherbal Tablets

To create approximately 25 tablets, a mixture of separately weighed 200 grams of powdered herbs was prepared. Following the individual weighing, the powdered extracts were thoroughly pulverized using a mortar and pestle. After achieving a uniform blend of all particles, the mixture underwent sieving through a no. 85 sieve. Subsequently, the sieved powder material was utilized for compression. Then 25 tablets were prepared for auxiliary assessment using tablet punching appliance.[12]

Animals

Male rats (150 – 200 g) used in study were maintained under typical ecological stricture with unrestricted food/water. Sanction for the experimental protocol was taken from IAEC.

Acute Toxicity Studies

It was conducted as per OECD guideline 423. All animals fed with polyherbal formulation (PHF) by oral route. NO sign and symptoms were observed at 2000 mg/kg.[13]

Methods

Triton WR 1339 (Isooctyl Polyoxyethylene Phenol) induced hyperlipidemia model[14]

Induction of hyperlipidemia using triton WR-1339, which accelerates hepatic cholesterol synthesis, is considered a crucial method for evaluating the effects of hypolipidemic drugs. Male Sprague–Dawley rats (200 – 250 g) were distributed into five groups with 6 animals in each. Normal control, received normal saline (1 week). The second group was administered Triton WR-1339 unraveled in 0.9% saline through i.p. route. 3rd and 4th group received PHF at 200 and 400 mg/kg/p.o/week. The 5th treated with atorvastatin in 0.5% carboxymethyl cellulose at 10 mg/kg/p.o/once a week.

On seventh day, all groups were subjected to 18 hours fasting with *ad libitum* followed by 400 mg/kg/i.p Triton WR 1339 immediately after administration of respective drugs.

On 8th day, blood was withdrawn *via* retro-orbital sinus pierced. It was centrifuged for 10 minutes at 2000 rpm, and the resulting serum was used for tests. Following blood collection, animals were sacrificed, and livers were carefully harvested for further analysis.

HCD induced atherogenesis[15]

Male albino rats (200–250 g) are categorized into five groups, each comprising six animals. All groups, except the normal control group, were subjected to a high cholesterol (HC) diet for 30 days, with a standard pellet diet. The treatment group received PHF at various doses.

- *Normal control group*

Received oral administration of normal saline for the entire 30-day period.

- *High cholesterol diet (HCD) positive control group*

Given for 30 days.

- *Polyherbal formulation group-1*

200 mg/kg/PO/once a day/30 days

- *Polyherbal formulation group-2*

400 mg/kg/PO/once a day/30 days

- *Atorvastatin group*

Group treated with atorvastatin suspension:10 mg/kg/PO/30 days.

After a 30-day experimental duration, blood was obtained through retro-orbital puncture and then subjected to centrifugation for 10 minutes at 2000 rpm. The resulting serum samples were utilized for conducting various biochemical tests. Following blood collection, the animals were euthanized, and livers were collected for further analysis.

Biochemical analysis of serum

The serum samples underwent comprehensive analysis to assess various biochemical parameters.

Furthermore, low density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were determined by employing the friedwald rule.

The atherogenic index (AI) was also determined utilizing a formula specifically designed for this objective.[16]

Biochemical analysis of liver[17]

Liver homogenization was carried out using a total of 20 volumes of cold chloroform: methanol mixture (2:1 v/v). The resulting contents underwent filtration through Whatman filter paper. The residue was subjected to a second extraction with smaller volumes of the chloroform: methanol mixture (2:1 v/v) and underwent another round of filtration. Filtrates from both rounds were combined, and 0.3 mL of glass-distilled water was introduced. The mixture was thoroughly mixed by vortexing and kept for phase separation at 25 to 30ºC.

Subsequently, upper phase was carefully collected using a pipette, and lower phase underwent a wash with 0.4 mL of Folch's pure upper phase solvent, composed of chloroform: methanol: water in a ratio of 3:48:47. The mixture was centrifuged at 3000 rpm for 10 minutes. The upper phase was discarded, and the lower phase was retained for the estimation of various biochemical parameters.

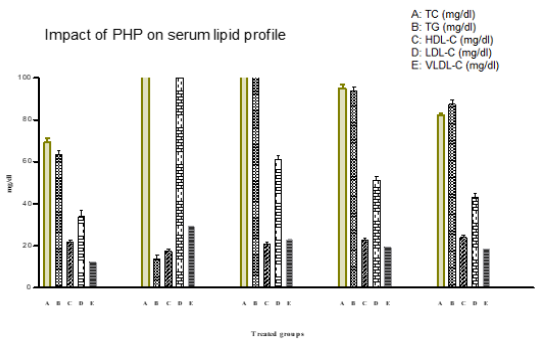

Figure 1: Impact of PHF on lipid profile in the serum

Assessment of hepatic HMG CoA reductase activity[18]

It was assessed indirectly through HMG CoA/Mevalonate share. A tissue homogenate is prepared by homogenizing 1-gm of liver tissue with 10 mL of saline arsenate. Alike volumes of prepared 10% tissue homogenate shared with diluted $HClO_4$. and permitted to rest for 5 minutes, centrifugated at 2000 rpm for 10 minutes.

Subsequently, 1-mL of filtrate delighted with 0.5 mL of alkaline hydroxylamine, mixed well, and, after 5 minutes, 1.5 mL of $FeCl_3$ added. Mixture was mixed, and was recorded after 10 minutes at 540 nm.

Statistical Analysis

Data was analysed by ANOVA, trailed by the Tukey test for post-hoc comparisons. Statistical momentousness is considered at $p < 0.05$. Results presented as Mean ± SEM, n = 6.

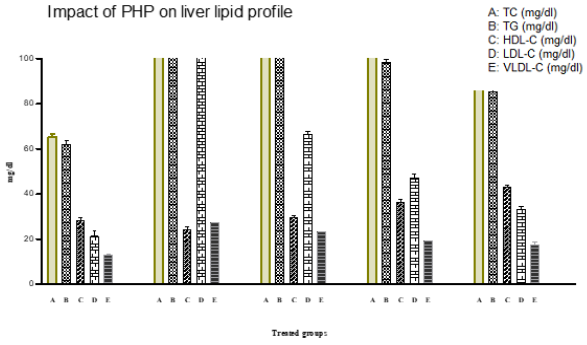

Figure 2: Impact of PHF on liver lipid profile

RESULTS

Consequences of PHF on Triton-Initiated Hyperlipidemia

Outcomes unveiled a substantial reduction in serum and liver biochemical indicators among rats receiving PHF when compared to their Triton-treated counterparts (Figures 1-4).

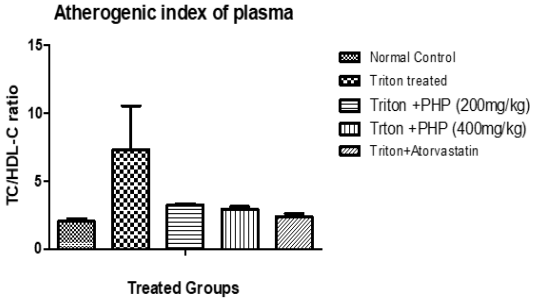

Figure 3: Atherogenic index of plasma

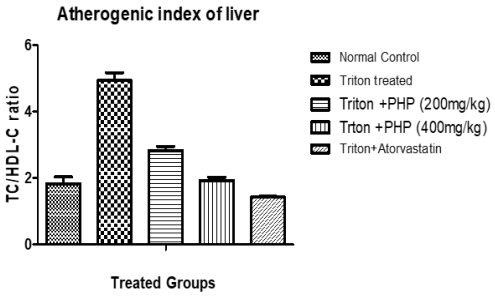

Figure 4: Atherogenic index of liver

Table 1: Impact of PHF on serum lipid profile in HCD-fed rats

Intervention	*TC (mg/dl)*	*TG (mg/dl)*	*HDL-C (mg/dl)*	*LDL-C (mg/dl)*	*VLDL-C (mg/dl)*	*Atherogenic index*
Normal	48.21 ± 1.06	44.32 ± 1.44	26.44 ± 0.34	10.12 ± 1.02	9.20 ± 0.29	0.9 ± 0.02
Triton treated	61.46 ± 1.95**	55.78 ±1.73*	22.10 ± 1.02*	30.33 ± 2.10**	11.22 ± 0.45*	2.01 ± 0.22*
HCD + PHF (200 mg/kg)	58.34 ± 0.41$^{\#}$	46.67 ±1.54$^{\#}$	26.82 ± 0.99$^{\#}$	17.29 ± 1.82$^{\#}$	10.21 ± 0.22$^{\#}$	1.02 ± 0.12$^{\#}$
HCD + PHF (200 mg/kg)	48.13 ± 1.92$^{\#\#}$	44.34 ±1.79$^{\#\#}$	31.23 ± 1.23$^{\#}$	9.13 ± 1.78$^{\#\#}$	9.89 ± 0.43$^{\#}$	0.64 ± 0.6$^{\#\#}$
HCD + Atorvastatin	53.97 ± 1.11$^{\#}$	48.22 ±1.02$^{\#}$	33.43 ± 1.02$^{\#\#}$	11.38 ± 1.02$^{\#\#}$	9.22 ± 0.20$^{\#}$	0.71 ± 0.02$^{\#\#}$

Table 2: Impact of PHF on liver lipid profile in HCD fed rats

Intervention	*TC (mg/dl)*	*TG (mg/dl)*	*HDL-C (mg/dl)*	*LDL-C (mg/dl)*	*VLDL-C (mg/dl)*	*Atherogenic index)*
Normal	64.47 ± 1.41	55.22 ± 2.29	27.93 ± 1.11	26.26 ± 1.76	11.93 ± 0.44	1.27 ± 0.20
Triton treated	111.36 ± 2.44**	93.29 ± 2.03**	25.67 ± 0.84*	69.49 ± 2.48**	20.12 ± 0.34*	3.93 ± 0.17**
HCD + PHF (200 mg/kg)	89.44 ± 3.11$^{\#\#}$	79.30 ± 1.71$^{\#\#}$	29.20 ± 1.20$^{\#}$	44.22 ± 1.89$^{\#\#}$	17.02 ± 1.02$^{\#}$	1.78 ± 0.28$^{\#\#}$
HCD + PHF (200 mg/kg)	78.22 ± 1.12$^{\#\#}$	58.49 ± 1.29$^{\#\#}$	32.83 ± 2.01$^{\#}$	30.12 ± 2.11$^{\#\#}$	18.32 ± 0.24$^{\#\#}$	1.01 ± 0.32$^{\#\#}$
HCD + Atorvastatin	88.42 ± 1.22$^{\#\#}$	63.29 ± 1.22$^{\#\#}$	33.43 ± 1.11$^{\#}$	40.64 ± 1.33$^{\#\#}$	13.21 ± 1.23$^{\#\#}$	1.98 ± 0.06$^{\#\#}$

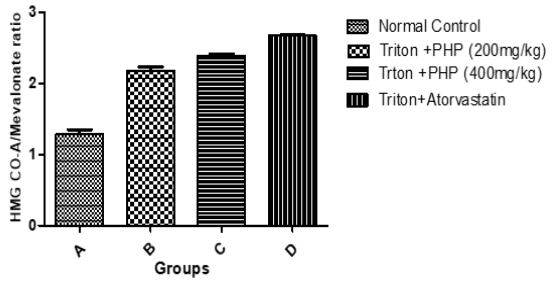

Figure 5: Effects of PHF on HMG-CoA enzyme reductase activity on triton WR 1339.

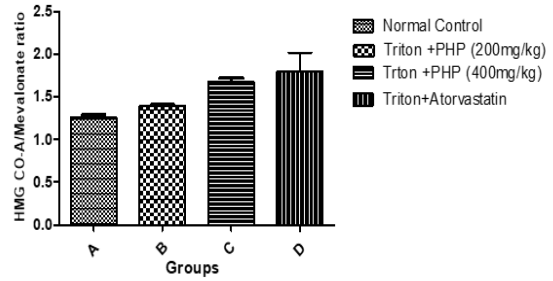

Figure 6: Effects of PHF on HMG-CoA enzyme reductase activity on HCD.

Consequences of PHF on HFD- Initiated Hyperlipidemia

Studied doses of PHF demonstrated a significant lowering effect on both serum and liver biochemical parameters as compared to rats that received a high cholesterol diet (HCD) (Tables 1 and 2).

Effect of PHF on HMG-CoA Reductase Activity

PHF (200 and 400 mg/kg) resulted in a noteworthy augmentation of the HMG-CoA/Mevalonate ratio in hyperlipidemic rats, as assessed in contrast to the normal group (Figures 5 and 6).

DISCUSSION

The present study delved into the antihyperlipidemic effect of a polyherbal formulation comprising extracts from *A. excelsa* Roxb, *C. citratus*, and *A. sativum* in rats induced with hyperlipidemia using triton WR-1339 and a high-fat diet. Results exhibited a noteworthy lessening in serum and liver lipid levels, emphasizing budding therapeutic worth of PHF in mitigating hyperlipidemia.[19]

The observed decline in lipid profile parametes and Atherogenic index in both models highlighted the formulation's ability to amend lipid metabolism. Conversely, there was a momentous enlarge in HDL–C levels, a crucial marker of cardiovascular health, following polyherbal treatment.

Notably, these findings align with traditional knowledge, as *A. excelsa* Roxb, *C. citratus*, and *A. sativum* have individually demonstrated antihyperlipidemic properties in various studies.[20] The synergistic effect of these botanicals in the polyherbal formulation enhances its potential as an effective intervention against hyperlipidemia. Additionally, the study extends support to the use of herbal remedies in managing lipid disorders, aligning with previous research emphasizing the significance of natural products in cardiovascular health.[21]

Furthermore, an exploration into HMG-CoA reductase clarifies promising mechanistic imminent into an antihyperlipidemic deed of PHF. The observed increase in HMG-CoA/Mevalonate ratio suggests a downregulation of HMG-CoA reductase action, a pivotal enzyme in cholesterol biosynthesis.[22] This mechanism aligns with traditional use of these herbs and provides a scientific basis for their antihyperlipidemic effects.[23-25]

Despite these promising results, it is essential to acknowledge certain limitations, including the need for further studies to explore the long-term effects, optimal dosage, and potential adverse effects of the polyherbal formulation. Additionally, comparative studies with standard hypolipidemic drugs would provide a comprehensive understanding of its efficacy in comparison to existing treatments.

Findings emphasize noteworthy antihyperlipidemic potential of PHF, supporting its conventional use in managing hyperlipidemia. The observed lipid-lowering effects, modulation of HMG-CoA reductase activity, and enhancement of HDL–C levels collectively contribute to its therapeutic promise. Prospective research should focus on expounding underlying mechanisms with clinical trials to ascertain its safety and efficacy in human subjects.

CONCLUSION

The study's conclusion underscores PHF's potential as a natural and effective strategy for managing hyperlipidemia. Observed lipid-lowering effects and intonation of HMG-CoA reductase activity contributed to its therapeutic promise, paving way for future research and potential clinical applications in field of cardiovascular health.

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