Determination of chemical composition and investigation of potential of triphala powder in hypercholesterolemia in men in controlled randomized trial

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Abstract: Hypercholesterolemia is a condition with elevated cholesterol and lipid profile. It is the leading reason behind myocardial infarction and coronary heart disease. It is observed in young people as well due to a sedentary lifestyle. Triphala powder has a hypolipidemic and anti-hypercholesterolemia effect. This study was designed to investigate the effect of triphala powder against hypercholesterolemia. This study also examined Triphala powder's chemical composition. Total phenolic and flavonoid content were examined. Encapsulated 400 mg and 600 mg Triphala powder were given to treatment groups I and II. Lipid profile parameters were measured and compared at 0 weeks and 10th weeks in all groups. All results were analyzed using ANOVA in IBM SPSS Statistics 20. Results of proximate analyses have shown that Okra pod powder contains moisture 12.27%, ash 11.25%, nitrogen-free extract 45.93%, crude protein 13.37%, crude fat 2.95%, and crude fiber 14.23%. Mineral analysis showed that iron and manganese are major minerals in triphala powder. Triphala powder showed a significant reduction in lipid profile parameters in hypercholesterolemia. All results are taken significantly at $p<0.05$.

Keywords: Triphala powder, Amla (*Emblica officinalis*), Harad (*Terminalia chebula*) and baheda (*Terminalia belirica*), hypercholesterolemia, hypolipidemic effect, mineral analysis, phytochemical analysis, lipid profile.

INTRODUCTION

Triphala powder is an important and wonderful formulation of ayurvedic medicine. It is often described as an elixir for life. It has many health benefits including lowering the levels of cholesterol and triglycerides, improving bowel movements and having anti-carcinogenic and anti-aging properties. It is used around the globe as a medicinal plant (Sharma, 2015). Hypercholesterolemia is a condition with elevated total plasma LDL-cholesterol levels than normal values (Ibrahim *et al*., 2022). Hypercholesterolemia is a dangerous condition as it can lead to myocardial infarction and coronary heart disease even at a young age (Santos *et al*., 2016). Atherogenesis i.e., plaque formation in the arteries is another major risk associated with hypercholesterolemia. An increasing number of people with hypercholesterolemia is due to their sedentary lifestyle and unhealthy eating habits (Larosa, 2003). Triphala powder is the combination of three important ayurvedic plants including Amla (*Emblica Officinalis*), Harad (*Terminalia chebula*), and based (*Terminalia belirica*). Triphala powder is very well known for its medicinal, anti-hypercholesterolemic, and antioxidant potential against many diseases. This study has investigated the anti-hypercholesterolemic potential of Triphala powder along with determining its chemical composition, mineral analysis, and phytochemical analysis.

MATERIALS AND METHODS

Collection of raw material and preparation of triphala powder

Amla (*Emblica Officinalis*), Harad (*Terminalia chebula*), and based (*Terminalia belirica*) were purchased from the market in dried form. They were cleaned and double-washed. First with tap water and then with distilled water. Then after drying, they were ground separately into powder form and refined. After that, all three powders were mixed in equal amounts and encapsulated with 400mg and 600mg. Gelatin capsules were used for

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encapsulation of triphala dose and gelatin capsules purchased from Halal capsule Pvt. Ltd. found to be safe for human consumption (Touchette & Cox, 2022).

Chemical composition of triphala powder
Proximate profile of triphala powder
AOAC was used to analyze triphala powder for moisture, ash, carbohydrates, crude protein, crude fiber, and NFE (Garcia-Amezquita *et al*., 2018).

Minerals determination
Atomic absorption spectrometry evaluated zinc, magnesium, iron, phosphorus, potassium, sodium and other minerals (Hernández *et al*., 2005).

Phytochemical features of triphala powder
Triphala powder phytochemical analysis for total phenolic and total flavonoid concentration (M'hiri *et al*., 2015) assessed TPC and TFC in mg Gallic acid equivalent and Quercetin equivalent.

Investigation of therapeutic potential of triphala powder against hypercholesterolemia in men
Selection of hyperlipidemic subjects
60hypercholesterolemia patients aged 30-60were randomly picked from the population.

Exclusion criteria
The study excluded hypercholesterolemic patients receiving cholesterol-lowering medicines and those with significant chronic problems.

Inclusion criteria
At research initiation, all hypercholesterolemia male subjects had blood biochemical analysis to examine their lipid profile.

Study duration
The study was conducted for 10 weeks.

Treatment groups and treatment plan
Men were split into three groups of 20each, and the following things happened to each group. The people in the control group got nothing. Triphala powder in capsule form was given to people in treatment group I and treatment group II.

Ethical approval
Ethical approval was taken from the Biosafety Committee of the Institution vide reference No.-----

STATISTICAL ANALYSIS

ANOVA under a Completely Randomised Design (CRD) was used to determine significance ($p < 0.05$) (Larson, 2008). IBM SPSS Statistics 20 performs all analyses.

RESULTS

This study examined Triphala powder's efficacy in lowering lipid profile in hypercholesterolemia.

Proximate composition of triphala powder
Triphala powder was characterized for moisture, ash, crude fibre, crude proteins, fat, and nitrogen-free extract (NFE) (table II). Results Proximate composition was crucial in determining raw material value. Proximate analysis showed that Triphala powder contains 13.37 ± 0.42 protein, 14.23 ± 1.02 of fiber, 11.25 ± 0.23 of ash and 12.27 ± 0.31 is moisture while nitrogen free extract is 45.93 ± 2.71.

Mineral analysis
In this study, Triphala powder was analysed for mineral concentration, including Fe, Mn, Cu, and Zn (table III). Fig. 1 demonstrates that Triphala powder contains mostly iron and manganese.
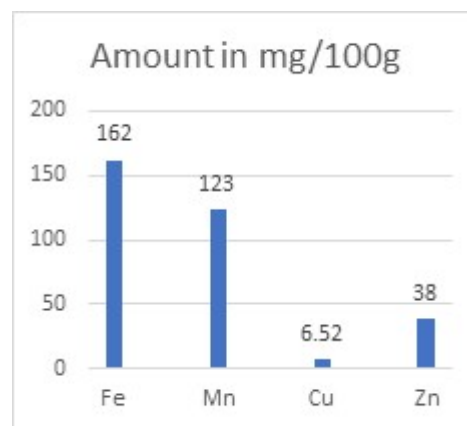
Fig. I: Mineral Composition of Triphala Powder on Dry Weight Basis. X-axis showed minerals and y-axis showed amount in mg.

Phytochemical characters of triphala powder
Antioxidant activity of phenolic compounds was examined. Triphala powder's antioxidant properties lowered lipids and reduced atherosclerosis. Triphala powder was TPC and TFC analysed in methanolic extract. Triphala powder contains a lot of TPC and TFC (table III).

Investigation of antihyperlipidemic potential of triphala powder
This study examined Triphala powder's nutraceutical and therapeutic effects on 60 hypercholesterolemia male patients. The control group (G0) received no treatment, whereas treatment groups-I (G1) and II (G2) received 400 mg/d and 600 mg/d Triphala powder in capsules for 10 weeks. Each subject's HDL, LDL, triglyceride and total cholesterol were measured before and after the experiment.

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Table 1: Treatment groups and treatment plan

Treatment Groups	Title	Treatment
G_0	Hypercholesterolemia Control	No treatment
G_1	Hypercholesterolemia treated group 1	Capsule with Triphala powder of 400 mg per day
G_2	Hypercholesterolemia treated group 2	Capsule with Triphala powder of 600mg

Table 2: Mean±S.D for proximate % composition of triphala powder on dry weight basis

Proximate analysis	Composition (%)
Moisture	12.27±0.31
Ash	11.25±0.23
Nitrogen Free Extract	45.93±2.71
Crude protein	13.37±0.42
Crude Fat	2.95±0.32
Crude fiber	14.23±1.02

Table 3: Mean±S.D for Phytochemical Analysis of Triphala Powder on Dry Weight Basis

Antioxidant	Antioxidant content
TPC (mg GAE/g)	73.29±3.1
TFC mg QE/g)	41.23±7.1

Table 4: Mean±S.D for serum cholesterol level of male subjects

Duration	G_O	G_1	G_2
0 week	271.03±11.47	267.15±12.78	271.32±18.52
10th week	273.21±12.44	251.12±6.49	262.34±16.36

Results are taken significant at *$p<0.05$. G_0 = No Triphala powder, G_1=400 mg/day Triphala powder, G_2= 600 mg per day of Triphala powder

Table 5: Mean±S.D for triglycerides level in male subjects

Duration	G_O	G_1	G_2
0 week	169.30±16.72	175.20±17.56	173.20±18.23
10th week	171.70±19.87	143.30±58.94	154.40±18.71

Table 6: Mean±S.D for HDL level in male subjects

Duration	G_O	G_1	G_2
0 week	37.70±6.78	39.87±11.23	41.58±9.57
10th week	37.60±7.23	46.28±10.97	49.53±8.23

Table7: Mean±S.D for LDL level in male subjects

Duration	G_O	G_1	G_2
0 week	139.60±13.27	137.60±12.35	141.21±12.80
10 week	1420±16.17	131.23±17.17	129.23±11.40

Changes in serum cholesterol level of male subjects
Table IV shows baseline and 10-week serum cholesterol levels. Triphala powder capsules reduced serum cholesterol in both treatment groups ($p<0.05$). Treatment group-I reduced total cholesterol from 267.15±12.78 mg/dl to 251.12±6.49 mg/dl, whereas treatment group II reduced serum cholesterol from 271.32±18.52 mg/dl to 262.34±16.36 mg/dl. Treatment group I reduced cholesterol better than group II. The control group increased cholesterol levels.

Reduction in serum triglycerides level of male subjects
Serum triglyceride levels of all groups on 0 week and 10th week are shown in table V. Both experimental groups have shown significant reduction in triglyceride levels from 175.20±17.56 mg/dl to 143.30±58.94mg/dl in G_1 and from 173.20±18.23 mg/dl to 154.40±18.71 mg/dl in G_2. Treatment group I showed a better reduction in triglycerides level than treatment group II. Triglyceride levels in the control group showed a minor increase in this 10th week study. All results are taken significant at $p<0.05$.

Positive changes in the serum HDL levels

Serum HDL results of all groups are shown in table VI. As compared to the G_0 group, HDL was increased in the experimental group G_1 and G_2. G_1 shows a change in HDL from 39.87 ± 11.23mg/dl to 46.28 ± 10.97mg/dl and G_2 shows an increase in HDL from 41.58 ± 9.57mg/dl to 49.53 ± 8.23mg/dl. Increase in HDL of treatment group II is higher than treatment group-I. While on the other hand, G_0 shows no significant change in the level of serum HDL as compared to G_1 and G_2.

Reduction in the serum LDL levels

Table VII shows how Triphala powder affects serum LDL. After 60 days of Triphala powder, the experimental group declined significantly. G1 and G2 showed a decrease in serum LDL from 137.60 ± 12.35mg/dl to 131.23 ± 17.17mg/dl and from 141.21 ± 12.80mg/dl to 129.23 ± 11.40mg/dl. G2 reduced LDL better than G1. G0 indicates no significant LDL increases compared to Triphala powder groups.

DISCUSSION

The study was designed to see the impact of Triphala powder on lipid profile parameters. Proximate analysis, mineral determination and phytochemical determination was also done.

Proximate analysis showed about 45% NFE and the remainder protein, fat, fiber and ash. Proximate analysis determines plant nutrition. Our six ingredients make up most of Triphala powder and our findings matched prior investigations (Pavani *et al*., 2020).

Iron and manganese predominate in mineral composition. Manganese, like iron, is a micronutrient and component of numerous enzymes and coenzymes that support metabolism. These findings match a prior Triphala powder mineral content analysis (Malik *et al*., 2022).

Phenolic chemicals are vital components of plants that possess antioxidant properties and control oxidative stress in plants. These phenolic compounds possess hydroxyl groups that scavenge free radicals. These antioxidant properties of phytochemicals in Triphala powder are really helpful in lowering the lipid profile especially triglycerides and LDL levels in the serum as results have shown reduction in serum triglycerides levels too. These findings are close to the findings of another study that measured TPC and TFC in a methanolic extract of Triphala powder (Malik *et al*., 2020).

Results have shown a reduction in cholesterol level which was more prominent in treatment group II. These findings were like another study that investigated the effect of Triphala powder against hypercholesterolemia in rats induced with hypercholesterolemia with a high-fat diet.

The study reported that this cholesterol-lowering effect is due to the 20% tannins present in Triphala powder (Saravanan *et al*., 2007).

Results of the study have shown a reduction in triglycerides level using Triphala powder. This lowering of triglycerides was also observed in a study that reported the lipid profile lowering effect of Triphala powder is due to the ability of Amla to reduce the bile reabsorption and increase the excretion of fat outside the body (Nalini & Kapoor, 1999).

Study has shown an increase in serum HDL levels. Treatment group II showed higher increase in HDL level treatment group I. These results were similar to the study designed to investigate the effect of Triphala powder *in vitro* study which reported that the hypolipidemic and HDL-increasing effect of Triphala powder is due to 3-hydroxy-3methyl-glutaryl Coenzyme reductase activity (Mudjupa & Rinthong, 2020).

LDL levels of treatment groups decreased when given Triphala powder. Reduction in LDL level is due to phytochemical, tannins and to 3-hydroxy-3methyl-glutaryl Coenzyme reductase activity. These results were supported by previous research that explained that the LDL-lowering effect of Triphala powder is due to the tannins and antioxidant potential of Triphala powder in (Mudjupa & Rinthong, 2020) and (Nalini & Kapoor, 1999).

CONCLUSION

Amla, harad, baheda are gems of ayurvedic medicine and they are taken as a wonder in the ayurvedic world. Combinations of these in powder and extract form are investigated in many studies for many health benefits. Triphala powder has proven its hypolipidemic, anticarcinogenic, antioxidants and antidiabetic potential in many studies. This study has also proven its therapeutic effect against hypercholesterolemia when investigated in lipid profile parameters. Triphala powder also contains a generous number of secondary metabolites that also helps in lowering the lipids profile. Both doses of 400mg/d and 600 mg/d are proven beneficial in lowering the lipid profile parameters including triglycerides, cholesterol, and LDL levels.

ETHICAL APPROVAL

Ethical approval was taken from the Biosafety Committee of the institution vide Reference No.Ec-108.

ACKNOWLEDGMENTS

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