

A comparative study of Ashwagandha (*Withania somnifera*) root powder and Arjuna (*Terminalia arjuna*) bark powder the herbs of medicinal importance in Ayurveda on total serum cholesterol *In-vitro*

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Abstract

Hypercholesterolemia is one of the most common risk factors for atherosclerotic disease and cardiovascular diseases. Modern medicine is used to manage hypercholesterolemia, but it is associated with long term adverse effects. Ayurveda is a widely practiced medicinal system in the Indian subcontinent for centuries & is a safer herbal system of treatment.

In present studies Arjuna (*Terminalia arjuna*) and Ashwagandha (*Withania somnifera*) were evaluated for their cholesterol-reducing activity on discarded non-infectious pooled serum samples. Hence, both Arjuna and Ashwagandha can be used as safer and more effective alternatives for the treatment of hypercholesterolemia. The outcomes of the study conducted were encouraging.

Keywords: Ashwagandha; Arjuna; Hypercholesterolemia; Herbal; Ayurveda

1. Introduction

Hypercholesterolemia is one of the most common risk factors for atherosclerotic and cardiovascular diseases.^{1,2} Over past few decades, the prevalence of hypercholesterolemia has increased in the Indian population. The major reasons being a sedentary lifestyle, unhealthy eating habits, and reduced physical activity. It was reported that hypercholesterolemia was found in both the urban and rural populations in India in almost 25-30% and 15-20% of populations, respectively.³ Cholesterol is a part of cellular membranes and is used for the synthesis of steroid hormones, bile salts, and vitamin D. Some of the non-modifiable risk factors include age, gender, and genetics while modifiable risk factors include smoking, lifestyle modifications, and healthy eating habits etc. The modifiable risk factors can reduce the comorbidity and mortality associated with hypercholesterolemia.¹

Hypercholesterolemia progresses to atherosclerotic disorders, and lowering cholesterol levels is beneficial in preventing atherosclerotic diseases and cardiovascular diseases.^{2,3}

Strategies used to counter hypercholesterolemia include the modern system of medicine, but are associated with adverse effects, and therefore there is a need to find additional safer approaches.^{1,4} Ayurveda is an alternative to the modern system of medicine and it implies the use of naturally available herbs and medicinal plants for the treatment of hypercholesterolemia¹. Ayurveda applications has resulted in the successful management of hypercholesterolemia.⁵

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The medicinal plants and herbs are available in various preparations and drugs acting in one way or another to reduce cholesterol levels and are being used for centuries in India. Few of the common medicinal plants used for hypercholesterolemia management include *Allium sativum* (garlic), *Commiphora mukul* (guggulu), *Cinnamomum zeylanicum* (cinnamon) and *Ocimum sanctum* (Tulsi) among others.⁶⁻⁸

Withania somnifera (Ashwagandha) is a herb used in the Ayurveda system of medicine for the management of the respiratory system, central nervous system, cardiopulmonary system, and reproductive system disorders.^{2,9} The medicinal properties of Ashwagandha include antitumor, antistress, anti-inflammatory, antioxidant, hemopoietic and immunomodulatory activities.¹⁰

There is not sufficient information available about Ashwagandha's effectiveness in lowering cholesterol and hence, Ashwagandha can be a prospective plant for hypercholesterolemia management. In the present in-vitro study, we have evaluated the total cholesterol reducing activity of Ashwagandha root powder soaked in distilled water (d/w) and Ashwagandha root powder soaked in cow urine (c/u) comparing it with soaked powder of arjuna bark (reference standard) in distilled water (d/w) & cow urine (c/u) respectively.

2. Material and methods

2.1. Chemicals and Reagents

Analytical grade materials were employed throughout the study. A cholesterol dynamic extended stability testing (CHOD-PAP) kit was used with standard cholesterol value at 200 mg / dL. Branded Arjuna bark powder and Ashwagandha root powder were used in the study. Purified branded and distilled cow urine (c/u) and distilled water (d/w) were used in the study.

2.2. Principle

CHOD-PAP method is a colorimetric assay method. Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol & 4-aminoantipyrine by the catalytic action of peroxidase to form a red-colored quinoneimine dye complex. The intensity of the color formed is directly proportional to the amount of cholesterol present in the sample.

2.3. Method

Discarded serum samples (pooled, non-infectious, n=260) were used for the *in vitro* evaluation of ashwagandha powder and Arjuna powder in the study. Arjuna served as the reference standard in the study. Preparations were prepared as follows,

- A1: Arjuna-soaked sample (300 mg powder in D/W for 12 hrs.)
- A2: Arjuna-soaked sample (300 mg powder in C/U for 12 hrs.)
- D1: Ashwagandha-soaked sample (300 mg powder in D/W for 12 hrs.)
- D2: Ashwagandha-soaked sample (300 mg powder in C/U for 12 hrs.)

Arjuna and Ashwagandha powders both branded (300 mg) were soaked in D/W or C/U for 12 hours, respectively. After 12 hours the preparations were filtered and the filtrate was used for further studies. Discarded pooled non-infectious serum samples were taken and treated with respective filtrates for 2 hr., 4 hr., and 6 hr. After completion of treatment absorbance of quinoneimine so formed is directly proportional to cholesterol concentration in the specimen. CHOD-PAP kit was used for cholesterol standard 200 mg / dL, the estimation of cholesterol content in the discarded pooled serum was done with the absorbance being measured at 505 nm with the EM 200 fully automated system & VITROS 5600 Autoanalyzer.

2.4. Statistical analysis

Statistical analysis was performed using SPSS 15.0 software. The data were all reported as mean $\pm$ SD. Paired t-test and unpaired t-test were used to compare means and were considered statistically significant if $p < 0.05$ or less.

3. Results

Table 1 depicts the reduction in mean total cholesterol in serum (discarded, non-infectious & pooled) after treatment with Arjuna powder soaked in d/w and Ashwagandha powder soaked in d/w. We found baseline mean cholesterol levels

in serum to be 183.2 ± 10.5 mg/dL in both groups. The mean cholesterol levels were significantly reduced after 2 hr, 4hr, and 6 hr after treatment of serum with Arjuna bark powder soaked in d/w as compared to baseline mean cholesterol levels. Also, the mean cholesterol levels were significantly reduced after 2 hr., 4 hr., and 6 hr. after treatment with Ashwagandha root powder soaked in d/w as compared to baseline mean serum cholesterol levels.

Table 2 depicts the reduction in mean total cholesterol in serum (discarded, non-infectious & pooled) after treatment with Arjuna bark powder soaked in C/U and Ashwagandha root powder soaked in C/U. We found baseline mean cholesterol levels in serum to be 183.2 ± 10.5 mg/dL in both groups. The mean cholesterol levels were significantly reduced after 2 hr., 4hr, and 6 hr. after treatment of serum with Arjuna bark powder soaked in C/U as compared to baseline mean cholesterol levels. Also, the mean cholesterol levels were significantly reduced after 2 hr., 4 hr., and 6 hr. after treatment with Ashwagandha root powder soaked in C/U as compared to baseline mean serum cholesterol levels.

Table 1 Mean total serum cholesterol reduction after treatment with Arjuna powder soaked in d/w and Ashwagandha powder soaked in d/w

Time (Hr)	Mean total cholesterol levels (mg/dL)		P value
	Arjuna bark powder d/w (n=260)	Ashwagandha root powder d/w (n=260)	
0	183.2 ± 10.5	183.2 ± 10.5	
2	153.4 ± 10.2	156.3 ± 10.5	0.001
P value	<0.0001	<0.0001	
4	143.5 ± 10.7	145.2 ± 10.5	0.055
P value	<0.0001	<0.0001	
6	101.9 ± 14.6	106.2 ± 10.5	<0.0001
P value	<0.0001	<0.0001	

Table 2 Mean total serum cholesterol reduction after treatment with Arjuna powder soaked in C/U and Ashwagandha powder soaked in C/U

Time (Hr)	Mean total cholesterol levels (mg/dL)		P value
	Arjuna bark powder C/U (n=260)	Ashwagandha root powder C/U (n=260)	
0	183.2 ± 10.5	183.2 ± 10.5	
2	151.5 ± 10.5	154.2 ± 10.5	0.002
P value	<0.0001	<0.0001	
4	142.8 ± 10.3	143.3 ± 10.5	0.550
P value	<0.0001	<0.0001	
6	98.7 ± 10.4	104.3 ± 10.4	<0.0001
P value	<0.0001	<0.0001	

4. Discussion

The current in-vitro study deals with the evaluation of the cholesterol-reducing activity of Arjuna bark and Ashwagandha root filtrates on the discarded pooled serum samples. Cardiovascular diseases are the leading cause of mortality globally. As atherosclerosis is a risk factor for cardiovascular diseases, hypercholesterolemia or dyslipidemia triggers the progression of atherosclerosis. Hypercholesterolemia can be due to other existing comorbid conditions like diabetes, obesity, hypertension, and certain risk factors like smoking, unhealthy eating habits, and a sedentary lifestyle, among others.^{1,3} We have determined the time-dependent cholesterol-reducing activity of Arjuna and Ashwagandha. The results showed a significant cholesterol-reducing activity for both Arjuna and Ashwagandha.

Ayurveda has been a widely practiced medicinal system in the Indian subcontinent for ages. Previous studies have reported the potential of Ayurvedic medicinal plants in the regulation of cholesterol levels.¹

Arjuna is one of the widely used medicinal plants in Ayurveda. A study on chronic heart disease reported that significant reductions in low-density cholesterol, and lipid peroxide levels after treatment with Arjuna bark. In the same study, no significant effect was observed on the high-density lipoproteins and triglyceride levels after Arjuna bark treatment. Authors attributed the cholesterol-lowering effect to the presence of potent antioxidant activity.⁶ Major phytochemicals found in Arjuna bark are triterpenoids, glycosides, flavonoids, tannins, β -sitosterols, and minerals and trace elements.¹³ A study demonstrated the β -sitosterols and soluble fibers present in the Arjuna are responsible for the hypocholesterolemic activity as they significantly reduced the total cholesterol, low-density lipoprotein, and lipid peroxide levels.⁶

Ashwagandha is used as a digestive tonic, brain stimulant tonic, antiaging activity, immune modulatory activity, strength provider & helps in the treatment of syncope etc.¹¹. Ashwagandha has alkaloids, steroidal glycosides and lactones as their major active phytoconstituents.^{10,11} The active ingredients present in Ashwagandha especially the root part was attributed to the lipid-lowering activity of Ashwagandha in a study on lipid profile of rats after treatment with Ashwagandha Rishta for 51 days.¹¹ In the study, the author reported the reduction of total cholesterol, low-density cholesterol, and also triglyceride levels in serum after treatment with Ashwagandha Rishta in rats. Ashwagandha has antioxidant properties and oxidative stress reduction which directly benefits the lowering of cholesterol levels. Oxidative stress is a major risk factor for the progression of coronary heart disease, hypertension, cancer, inflammation, and atherosclerosis among others.¹²

5. Conclusion

The results of the current in vitro study clearly indicate the total cholesterol-reducing activity of Ashwagandha which is probably attributable to the presence of alkaloids, and glycosides in Ashwagandha root. The triterpenoids, glycosides and flavonoids in Arjuna bark are responsible for the hypo-cholesterolemic activity of Arjuna. Both Arjuna and Ashwagandha need further evaluation for establishing them as effective alternatives for the treatment of hypercholesterolemia.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

References

- [1] Gyawali, D., Vohra, R., Orme-Johnson, D., Ramaratnam, S., & Schneider, R. H. (2021). A Systematic Review and Meta-Analysis of Ayurvedic Herbal Preparations for Hypercholesterolemia. *Medicina*. <https://doi.org/10.3390/medicina57060546>
- [2] Visavadiya, N. P., & Narasimhacharya, A. V. R. L. (2007). Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. *Phytomedicine*, 14(2-3), 136-142. <https://doi.org/10.1016/j.phymed.2006.03.005>
- [3] Gupta, R., Rao, R. S., Misra, A., & Sharma, S. K. (2017). Recent trends in epidemiology of dyslipidemias in India. *Indian Heart Journal*, 69(3), 382-392. <https://doi.org/10.1016/j.ihj.2017.02.020>
- [4] Satoskar RS, Nirmala NR, Raakhi KT, & Bhandarkar SD. (2017). *Pharmacology and pharmacotherapeutics* (25th ed.). Popular prakashan publication.
- [5] Singh BB, Vinjamury SP, Der-Martirosian C, Kubik E, Mishra LC, Shepard NP, Singh VJ, Meier M, & Madhu SG. (2007). Ayurvedic and collateral herbal treatments for hyperlipidemia: a systematic review of randomized controlled trials and quasi-experimental designs. *Altern Ther Health Med*, 13(4), 22-28.

- [6] Gupta, R., Singhal, S., Goyle, A., & Sharma, V. N. (2001). Antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree-bark powder: a randomised placebo-controlled trial. The Journal of the Association of Physicians of India, 49, 231–235.
- [7] Silagy, C., & Neil, A. (1994). Garlic as a lipid lowering agent--a meta-analysis. Journal of the Royal College of Physicians of London, 28(1), 39–45.
- [8] Szapary, P. O., Wolfe, M. L., Bloedon, L. A. T., Cucchiara, A. J., DerMarderosian, A. H., Cirigliano, M. D., & Rader, D. J. (2003). Guggulipid for the treatment of hypercholesterolemia: a randomized controlled trial. JAMA, 290(6), 765–772. <https://doi.org/10.1001/JAMA.290.6.765>
- [9] Mishra, L. C., Singh, B. B., & Dagenais, S. (2000). Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. Alternative Medicine Review : A Journal of Clinical Therapeutic, 5(4), 334–346.
- [10] Bano, A., Sharma, N., Dhaliwal, H., & Sharma, V. (2015). A Systematic and Comprehensive Review on *Withania somnifera* (L.) Dunal- An Indian Ginseng. British Journal of Pharmaceutical Research, 7(2), 63–75. <https://doi.org/10.9734/BJPR/2015/17102>
- [11] Rahman, T., Salahuddin Bhuiya, M., Hasan, R., & Choudhuri, M. S. K. (2020). Effect of Ashwagandharishta on the lipid profile of male and female rats. In Jahangirnagar University J. Biol. Sci (Vol. 9, Issue 2).
- [12] Tiwari, P., & Patel RK. (2010). Comparison of Anti-Hyperlipidemic Activity in Ashwagandharishta Prepared By Traditional and Modern Methods. Asian Journal of Research in Chemistry, 3(3), 574–577.
- [13] Dwivedi, S., & Chopra, D. (2014). Revisiting *terminalia arjuna*-an ancient cardiovascular drug. Journal of Traditional and Complementary Medicine, 4(4), 224–231. <https://doi.org/10.4103/2225-4110.139103>.