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Hypolipidemic Effect a Comparison of Bacopa monnierileaf Powder (Brahmi) and With ania Somnifera Root Powder (Ashwagandha) Herbs of Medicinal Value in Ayurveda : An in-Vitro Study

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Abstract
Hypercholesterolemia, the sixth-highest risk factor for death worldwide, is one of the most common risk factors for atherosclerotic & cardiovascular disease. Elevated cholesterol levels, which are widespread in both developed and developing nations, are the root cause of one-third of ischemic heart disease. Modern medicine can control hypercholesterolemia, however there are long-term unfavourable side effects. The Ayurvedic medical system has been used extensively to treat illnesses on the Indian subcontinent for ages.
Herbal remedies frequently support current medical practises, particularly when it comes to providing safer alternative & well-tolerated treatments for chronic illnesses like Hypercholesterolemia.The research also providedencouraging results for the anti-hypercholesterolemic properties of Brahmi and Ashwagandha.

Keywords: Cholesterol, Brahmi, Ashwagandha Hypercholesterolemia, safer alternative.

Introduction:

The sixth-highest risk factor for death in the world is high cholesterol. [1]People with high cholesterol levels are becoming more common both globally and in India. [2] Genetics, sedentary habits, and high-saturated-fat diets can all raise cholesterol levels. Heart disease, stroke, and other vascular illnesses are all made more likely by high cholesterol. One-third of ischemic heart disease cases worldwide are caused by elevated blood cholesterol. The primary cause of atherosclerosis and the related heart problems is hypercholesterolemia. People with high cholesterol levels are more prone to develop coronary heart disease at various stages of the illness. The main goal of therapy is to lower blood cholesterol[2].

Many national and international pharmaceutical companies are developing and actively marketing a variety of cutting-edge synthetic medications as a result of the rise in the number of hypercholesterolemic patients around the world. However, excessive use of synthetic drugs has exacerbated negative drug effects and made people more resistant to them. This has inspired a lot of individuals to go back to using herbal medicines, which are natural and far safer [3].

Herbal remedies frequently supplement current medical practises, particularly when it comes to treating chronic ailments because they are secure and well-tolerated. Traditional medicine in Western nations, particularly Canada, the USA, and Britain, is experiencing a significant resurgence because many chronic illnesses now lack viable conventional remedies [4].

The addition of specific nutrients with positive health effects is made possible by nutraceuticals, which are items that fall in the middle of meals and pharmaceuticals. Plant-based nutraceuticals may help to improve the plasma lipid profile. [5] Studies have been done on the cholesterol-lowering potential of Anogeissus latifolia gum ghatti, Sida rhomboidea, soy protein, grape seeds, garlic, ginger, and citrus peel extracts. [6] For instance, numerous herbal extracts are being studied to see if they might lower cholesterol levels.

The predictive and causal features of dyslipidemia and oxidative stress, as well as the efficacy of antioxidant treatment to treat abnormal lipid parameters, are supported by a large body of research [7]. Brahmi, also known as Bacopa monnieri(BM) belonging to Scrophulariaceae family, is a leaf or entire plant extract used in Ayurveda as a possible brain tonic. The brain chemicals involved in memory, learning, and thought are improved as well, which improves people's cognitive ability. [8]

The family to which Withania somnifera (WS) belongs is Solanaceae. It is an evergreen plant with important medicinal qualities that is also known as winter cherry and ashwagandha [9]. The antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, and other disease-fighting characteristics of W. somnifera are used in medicine [9]. The roots of W. somnifera include various types of alkaloids, flavonoids, anolides, reducing sugars, amino acids, steroids, volatile oil, starch, glycosides, hentriacontane, dulcitol, and withaniol [9].

Despite these beneficial effects, there is very little proof that the herbal medications indicated above have a hypolipidemic effect. In order to compare and assess the total cholesterol-lowering effects of Bacopa monnieri leaf powder (Brahmi) and Withania somnifera root powder (Ashwagandha) filtrate , the current study was conducted on discarded pooled healthy serum samples to check their activity on hyperlipidemia.

Materials and Methods:

Standard preparation:

Cholesterol standard was obtained from Erba chem Transasia kit (Trinder's method, endpoint) with standard cutoff value 200 mg/dL.

Sample preparation:

Herbal filtrate of ashwagandha *(Withania somnifera)* branded root powder in distilled water (d/w) & cow's urine (c/u) was taken and added to the discarded healthy pooled serum samples collected aseptically and a kinetic study was performed with it.

Herbal filtrate of Brahmi *(Bacopa monnieri*) branded leaf powder in distilled water (d/w) & cow's urine (c/u) was taken and added to the discarded healthy pooled serum samples collected aseptically and a kinetic study was performed with it

Following samples were obtained:
PS: Pooled sample
B1:- Brahmi soaked sample (300 mg powder in d/w for 12 hrs)
B2:- Brahmi 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 powder mg in c/u for 12 hrs)

Aliquots were drawn at an interval of 2 ,4 ,6 hours from each tube maintained in hot water bath at 37^{0} C.
Method: CHOD -PAP :**Enzymatic Colorimetric Determination of Serum Cholesterol,** is intended for the in- vitro quantitative determination of total cholesterol in serum and plasma on both automated and manual systems.

Statistical analysis:
Statistical analysis was performed using SPSS 15.0 software. The means of all the samples were determined followed by paired t-test and unpaired t-test. Means were compared and considered statistically significant if $p<0.05$.

Result
Table 1 shows the results of the CHOD-PAP method used in the present study to demonstrate cholesterol reducing activity of Brahmi powder soaked in d/w and Ashwagandha powder soaked in d/w. The baseline (0 Hr) cholesterol values were 183.2±10.5 mg/dL in both groups. In the Ashwagandharoot powder soaked in the d/w group, a significant reduction in cholesterol activity was found after 2 hr (156.3±10.5 mg/dL), 4 hr (145.2±10.5 mg/dL), and 6 hr (106.2±10.5 mg/dL). In the Brahmi powder soaked in the d/w group a significant reduction in cholesterol activity was found after 2 hr (149.4±10.2 mg/dL), 4 hr (140.4±10.5 mg/dL), and 6 hr (110.0±10.4 mg/dL).
Hence , the cholesterol-reducing activity of both ashwagandha root powder &Brahmi leaf powder (both branded) soaked in d/w at 2 hr and 4 hr & 6 hr showed encouraging & satisfactory results .

Table 1: Mean serum total cholesterol reduction after treatment with Ashwagandharoot powder soaked in d/w and Brahmi leaf powder soaked in d/w (distilled water)

Time (Hr)	**Mean total cholesterol levels (mg/dL)**		**P value**
	Ashwagandha d/w (mg/dL) (n=260)	**Brahmi d/w (mg/dL) (n=260)**	
0	183.2±10.5	183.2±10.5	
2	156.3±10.5	149.4±10.2	<0.0001

P value	<0.0001	<0.0001	
4	145.2±10.5	140.4±10.5	0.001
P value	<0.0001	<0.0001	
6	106.2±10.5	110.0±10.4	0.032
P value	<0.0001	<0.0001	

Table 2 shows the results of the CHOD-PAP method used in the present study to demonstrate cholesterol reducing activity of Brahmi leaf powder soaked in C/U and Ashwagandharoot powder soaked in C/U (cow's urine). The baseline (0 Hr) cholesterol values were 183.2±10.5 mg/dL in both groups. In the Ashwagandha powder soaked in the C/U group, a significant reduction in cholesterol activity was found after 2 hr (154.2±10.5 mg/dL), 4 hr (143.3±10.5 mg/dL), and 6 hr (104.3±10.4 mg/dL). In the Brahmi powder soaked in the C/U group a significant reduction in cholesterol activity was found after 2 hr (148.2±10.5 mg/dL), 4 hr (138.2±10.5 mg/dL), and 6 hr (104.2±10.5 mg/dL).
Hence , the cholesterol-reducing activity of both ashwagandha root powder & Brahmi leaf powder (both branded) soaked in C/U at 2 hr and 4 hr & 6 hr showed encouraging & satisfactory results .

Table 02: Mean serum total cholesterol after treatment with Ashwagandha root powder soaked in C/U and Brahmi leaf powder soaked in C/U.

Time (Hr)	**Mean total cholesterol levels (mg/dL)**		**P value**
	Ashwagandha C/U (mg/dL) (n=260)	**Brahmi C/U (mg/dL) (n=260)**	
0	183.2±10.5	183.2±10.5	
2	154.2±10.5	148.2±10.5	<0.0001
P value	<0.0001	<0.0001	
4	143.3±10.5	138.2±10.5	<0.0001
P value	<0.0001	<0.0001	
6	104.3±10.4	104.2±10.5	<0.0001
P value	<0.0001	<0.0001	

Discussion

Hence ,cholesterol is neither unhealthy nor harmful. Instead, it is a substance that is necessary for the structure of every cell as well as for the efficient operation of the neurological system and the brain. [15] Although a high blood cholesterol level is not an illness, it can cause cardiac ailments. The body's cholesterol levels have risen in recent years as a result of lifestyle changes, stress exposure, decreased physical activity, and bad eating habits[16]. In the current study, we assessed the ability of Brahmi powder and Ashwagandha powder to lower cholesterol.
In comparison to the contemporary medical system, ayurvedic herbal treatments are safer to use and effective at lowering cholesterol. It is suggested that many herbs, including , garlic, cinnamon, tulsi, basil, ginger, fenugreek, and Indian gooseberry, can treat

hypercholesterolemia[17]. On the pooled healthy serum samples that were discarded, an in vitro research was conducted using the CHOD-PAP (cholesterol dynamic extended stability testing) method. It is a colorimetric technique, and the amount of cholesterol found in the serum is correlated with the colour intensity that results. In our investigation, the treatment of Ashwagandha & Brahmi powder on serum was done using the d/w and the c/u, respectively. The action of Brahmi powder and Ashwagandha powder is increased or potentiated by the cow urine, which functions as a bioenhancer.[18]

Many of the herbs utilised in the Ayurvedic medical system include saponins. By encouraging the liver to produce bile from plasma cholesterol, the saponins lower the levels of plasma cholesterol in the blood. This is frequently caused by decreased gastrointestinal absorption of cholesterol, which the liver needs to produce bile20. High-density lipoprotein, the good cholesterol, may be increased by flavonoids, whereas low-density lipoprotein oxidation, the bad cholesterol, may be decreased[21]. Oxidized low-density lipoprotein contributes to the development of plaque and atherosclerotic disease.

Hypercholesteremia develops as a result of oxidative stress, which is exacerbated by reactive oxygen species scavenging activity[18,19]. Saponins, flavonoids, and phytosterols are the active phytoconstituents found in Brahmi. These phytoconstituents, which can be found in Brahmi powder soaked in d/w or c/u, may be the cause of the cholesterol-lowering effects shown in our study[11],[23]. Our results are consistent with earlier research documenting Brahmi's ability to prevent hypercholesteremia in rats fed a high-fat diet. [11]

In addition to its use as a digestive tonic and brain stimulant, ashwagandha has immunological modulatory, anti-aging, syncope-treating, and cardiovascular protecting properties. [24] The three main active phytoconstituents of ashwagandha are lactones, steroidal glycosides, and alkaloids. [25] In a study on the lipid profile of rats following treatment with Ashwagandha Rishta for 51 days, the active components in Ashwagandha, particularly the root part, were ascribed to the herb's ability to decrease cholesterol. [24] In a study, the author noted that therapy with Ashwagandha Rishta in rats resulted in lower serum levels of triglycerides, low-density cholesterol, and total cholesterol. Due to the antioxidant qualities of ashwagandha, oxidative stress reduction directly contributes to decreasing cholesterol levels.

The development of coronary heart disease, hypertension, cancer, inflammation, and atherosclerosis, among other conditions, are all significantly influenced by oxidative stress. [26]

Conclusion

Ayurveda is a practise that dates back thousands of years and is safer and more holistic than contemporary medicine. In the current study, we have shown that Brahmi powder and Ashwagandha powder have cholesterol-lowering properties. Therefore, we suggest that Brahmi and ashwagandha, which are frequently used as a mental tonic, strength-providers, etc., need additional research to determine their impact on cholesterol levels. Preclinical and

clinical research should be done in the future to verify the anti-hypercholesterolemic effects of Brahmi and Ahwagandha herbs.

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References:

1. Pirillo A, Casula M, Olmastroni E, Norata GD, Catapano AL. Global epidemiology of dyslipidaemias. Nat Rev Cardiol. 2021 Oct;18(10):689-700.
2. High blood cholesterol What you need to know. Available from https://www.nhlbi.nih.gov/files/docs/public/heart/wyntk.pdf. Last accessed 13th July 2022.
3. Rouhi-Boroujeni H, Heidarian E, Mohammadizadeh F, Rafieian-Kopaei M. Herbs with anti-lipid effects and their interactions with statins as a chemical anti- hyperlipidemia group drug: A systematic review. Atherosclerosis. 2015 Jul; 11(4): 244–251.
4. MacLennan AH, Wilson DH, Taylor AW. Prevalence and cost of alternative medicine in Australia. Lancet. 1996. 347:569–573.
5. Mannarino MR, Ministrini S, Pirro M. Nutraceuticals for the treatment of hypercholesterolemia.EurJIntern Med. 2014; 25:592-9.
6. Parvathi KM, Ramesh CK, Krishna V, Paramesha M, Kuppast IJ. Hypolipidemic activity of gum ghatti of Anogeissus latifolia. Pharmacogn Mag. 2009; 5:11.
7. Yang RL, Shi YH, Hao G, Li W, Le GW. Increasing Oxidative Stress with Progressive Hyperlipidemia in Human: Relation between Malondialdehyde and Atherogenic Index. J Clin Biochem Nutr. 2008 Nov;43(3):154-8.
8. 8 Scharfe H. Kharosti and Brahmi. Journal of the American Oriental Society 2002; 122 (2): 391–393.
9. Laylani L, Saleh A. Alcoholic extract effect of Withania somnifera roots on cholesterol diet induced hyperlipidemia in male rabbits. Iraqi Journal of Science, 2018; 59(1B): 267-270.)
10. Huxley R, Lewington S, Clarke R. Cholesterol, coronary heart disease and stroke: a review of published evidence from observational studies and randomized controlled trials. Semin Vasc Med. 2002 Aug;2(3):315-23.
11. Kamesh V, Sumathi T. Antihypercholesterolemic effect of Bacopa monniera linn. on high cholesterol diet induced hypercholesterolemia in rats. Asian Pac J Trop Med. 2012 Dec;5(12):949-55.
12. Kirtikar K, Basu D.(eds.) Indian medicinal plants. Dehradun: International Book Distributors;1994.
13. Hou CC, Lin SJ, Cheng JT, Hsu FL. Bacopaside III, bacopasaponin G and bacopaside (bacoside) A, B and C from Bacopa monnieri. J Nat Prod 2002; 65: 1759-1763.
14. Assmann G, Antonio M, Gotto Jr. HDL Cholesterol and protective factors in atherosclerosis. Circulation 2004; 109: 8 -14.

15. Valenza M, Chen JY, di Paolo E, Ruozi B, Belletti D, Ferrari Bardile C, et al. Cholesterol-loaded nanoparticles ameliorate synaptic and cognitive function in Huntington's disease mice. EMBO Mol Med . 2015 Dec [cited 2022 Nov 3];7(12):1547–64. Available from: https://pubmed.ncbi.nlm.nih.gov/26589247/
16. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. 2015 Jan 27 [cited 2022 Nov 2];131(4):e29–39. Available from: https://pubmed.ncbi.nlm.nih.gov/25520374/
17. Gyawali D, Vohra R, Orme-Johnson D, Ramaratnam S, Schneider RH. A Systematic Review and Meta-Analysis of Ayurvedic Herbal Preparations for Hypercholesterolemia. Medicina (B Aires) . 2021 [cited 2022 Oct 28]; Available from: https://doi.org/10.3390/medicina57060546
18. Randhawa G. Cow urine distillate as bioenhancer. J Ayurveda Integr Med 2010 [cited 2022 Oct 29];1(4):240. Available from: /pmc/articles/PMC3117312/
19. Srinath S. Memory Enhancing Medicinal Herbs. Journal of Pharmaceutical Sciences and Research 2014 [cited 2022 Nov 2];6(10):331–3. Available from: http://www.thorne.com/altmedrev/.fulltext/9/1/79.pdf.
20. Li H, Wang QJ, Zhu DN, Yang Y. Reinioside C, a triterpene saponin of Polygala aureocauda Dunn, exerts hypolipidemic effect on hyperlipidemic mice. Phytother Res 2008 [cited 2022 Nov 3];22(2):159–64. Available from: https://pubmed.ncbi.nlm.nih.gov/18167051/
21. Akila M, Devaraj H. Synergistic effect of tincture of Crataegus and Mangifera indica L. extract on hyperlipidemic and antioxidant status in atherogenic rats. Vascul Pharmacol . 2008 Oct [cited 2022 Nov 3];49(4–6):173–7. Available from: https://pubmed.ncbi.nlm.nih.gov/18755296/
22. Warnholtz A, Mollnau H, Oelze M, Wendt M, Münzel T. Antioxidants and endothelial dysfunction in hyperlipidemia. Curr Hypertens Rep . 2001 [cited 2022 Nov 3];3(1):53–60. Available from: https://pubmed.ncbi.nlm.nih.gov/11177709/
23. Hou CC, Lin SJ, Cheng JT, Hsu FL. Bacopaside III, bacopasaponin G, and bacopasides A, B, and C from Bacopa monniera. J Nat Prod 2002 Dec 1 [cited 2022 Nov 3];65(12):1759–63. Available from: https://pubmed.ncbi.nlm.nih.gov/12502309/
24. 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).
25. 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
26. 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.

