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A Comparative Study of Brahmi Leaves (*Bacopa Monnieri)* Powder and Arjuna Bark (*Terminalia Arjuna)* Powder of Medicinal Importance in Ayurveda on Serum Cholesterol In-Vitro

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Abstract: Ayurveda is an age-old system of medicine widely practiced in India. It has remedies mentioned in the ancient scriptures for many diseases. Hypercholesterolemia is a major contributing factor to cardiovascular diseases and other comorbidities. In the present study, we have evaluated the cholesterol-reducing activity of Brahmi powder and Arjuna powder in discarded serum samples. Results demonstrated a significant reduction of cholesterol levels in pooled serum samples at 2 hr, 4hr, and 6 hr .We concluded that the Brahmi herb and Arjuna herb, widely used as a mental tonic and cardiotonic, respectively can be utilized for the treatment of hypercholesterolemia & Dyslipidaemia .
Keywords: Arjuna, Brahmi, Hypercholesterolemia, Dyslipidaemia.

I. INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death globally. Hypercholesterolemia, high blood pressure, and smoking are some of the major factors that increase the risk of CVD[1]. Hypercholesterolemia is characterized by increased levels of peripheral lipid profile. A high cholesterol level is attributed to lifestyle changes, unhealthy eating habits, lack of physical activity, and continuous exposure to increased stress [2]. Guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) have recommended peripheral low-density lipid cholesterol (LDL-C) levels as optimal (<100 mg/dL), suboptimal (100-129 mg/dL), borderline (130-159 mg/dL) and high (>160 mg/dL). [3]

Importantly, the guidelines suggested targeting LDL-C for the management of hypercholesterolemia in reducing CVD-associated comorbidity and mortality[1,3.] Hypercholesterolemia also incurs healthcare costs often due to recurrent hospitalizations or hospital visits. [4,5]

Modern health care system implies the use of modern medical therapy for the management of hypercholesterolemia.[3]

In the quest to discover newer approaches to the management of hypercholesterolemia, Ayurveda an age-old natural system of healing from India has the answers within it. The research focused on proving the claims of Ayurveda have accelerated recently, and this has led to promising Ayurvedic remedies in the management of hypercholesterolemia. [6] In the classical texts in Ayurveda main herbs described for hypercholesterolemia or dyslipidemia or “medovriddhi” or “medodushti” management are the Arjuna (*Terminalia arjuna*), Garlic (*Allium sativum),* tulsi (*Ocimum* sanctum), cinnamon (*Cinnamomum* zeylanicum) and Guggulu (*Commiphora mukul*) among many others. [7-9]

The Ayurvedic formulations comprise single herbs or more than one herb, in various forms like powders, juices, decoctions, extracts, or tablets. Apart from the above-mentioned herbs, many other herbs can be utilized for cholesterol management including Brahmi, karela, or ashwagandha. The bark of arjuna also helps to lower blood lipid levels and prevents the hardening of blood vessels by reducing lipid accumulation in the arteries, preventing atherosclerosis.[10]

Brahmi (*Bacopa monniera*) is regarded as an excellent metal tonic in Ayurveda enhancing the thinking, learning, memory, and cognitive function in humans.[11] Apart from being used as a mental tonic, Brahmi is being used alone or in combination with other herbs in the treatment of asthma, mental disorders, nervine tonic, as a diuretic and in as a cardiotonic.[12]

It also helps in reducing the scavenging activity of reactive oxygen species (ROS) by reducing the key enzymes involved in ROS generation.[13] In the present research work, we have evaluated the high cholesterol-reducing potential of Brahmi powder soaked in distilled water and also soaked in distilled cow urine.

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II. MATERIALS AND METHODS

A. *Chemicals and Reagents*

Chemical used for the analysis were pure & of analytical grade. The investigation only utilised analytical-grade compounds. A cholesterol dynamic extended stability testing method (CHOD-PAP) kit was used for the evaluation of cholesterol. Branded Arjuna bark powder and Brahmi leaves powder were used in the study. Purified branded and distilled cow urine (C/U) and distilled water (D/W) were used in the study.

B. *Principle*

CHOD-PAP method is a colorimetric assay method. Cholesterol esterase hydrolyses esterified cholesterols 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-coloured quinonimine dye complex. The intensity of the color formed is directly proportional to the amount of cholesterol present in the sample.

C. *Methods*

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

A1: Arjuna-soaked sample (300 mg in D/W for 12 hrs)
A2: Arjuna-soaked sample (300 mg in C/U for 12 hrs)
B1: Brahmi-soaked sample (300 mg in D/W for 12 hrs)
B2: Brahmi-soaked sample (300 mg in C/U for 12 hrs)

Arjuna and Brahmi powders (300 mg) were soaked in D/W or C/U for 12 hours, respectively. After 12 hours the formulations were filtered and the filtrate was used for further studies. Discarded pooled non-infectious serum samples were taken and treated with respective above filtrates for 0 hr, 2 hr, 4 hr, and 6 hr. After completion of treatment absorbance of quinonimine so formed is directly proportional to cholesterol concentration in the specimen. CHOD-PAP kit standard was used for the estimation of original cholesterol content in the pooled serum. The absorbance was measured at 505 nm on the VITROS 5600 Autoanalyzer.

D. *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 $p<0.05$.

III. 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 Arjuna powder soaked in d/w. The baseline (0 Hr) cholesterol values were 183.2±10.5 mg/dL in both groups. In the Arjuna powder soaked in the d/w group, a significant reduction in cholesterol activity was found after 2 hr (153.4±10.1 mg/dL), 4 hr (143.4±10.7 mg/dL), and 6 hr (101.9±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±58.4 mg/dL). The cholesterol-reducing activity of Brahmi powder soaked in d/w at 2 hr and 4 hr demonstrated a potent significant reduction as compared to Arjuna powder soaked in d/w. At 6 hr the cholesterol-reducing activity of Arjuna powder soaked in d/w was more potent as compared to Brahmi powder soaked in d/w.

Table 1: Mean serum cholesterol reduction after treatment with Arjuna powder soaked in d/w and Brahmi powder soaked in d/w.

Time (Hr)	Mean cholesterol levels		P value
	Arjuna d/w (mg/dL) (n=260)	Brahmi d/w (mg/dL) (n=260)	
0	183.2±10.5	183.2±10.5	
2	153.4±10.1	149.4±10.2	<0.0001
P value	<0.0001	<0.0001	
4	143.4±10.7	140.4±10.5	0.001
P value	<0.0001	<0.0001	
6	101.9±10.5	110.0±58.4	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 powder soaked in C/U and Arjuna powder soaked in C/U. The baseline (0 Hr) cholesterol values were 183.2±10.5 mg/dL in both groups. In the Arjuna powder soaked in the C/U group, a significant reduction in cholesterol activity was found after 2 hr (151.5±10.4 mg/dL), 4 hr (142.8±10.3 mg/dL), and 6 hr (98.7±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). The cholesterol-reducing activity of Brahmi powder soaked in C/U at 2 hr and 4 hr demonstrated a potent significant reduction as compared to Arjuna powder soaked in C/U. At 6 hr the cholesterol-reducing activity of Arjuna powder soaked in C/U was more potent as compared to Brahmi powder soaked in C/U.

Table 02: Mean serum cholesterol after treatment with Arjuna powder soaked in C/U and Brahmi powder soaked in C/U.

Time (Hr)	Mean cholesterol levels		P value
	Arjuna C/U (mg/dL) (n=260)	Brahmi C/U (mg/dL) (n=260)	
0	183.2±10.5	183.2±10.5	
2	151.5±10.4	148.2±10.5	<0.0001
P value	<0.0001	<0.0001	
4	142.8±10.3	138.2±10.5	<0.0001
P value	<0.0001	<0.0001	
6	98.7±10.4	104.2±10.5	<0.0001
P value	<0.0001	<0.0001	

IV. DISCUSSION

Cholesterol is neither bad nor unhealthy. Instead, it is an essential compound for every cell structure, and for the proper functioning of the brain and nervous system.[14] A high cholesterol level in the blood is not a disease but can lead to heart diseases. Nowadays, due to changes in lifestyle, exposure to stress, less physical activities & unhealthy food habits, cholesterol levels have increased in the human body [2]. In the present study, we evaluated the cholesterol-reducing activity of Brahmi powder and Arjuna powder.

Ayurvedic herbal remedies are effective in reducing cholesterol and as well as safer to use with respect to the modern system of medicine. Many herbs such as arjuna, garlic, cinnamon, tulsi, ginger, fenugreek, and Indian gooseberry are implied for their hypercholesterolemia[1]. *In vitro* study was performed on the discarded pooled serum samples, CHOD-PAP (cholesterol dynamic extended stability testing) method was utilized. It is a colorimetric method where the intensity of color produced is proportional to the amount of cholesterol present in the serum. In our study, the d/w and c/u were used as vehicles for the treatment of Brahmi and Arjuna powder on serum, respectively. The cow urine acts as a bioenhancer thereby increasing or potentiating the effect of Brahmi powder and Arjuna powder[15].

Saponins are present in many herbs used in the Ayurveda system of medicine. The saponins cause a reduction in plasma cholesterol levels by inducing the production of bile from plasma cholesterol in the liver. This is often due to decreased absorption of cholesterol from the intestine and required by the liver for the synthesis of bile[16]. Flavonoids have the potential to increase the good cholesterol i.e. high-density lipoprotein and decrease the bad cholesterol i.e. low-density lipoprotein oxidation[17]. Oxidized low-density lipoprotein is involved in plaque formation thus progressing to atherosclerotic disease. [18] Oxidative stress leads to the progression of hypercholesteremia; reactive oxygen species scavenging activity increases oxidative stress[13,15]. Brahmi contains saponins, flavonoids, and phytosterols as active phytoconstituents. These phytoconstituents are present in the Brahmi powder soaked in d/w or Brahmi powder soaked in c/u may be responsible for cholesterol-reducing activity in our study[19,20]. Our findings are in line with the previous studies reporting the anti- hypercholesterolemic effect of Brahmi in high-fat diet-induced rats[20].

Arjuna contains triterpenoid saponins, tannins, sterols, and flavonoid polyphenols among others. Tannins and flavones exhibit radical scavenging activity, the sterols in Arjuna induce the elimination of fats and cholesterols in faeces and thus reduce the plasma levels of plasma cholesterol [21]. As discussed above saponins and flavonoids produce cholesterol-reducing activity[12,16,17]. In our study, the Arjuna powder soaked in d/w and Arjuna powder soaked in C/U must have reduced the cholesterol in discarded pooled serum in our study. Authors in a study described the anti-atherogenic activity of the Arjuna (ethanolic fraction) in hypercholesterolemic rabbits [22]. Thus, both Brahmi and Arjuna powder can be potential therapies that need to be evaluated for their effects on cholesterol.

V. CONCLUSION

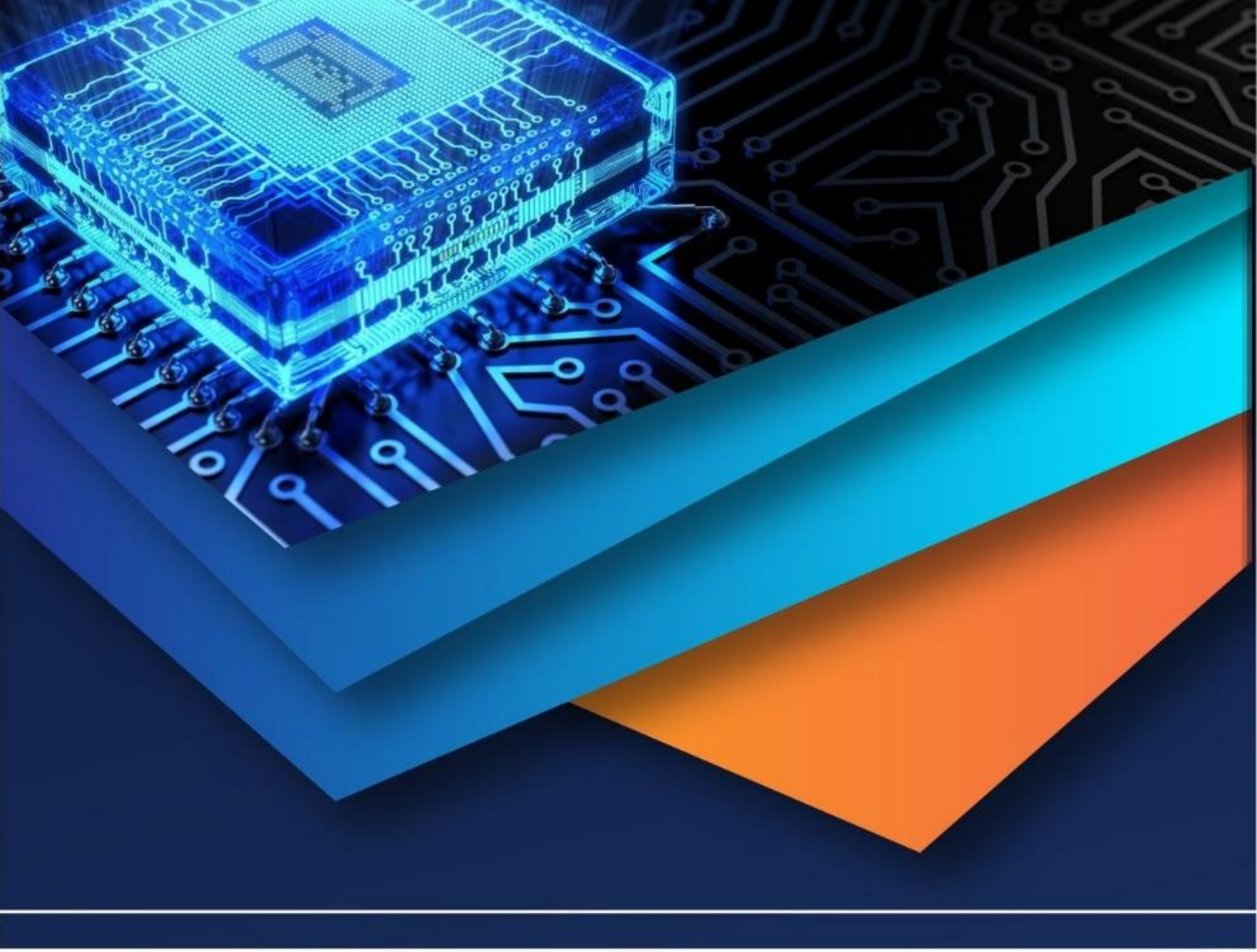
Ayurveda is an age-old system and has a holistic as well as a safer approach than the modern medicine system. In the present study, we have demonstrated the cholesterol-reducing activity of Brahmi powder and Arjuna powder. Thus, we propose the Brahmi herb, widely used as a mental tonic, need further evaluation for the effect on cholesterol levels. In the future, preclinical and clinical studies should be conducted to confirm the anti-hypercholesterolemic activity of Brahmi powder.

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