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RESEARCH ARTICLE

Hypolipidemic effect of Ashwagandha (*Withania somnifera*) and Arjuna (*Terminalia arjuna*): An *in vitro* study

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ABSTRACT

Background: High cholesterol is the sixth-highest risk factor for death in the globe. Herbal medications regularly complement modern medical care, especially by providing safe, well-tolerated therapies for chronic conditions. **Aims and Objectives:** The present study was undertaken to evaluate and compare the cholesterol-lowering effects of *Terminalia arjuna* bark and *Bacopa monnieri* leaves (Brahmi) extract in human blood samples diagnosed with hyperlipidemia. **Materials and Methods:** Herbal extract of arjuna (*T. arjuna*) and Ashwagandha (*Withania somnifera* [WS]) in distilled water (d/w) and cow's urine (c/u) [A1, A2, D1, and D2] was taken and added to the pooled serum samples collected aseptically and a kinetic study was performed with it. Cholesterol standard was obtained from Erba Chem Transasia kit (Trinder's method, endpoint) with a standard cutoff value of 200 mg/dL. **Results:** Baseline reading of total cholesterol in all samples was 189 mg/dL. After 2 h, the total cholesterol reading in A1 was 159 mg/dL, 157, 162, and 160 mg/dL in A2, D1, and D2, respectively. After 4 h, the total cholesterol reading in A1 was 149 mg/dL, 148, 151, and 149 mg/dL in A2, D1, and D2, respectively. After 6 h, the total cholesterol reading in A1 was 109 mg/dL, 104, 112, and 110 mg/dL in A2, D1, and D2, respectively. **Conclusion:** From the findings of the present study, it was found that aqueous extract of Ashwagandha (WS) and Arjuna (*T. arjuna*) was effective in reducing total cholesterol levels. It can be considered a potential therapeutic alternative in patients with hyperlipidemia but warrants further clinical studies.

KEY WORDS: Cholesterol; Serum; Ashwagandha; Arjuna; Hypolipidemic

INTRODUCTION

High cholesterol is the sixth-highest risk factor for death in the globe.[1] Both globally and in India, there are an increasing number of persons with high cholesterol levels.[2] High-saturated-fat diets, sedentary lifestyles, and genetics can all cause cholesterol levels to rise. Cholesterol increases the risk of vascular diseases including heart disease, stroke, and others. Worldwide, ischemic heart disease is one-third due to high blood cholesterol. Hypercholesterolemia is the main contributor to atherosclerosis and its accompanying cardiac issues. At different phases of the illness, coronary heart disease is more likely to develop in those with high low-density lipoprotein (LDL) cholesterol levels. Lowering blood LDL cholesterol is the major objective of therapy.[2]

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Due to the increase in the number of hypercholesterolemic patients globally, several national and multinational pharmaceutical businesses are inventing and aggressively selling a variety of cutting-edge synthetic drugs. However, overuse of synthetic pharmaceuticals has increased adverse drug reactions and led to the creation of an immune to them. A modest rise in the frequency of muscular soreness, weakness, and sadness has been linked to statin, the first-line pharmacological therapy. In addition, in individuals 70 years

of age or older, it did not lower the risk for atherosclerotic cardiovascular events. More effort is required to give every patient access to the success of therapeutic intervention since many individuals are still unable to achieve remission. The use of herbal therapy as an alternative to conventional treatment is widespread. The bioactive components from herbal medicines have been shown to be efficient and safe to enhance the lipid profile in a number of rigorous clinical trials. Many people have been motivated by this to return to using herbal remedies, which are natural and far safer.[3]

Herbal medications regularly complement modern medical care, especially by providing safe, well-tolerated therapies for chronic conditions. Because many chronic illnesses currently have no effective conventional therapies, traditional medicine is seeing a major resurgence in Western countries, especially Canada, the USA, and Britain.[4]

Nutraceuticals, which are products that lie between meals and medicines, enable the inclusion of certain nutrients with favorable effects on health. Nutraceuticals made from plants have been suggested to improve plasma lipid profiles.[5] *Anogeissus latifolia* gum ghatti, *Sida rhomboidea*, soy protein, grape seeds, garlic, ginger, and citrus peel extracts have all been studied for their ability to lower cholesterol.[6] In instance, many herbal extracts being investigated for decreasing cholesterol levels. Ashwagandha (*Withania somnifera* [WS] root) and Arjuna are two of them (*Terminalia arjuna*-ree bark).

Solanaceae is the family to which WS belongs. It is an evergreen plant that is also referred to as winter cherry and ashwagandha and has essential medicinal properties.[7] In medicine, WS is utilized for its antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, and other diverse disease-fighting properties.[7] Different types of alkaloids, flavonoids, anolides, reducing sugars, amino acids, steroids, volatile oil, starch, glycosides, hentriacontane, dulcitol, and withaniol are found in the roots of WS.[7]

Despite these, the evidence for hypolipidemic action of the abovementioned herbal drugs is very scarce. Thus, the present study was undertaken to evaluate and compare the cholesterol-lowering effects of *T. arjuna* bark and WS root (Ashwagandha) extract in human blood samples diagnosed with hyperlipidemia.

MATERIALS AND METHODS

Standard Preparation

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

Reference standard: Herbal extract of arjuna (*T. arjuna*) in distilled water (d/w) and cow's urine (c/u) was taken and added to the pooled serum samples collected aseptically and a kinetic study was performed with it.

Sample Preparation

Pooled serum (discarded and non-infectious) was taken and herbal aqueous extract and extract in cow's urine of Karela (*Momordica charantia*-fruit) were added to the collected sample.

The following samples were obtained

- PS: Pooled sample
- A1: Arjuna-soaked sample (300 mg in d/w for 12 h)
- A2: Arjuna-soaked sample (300 mg in c/u for 12 h)
- D1: Ashwagandha-soaked sample (300 mg in d/w for 12 h)
- D2: Ashwagandha-soaked sample (300 mg in c/u for 12 h).

Aliquots were drawn at an interval of 0, 2, 4, and 6 h from each tube maintained in hot water bath at 37°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.

System Used

Erba Chem EM 200 Autoanalyzer

1. Automation: Fully Automatic
2. Model: EM 200
    - Brand: Erba
    - Usage/application: Clinical

Erba EM 200 Fully Automated biochemistry analyzer, random access, and discrete clinical chemistry analyzer that enhances productivity and turnaround time. It has a throughput of 200 spectrophotometric tests per hour. Other instrument used was *vitro* s 5600 integrated system. Ethics committee permission was not needed as there was no involvement of human subjects/patients. However, the study was done in accordance with the declaration of Helsinki.

RESULTS

Baseline reading in all the samples was 189 mg/dL [Table 1]. After 2 h, the total cholesterol reading in A1 was 159 mg/dL, 157, 162, and 160 mg/dL; in A2, D1, and D2, respectively [Figure 1]. After 4 h, the total cholesterol reading in A1 was 149 mg/dL, 148, 151, and 149 mg/dL in A2, D1, and D2, respectively [Figure 2]. After 6 h, the total cholesterol reading in A1 was 109 mg/dL, 104, 112, and 110 mg/dL in A2, D1, and D2, respectively [Figure 3].

DISCUSSION

The pathogenesis of coronary heart disorders and myocardial ischemia has been linked to hypercholesterolemia and atherosclerosis that result from it. Since lowering cholesterol levels may reduce the risk of CVD, great efforts have been made to accomplish this goal.[8] In the current investigation, Brahmi (*Bacopa monnieri*) herbal aqueous extract and extract in cow's urine were utilized to assess its hypolipidemic impact by noticing a decrease in total cholesterol in a human blood sample of a hyperlipidemic patient. As a reference standard, Arjuna (*Arjuna terminalia*) herbal aqueous extract and cow's urine extract were used. In the current investigation, samples that had been soaked in arjuna or ashwagandha for 2, 4, and 6 h had significantly less total cholesterol than the control sample.

Because it enhances the elimination of cholesterol as well as bile acids through fecal sterol excretion, the active component withaferin A in WS is crucial to the cholesterol-lowering action. The greater fiber content in the WS root may be the cause of the improved fecal-neutral sterol excretion. Through enhanced resistance to diffusion in the aqueous luminal media, dietary fibers are helpful in reducing the uptake of exogenous cholesterol from micelles, according to several research on the dietary phytoconstituents of WS rhizome.[9] Phytosterols have a stronger affinity for micelles than for cholesterol because they are more hydrophobic. To lessen cholesterol absorption, the removes intestinal cholesterol from micelles. A corrective increase in endogenous cholesterol production results from the resulting reduction in hepatic cholesterol levels. The fiber content of WS may be responsible for the reduction in intestinal commute time for cholesterol and carbohydrate absorption, which in turn leads to a decrease in hepatic lipogenesis and a decrease in liver and plasma triglyceride concentrations. This decrease in triglyceride levels is a result of WS.[9] According to research conducted by Priya *et al*., *T. arjuna* medication for 1 month resulted in a favorable alteration in the whole lipid profile.[10] Research included in Khalil's thesis revealed that using statins plus arjuna bark powder for 3 months reduced triglycerides, LDL cholesterol, and total cholesterol by 15%, 11%, and 16%, respectively. Nitrites and lipoprotein levels only slightly decreased. As a result, it is possible that Arjuna and statins can reduce hyperlipidemia.[11]

The major strength of the present study is that it provides evidence for the effectiveness of herbal drugs in reducing total cholesterol levels. However, the present study was an *in vitro* study, therefore, the results of the present study need further in human studies to further substantiate the same.

Table 1: Changes in total cholesterol levels in the pooled sample (PS), Arjuna-soaked sample (300 mg in distilled water for 12 h)-A1, Arjuna-soaked sample (300 mg in distilled cow urine for 12 h)-A2, Ashwagandha-soaked sample (300 mg in distilled water for 12 h)-DL, Ashwagandha-soaked sample (300 mg in distilled cow urine for 12 h)-D2

Sample	Baseline	2 h	4 h	6 h
Cholesterol PS	189 mg/dL	187 mg/dL	186 mg/dL	183 mg/dL
A1	189 mg/dL	159 mg/dL	149 mg/dL	109 mg/dL
A2	189 mg/dL	157 mg/dL	148 mg/dL	104 mg/dL
D1	189 mg/dL	162 mg/dL	151 mg/dL	112 mg/dL
D2	189 mg/dL	160 mg/dL	149 mg/dL	110 mg/dL

PS: Pooled sample

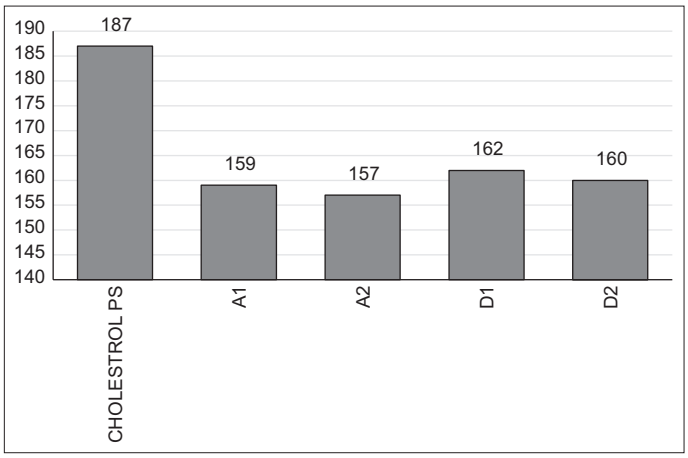

Figure 1: Readings of total cholesterol at 2 h

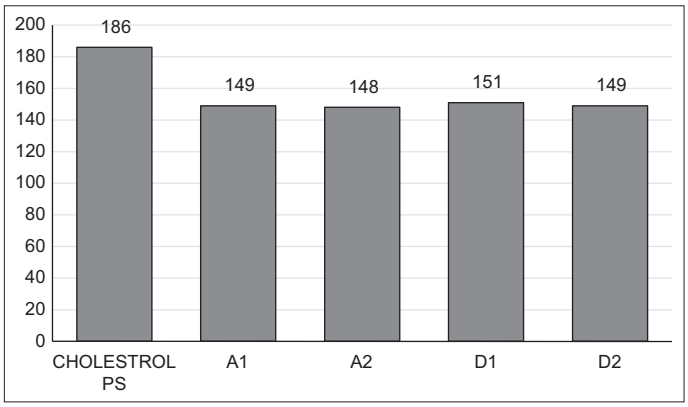

Figure 2: Readings of total cholesterol at 4 h

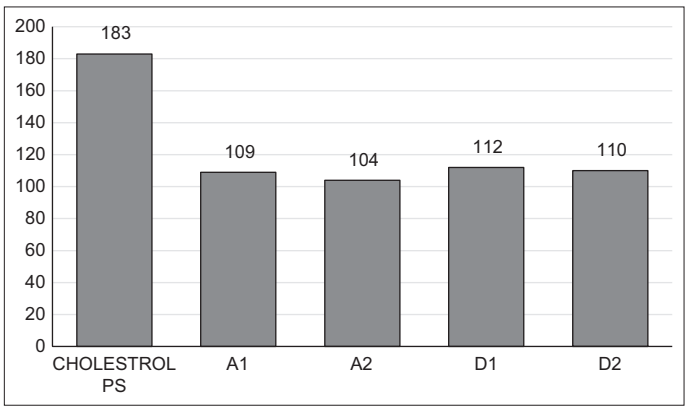

Figure 3: Readings of total cholesterol at 6 h

CONCLUSION

From the findings of the present study, it was found that an aqueous extract of Ashwagandha ([WS] root) and Arjuna (*T. arjuna*) was effective in reducing total cholesterol levels. It can be considered a potential therapeutic alternative in patients with hyperlipidemia but warrants further clinical studies.

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