Research Article

INTERNATIONAL JOURNAL of Indigenous Herbs and Drugs
ISSN 2456-7345

AN OPEN CLINICAL STUDY ON THE EFFICACY OF WITHANIA SOMNIFERA MOTHER TINCTURE IN THE MANAGEMENT OF HYPERLIPIDEMIA

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Article History:

Received on: 19-04-2018
Revised on : 15-05-2019
Accepted on : 23-06-2019

Keywords:

Open clinical study, hyperlipidemia, Withania somnifera.

Abstract

Withania somnifera has anti toxidant, anti inflammatory and anti diabetic activities. In the present open clinical study is that efficacy of Withania somnifera mother tincture in hyperlipidemia. Hyperlipidemia patients meeting the inclusion criteria were randomly assigned in to the treatment and placebo. **Methods**: 30 all randomized patients in treatment group took Withania somnifera mother tincture; whereas the patients in placebo group took placebo mother tincture for 12 weeks. The lipid profiles, blood pressure of the patients were evaluated at baseline, and after 6 and 12 weeks of the open clinical trial. **Results:** The final results showed that Withania somnifera mother tincture significantly p value 0.05 improved LDL, HDL, cholesterol, compared to the placebo group. It had no effect on systolic or diastolic blood pressure was not significant (P > 0.05) compared to placebo. The Withania somnifera showed an inhibitory effect on of hepatic enzymes and possible liver toxicity. No serious side effect was reported for Withania somnifera mother tincture administration. Therefore, Withania somnifera mother tincture could be considered as a supplement for treatment of dyslipidemia.

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INTRODUCTION

Various plant based formulations are known till date, which in one way or the other are known to have an effect on cholesterol level. Withania somnifera known as Ashwagandha is widely used in homoeopathy and ayurvedic system of medicine in India [1-3]. Ashwagandha is the main component of a variety of formulations prescribed for common diseases of respiratory and reproductive tracts. Mishra, L., Singh, B.B., Dagenais, S. etc., Several studies on this plant indicated that it possesses antiinflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic and rejuvenating properties besides positively influencing the endocrine, cardiopulmonary and central nervous systems. Elevated levels of plasma cholesterol and triglycerides have been implicated as causative factors in the development of atherosclerosis and coronary heart disease [4-7].

Atherosclerosis is a process of arteries hardening due to deposition of cholesterol in the arterial wall which causes narrowing of the arteries. Atherosclerosis and atherosclerosis associated disorders like coronary, cerebrovascular and peripheral vascular diseases are accelerated by the presence of hyperlipidemia. Tripathi, A.K., Shukla, Y.N., Kumar, S etc., no reports exist on its hypocholesteremic and antioxidant properties. We,

therefore, have attempted to investigate the effect of WS on the lipid and antioxidant profiles of hypercholesteremic Indian peoples [8,9].

CLASSIFICATION OF APOLIPOPROTEIN

- Chylomicrons – Triglyceride rich bearer of dietary fats.
- Very Low Density Lipoprotein (VLDL) – Triglyceride rich bearer of hepatic blended triglycerides (TG)
- Intermediate and Low Density Lipoprotein (IDL & LDL) – Cholesterol rich leftover particles got from lipolysis of triglycerides in VLDL
- High Density Lipoprotein (HDL) – Cholesterol rich molecule that transports cholesterol to liver for removal or reusing.

These lipoproteins move into the circulation system where they got hydrolyzed by endothelial lipoprotein lipase which hydrolyzes the triglyceride into glycerol and non esterifies unsaturated fats. After which the chylomicron remainders are invested in the liver and bundled with cholesterol, cholesterol esters and ApoB100 to shape VLDL. After the arrival of VLDL into the circulation system it will be changed over into IDL by the activity of lipoprotein lipase and hepatic lipase, where phospholipids and apolipoproteins moved back to HDL [10,11]. Besides, after the hydrolysis by hepatic lipase, IDL will be changed over to LDL and misfortune more apolipoproteins. Fringe cholesterol is come back to the liver by invert cholesterol transport pathway utilizing HDLs which are initially orchestrated by the liver what's more, discharged into the blood. In the blood, HDL cholesterol is esterifies by LCAT to cholesterol ester what's more, moved to VLDL and chylomicrons to return to the liver through LDL receptor. Cholesterol ester are moved to LDL particles by CETP and afterward exposed to LDL-receptors interceded endocytosis. At long last, cholesterol esters are hydrolyzed to cholesterol and removed from the body as bile corrosive [12,13].

MATERIAL AND METHODS

Preparation of Withania somnifera mother tincture

Withania somnifera mother tincture was obtained from Belgaum Pharmacy, Belagavi, Karnataka, India. The Withania somnifera mother tincture was kept at room temperature in darkness until use.

PREPARATION OF MOTHER TINCTURE BOTTLES

Extraction of Withania somnifera mother tincture was purchased and stored as mentioned above. Withania somnifera mother tincture and placebo were prepared into bottle in Department of homoeopathy pharmacy, Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi. The Withania somnifera mother tincture bottle was identified at the department of homoeopathy Pharmacy, Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi, Karnataka. The placebo mother tincture bottle was filled with neutral and inert additive substance whereas; each Withania somnifera mother tincture bottle was filled with 200µl/kg body weight mother tincture (recommended dose given for rats in 20 µl/100 g body weight) & per orally in de ionized water (180 µl) as vehicle for administration[13]. There were no in clinical studies on the anti hyperlipidemic effect of Withania somnifera mother tincture. In the abovementioned works, administration of Withania somnifera mother tincture was safe and effective.

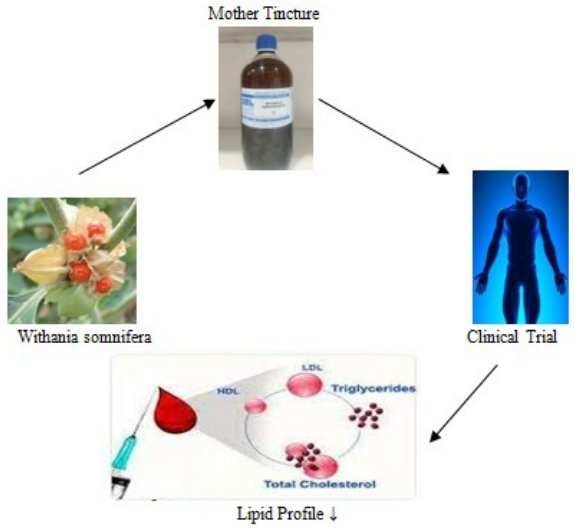

Figure 01: Graphical Abstract

STUDY DESIGN

The study was fully conducted in accordance with the Ethical Committee of the Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi and written informed consent was obtained from all patients before their inclusion in the open study. The study was an open

clinical study, three months, clinical trial which was carried out on 30 hyperlipidemic outpatients of Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi, Karnataka, India. The authors applied inclusion and exclusion criteria for patients to improve the quality of the results in this study.

INCLUSION CRITERIA

Male and female outpatients aged 25 to 65 years; incidence of hyperlipidemia with at least one of the following factors: cholesterol level >200 mg/dl or TG level higher than 150 mg/dl or LDL-C level higher than 130mg/dL or HDL-C level <40 mg/dl.

EXCLUSION CRITERIA

The patients who had a history of chronic or metabolic diseases such as diabetes, Ischemic heart disease, hypertension, tachycardia, peripheral vascular disease, coronary artery disease, thyroid dysfunction, hospitalized, cannot follow therapeutic lifestyle modification and pregnancy. In addition, the exclusion criterion was a recent change in dosage of antilipemic agents such as hydroxymethylglutaryl coenzyme A (HMG-COA) reductive inhibitor, or adding hypoglycemic agents such as first and second generation sulfonylureas or supplements or drugs known to affect the blood lipids, presence of side effects and unwillingness to participate in study.

Other exclusion criteria were: LDL level more than 190 in patients who need medical treatment (for healthy people or with one risk factor); LDL ≥ 160 in patients who need drug treatment (for those with two or more risk factors of the following:

- Smoking.
- Hypertension.
- Low HDL level (less than 40).
- History of coronary artery disease at an early age in the household (less than 55 years in males and in females under age 65 years old), 5. Age above 65 years old).

SAMPLE SIZE

To have a power of 90%, a two sided test was used, with a significance level of 0.05, and a 20% minimum detectable mean difference changes for LDL-C and SD 20.5% between treatment and placebo group. Finally, minimum sample size of 30 patients for each arm was calculated. Because of expected dropout, we considered 15 patients in each group.

The patients were randomly divided into the treatment (15 patients) treatment group and the placebo (15 patients) groups. Finally, 30 patients successfully completed an open clinical study.

INTERVENTIONS

Participants were randomized to 2 intervention groups of 15 patients. The patients in the treatment group were taking Withania somnifera mother tincture, for 12 weeks; whereas the patients in placebo group were taking placebo (mother tincture) for 12 weeks. Participants did not receive any other hypocholesterolemic drugs during the randomized open study. The patient's compliance and medication adherence were confirmed through checking with the patient and his/her caregiver along with a mother tincture count at each visit.

OUTCOME MEASURES

Lipid profile (Cholesterol, TG, HDL and LDL), blood pressure (SBP and DBP), BMI index and liver enzymes (ALT, AST, ALP) were measured at baseline, 6 weeks and 3 months after intervention in treatment and placebo group.

MASKING

The enrolled participants were assigned using a stratified randomization and all of them received Withania somnifera mother tincture or placebo mother tincture, which were prepared in the same way. For randomization, a randomized code number was obtained from Microsoft Excel for each pillbox (treatment and control groups). All mother tincture bottles had similar colour, shape, size, texture and odour. The mother tincture bottles were stored in a dark container and coded by a pharmacist. The participants and those assessing outcomes were blinded until all participants finished the protocol.

SAFETY

The patients were requested to inform investigators about any adverse events or complaints for all illnesses, and hospitalizations that occurred during the trial. The symptoms were checked and recorded at the beginning and at each visit by general physician, cardiologist. Also, possible side effects were checked and recorded via telephone call every week and the general physician/homoeopathy physician was responsible for continuing or discontinuing the drugs.

STATISTICAL ANALYSIS

Baseline characteristics were analyzed using independent t-test or $\chi 2$ tests. The significant differences at various time points were assessed by repeated measures of ANOVA. The variables were reported as mean and standard deviation (Mean ± SD). P value less than 0.05 was considered statistically significant.

Table 01: Demographic data of the patients in both study groups (M±SD)

Variable	Control group	Test group	P_{value}
Age Years	0.30 ±0.70 0.5 ±0.71	0.41 ±0.70 0.5 ±0.71	0.27 0.4
Gender Female Male	 0.3±0.65 0.51±0.70	 0.51 ±0.8 0.6 ±0.4	 0.290 0.101
Marital status (n) Married Single	 0.45 ±0.50 0.30 ±0.41	 0.3 ±0.61 0.70 ±0.64	 0.34 0.04
Level of education (n) Under graduate Post graduate Doctor of Philosophy	 0.41±0.70 0.11±0.31 0.22 ±0.51	 0.25±0.591 0.21±0.41 0.7±0.92	 0.211 0.217 0.152
Smoking Former current	 0.51 ±0.60 0.51±0.71	 0.33 ±0.61 0.6 ±0.82	 0.212 0.617

Table 02: The measurements of lipid profile between two groups (M±SD)

Variable	Control group	Test group	P Value
Cholesterol			
At base line	201.7±0.1	209 ±0.2	0.160
After 6 weeks	201.7 ±0.3	170.8 ±1.0	0.001
After 12 weeks	201.6 ±0.6	120.0 ±0.5	0.0001
TG			
At base line	150.5 ±0.81	150.8±0.3	0.20
After 6 weeks	151.8±1.51	129.7±4.3	0.001
After 12 weeks	151.1±1.50	94.1 ±1.0	0.001
LDL			
At base line	145.4 ±0.80	143.92±0.36	0.105
After 6 weeks	145.3 ± 1.0	134.3±1.8	0.001
After 12 weeks	145.2 ± 1.0	101.1 ±0.6	0.0001
HDL			
At base line	31.7 ±1.6	31.3 ±2.1	0.32
After 6 weeks	31.5 ± 1.5	37.1 ±2.2	0.001
After 12 weeks	31.3 ± 1.7	49.85 ± 2.1	0.0001

Table 3: The results of blood pressure in both groups (M±SD)

Variable	Control group	Test group	P Value
SBP (mm of Hg)			
At base line	138.2 ±0.60	138.4±2.1	0.20
After 6 weeks	138.1 ±0.4	128.3 ± 1.3	0.001
After 12 weeks	138.1 ±0.3	121.1 ± 0.1	0.0001
DBP (mm of Hg)			
At base line	88.4±0.8	88.5±0.6	0.28
After 6 weeks	88.6±0.1	82.7±0.4	0.001
After 12 weeks	88.1±0.2	79.1 ±0.1	0.0001

RESULTS

Among 30 type 2 hyperlipidemia patients with mean ± SD, age group cases were observed 0.30 ±0.70 in control group and 0.41 ±0.70 in a test group, P_{value} showed 0.27. Patient years mean ± SD were 0.50 ±0.71 in control group and test group was 0.5 ±0.71, P_{value} showed 0.4 (not significant). The female 30 hyperlipidemia patients were 0.3 ±0.65 in control group and 0.51 ±0.8 in test group, P_{value} is 0.290 and male patients were 0.51 ±0.70 in control group and 0.6 ±0.4 in test groups, P_{value} was 0.101. Marital status of married patients mean ± SD were 0.45 ±0.50 in control group and 0.3 ±0.61 in test group, P_{value} showed 0.34. In single patients values were 0.30 ±0.41 in control group and 0.70 ±0.64 in test group, P_{value} showed 0.04. Level of education in under graduate mean and SD values were 0.41 ±0.70 under control group, 0.25 ±0.591 under test group, P_{value} showed 0.211, post graduate mean and SD values were 0.11 ±0.31 under control group, 0.21 ±0.41 under test group, P_{value} showed 0.217 and doctor of

philosophy mean and SD values were 0.22 ±0.51 under control group, 0.7 ±0.92 under test group, P $_{value}$ showed 0.152. In former and current mean and SD were 0.51 ±0.60, 0.33 ± 0.61 and 0.51 ±0.71 and 0.6 ± 0.82, P $_{values}$ were 0.212 in control group and 0.617 in test group (Table 01).

In 30 hyperlipidemia patient mean ±SD of cholesterol is 201.7 ±0.1 in control group, 209 ±0.2 in test group, P $_{value}$ is 0.160 at baseline. After 6 weeks of the cholesterol mean and standard deviation values are 201.7 ±0.3 in control group, 170.8 ±1.0 in test group, p $_{value}$ is 0.001. After 12 weeks mean ±SD values 201.6 ±0.6 in control group, 120.0 ±0.5 in test group, P $_{value}$ is 0.0001. P value is very signification in cholesterol variable. Triglycerides base line mean ±SD values are 150.5 ±0.81 in control group, 150.8 ±0.3 in test group, P $_{value}$ is 0.20. After 6 weeks mean ± SD values were 151.8 ±1.51 in control group, 129.7 ±4.3 in test group, P $_{value}$ is 0.001. After 12 weeks mean ± SD values were 151.1 ±1.50 in control group, 94.1 ±1.0 in test group, P $_{value}$ is 0.001. In low density lipoprotein base line values were 145.4 ±0.80 in control group, 143.52 ±0.36 in test group, P $_{value}$ is 0.105. After 6 weeks mean ± SD values were 145.3 ± 1.0 in control group, 134.3 ± 1.8 in test group, P $_{values}$ 0.001. After 12 weeks mean ± SD values were 145.2 ± 1.0 in control group, 101.1 ± 0.6 in test group, P $_{value}$ is 0.0001. HDL base line mean ± SD values were 31.7 ±1.6 in control group, 31.03 ± 2.1 in test group, P $_{value}$ 0.32. After 6 weeks mean ± SD values were 31.5 ±1.5 in control group, 37.1 ±2.2 in test group, P $_{value}$ is 0.001. After 12 weeks mean ± SD value is 31.3 ± 1.7 in control group, 49.85 ± 2.1 in test group, P $_{value}$ is 0.0001 (Table 02).

Results of systolic blood pressure (mm of Hg) mean ± SD values were 138.2 ± 0.60 in control group, 138.4 ± 2.1 in control group, P value is 0.2. After 6 weeks mean ± SD values were 138.1 ±0.4 in control group, 128.3 ± 1.3 in test group, P value is 0.001. After 12 weeks mean ± SD values were 138.1 ± 0.3 in control group, 121.1 ± 0.1 in test group, P $_{values}$ is 0.0001. In Diastolic blood pressure mean ± SD values were 88.4 ±0.8 in control group, 88.5 ±0.6 in test group, P $_{value}$ is 0.28. After 6 weeks mean ± SD values were 88.6 ±0.1 in control group, 82.7 ±0.4 in test group, P $_{values}$ 0.001. After 12 weeks mean ± SD values were 88.1 ±0.2 in control group, 79.1 ±0.1 in test group, P $_{values}$ is 0.0001 (Table 03).

DISCUSSION

According to our data and previous research does not have a serious side effect in therapeutic doses. Also, in this study we observed that the serum level of liver enzymes like ALT, AST and ALKP were P value significant in test group. Some studies showed that green leaf lettuce contains water soluble, antioxidant compounds such as phenolic acids, flavonoids, anthocyanins, lactucin, vitamins A and C. Bhattacharya, S.K., Satyan, K.S., Ghosal et al. investigated antidyslipidaemic activity of Withania somnifera in high fructose diet induced dsyslipidaemic syrian golden hamsters [14].

Warnholts, A., Mollnau, H., Oelze, M., Wendt, M., Munzel, T. etc., some genotypes viz. HWS-08-14, HWS-08-18, HWS-1228, HWS-1229 and selection-2 B and varieties JA-20 and RVA-100. The above mentioned components may play a role in antihyperlipidemic activity of Withania somnifera mother tincture. Also, we hope that this open clinical study will help pave the way for other researchers to join in the attempt to bridge the gap between alternative medicine and evidence based medicine.

CONCLUSION

The results obtained in this study therefore suggest that the hypocholesteremic effect of Withania somnifera could be mediated through an increased bile acid synthesis for elimination of body cholesterol. The increased hepatic antioxidant activities in Withania somnifera homoeopathic mother tincture administered people indicate that fiber, phytosterols, polyphenols, flavonoids and vitamin C in Withania somnifera homoeopathic mother tincture mother tincture could contribute to amelioration of the hyperlipidemic conditions. However, further researches are required to clarify the mechanism of this effect.

REFERENCES

1. Vural H, Sabuncu T, Arslan SO, Aksoy N. Melatonin inhibits lipid peroxidation and stimulates the antioxidant status of diabetic rats. J Pineal Res 2001; 31: 193-198.
2. Visavadiya NP, Narasimhacharya AVRL. Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. *Phytomedicine*. 2007;14(2-3):136–142.

3. Godi R, Samaha FF. Dyslipidemia in type 2 diabetes mellitus. Curr Diab Rep 2007; 7(3): 228–234.
4. Brunzell JD, Ayyobi AF. Dyslipidaemia in the metabolic syndrome and type 2 diabetes mellitus. Am J Med 2003; 115: 24S-28S.
5. Kerckhoffs DAJM, Brouns F, Hornstra G, Mensink RP. Effects on the human serum lipoprotein profile of *β*-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *Journal of Nutrition*. 2002;132(9):2494–2505.
6. NRC [National Research Council]. 1996. Guide for the Care and Use ofLaboratory Animals. 7th ed. Washington DC: National Academy Press.
7. Wybenga DR, Pileggi VJ, Dirstine PH, Di Glorgio J. Direct manual determination of serum total cholesterol with a single stable reagent. Clin Chem 1970; 16: 980- 984.
8. Warnick GR, Nguyen T, Albers AA. Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol. Clin Chem 1985; 31: 217-22.
9. Bucolo G, David M. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 1973; 19: 476-82.
10. Tripathi YB, Singh BK, Pandey RS, Kumar M. BHUX: a patent herbal formulation to prevent atherosclerosis. *Evidence-Based Complementary and Alternative Medicine. 2005;* 2(2):217–221.
11. Friedewald WT, Levy RL, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
12. Claiborne A. Catalase activity. In: Greenwald, R.A (Ed.). CRC Handbook of Methods for Oxygen Radical Research 1985. CRC Press, Boca Raton, FL, pp. 283-284.
13. Arjmandi, B.H., Ahn, J., Nathani, S., Reeves, R.D., 1992. Soluble dietary fiber and cholesterol affect serum cholesterol concentration, hepatic portal venous shot-chain fatty acid concentrations and fecal sterol excretion in rats. J. Nutr. 122, 246–253.