Research Article

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DOUBLE BLIND RANDOMIZED CONTROLLED STUDY ON THE EFFICACY OF ASPARAGUS RACEMOSUS MOTHER TINCTURE IN HYPERLIPIDEMIA

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Abstract

Aim: Asparagus racemosus has anti toxidant, anti inflammatory and anti diabetic activities. In the present double blind randomized controlled study is that efficacy of asparagus racemosus mother tincture in hyperlipidemia. Hyperlipidemia patients meeting the inclusion criteria were randomly assigned in to the treatment and placebo. Methods: 50 patients in treatment group took asparagus racemosus mother tincture; whereas the patients in placebo group took placebo mother tincture for 12 weeks. The lipid profiles of the patients were evaluated at baseline, and after 6 and 12 weeks of the double blind randomized controlled study. Results: The final results showed that asparagus racemosus mother tincture significantly p value 0.05 improved LDL, HDL, cholesterol, compared to the placebo group. The asparagus racemosus showed an inhibitory effect on of hepatic enzymes and possible liver toxicity. No serious side effect was reported for asparagus racemosus mother tincture administration. Therefore, asparagus racemosus mother tincture could be considered as a supplement for treatment of dyslipidemia.

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INTRODUCTION

Asparagus racemosus is an important medicinal plant which is regarded as a 'rasayana' which means plant drugs promoting general well-being by increasing cellular vitality and resistance [1]. Asparagus racemosus helps in regulating cardiac disorders, hyperlipidemia and hypertension. In Thailand, traditionally the decorticated roots of the plant have been used as a remedy for diseases of spleen, liver and other internal organs, including preventing miscarriage [2-4]. Microscopicallythe inner parenchymatous zone of cortex is composed of 18-24 layers in the upper portion and 42-47 layers in the middle tuberous portion of the roots. Cells are thin-walled and composed of cellulosic fibres; with circular to oral outlines and distinct inter cellular spaces. In some roots 3-4 layers of cortex immediately adjacent to the endodermis are modified into a sheath of stone cells round the endodermis. Asparagus racemosus consists of a diverse range of molecules in which major constituent is steroidal saponins along with alkaloids, flavonoids, dihydrophenanthrene derivatives, furan derivatives and volatile constituents [5,6]. Elevated levels of plasma cholesterol and triglycerides have been implicated as causative factors in the development of

atherosclerosis and coronary heart disease [7,8]. Atherosclerosis is a process of arteries hardening due to deposition of cholesterol in the arterial wall which causes narrowing of the arteries [9,10]. 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 [11]. After which the chylomicron remainders are invested in the liver and bundled with cholesterol, cholesterol esters and ApoB100 to shape VLDL [12].

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 [13]. Besides, after the hydrolysis by hepatic lipase, IDL will be changed over to LDL and misfortune more apolipoproteins [14]. 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 [15-17].

MATERIAL AND METHODS

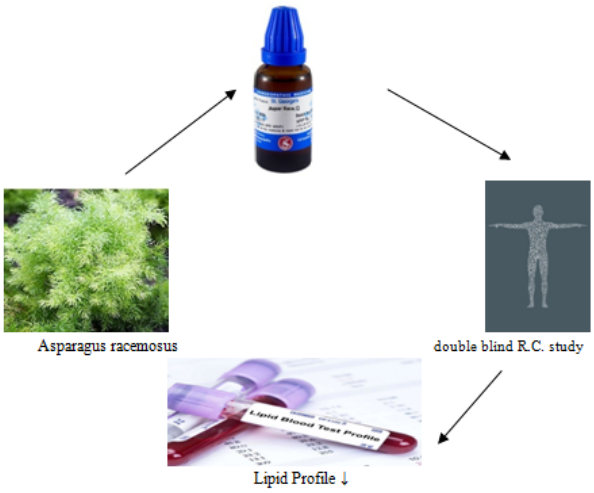
PREPARATION OF ASPARAGUS RACEMOSUS MOTHER TINCTURE

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

PREPARATION OF MOTHER TINCTURE BOTTLES

Extraction of asparagus racemosus mother tincture was purchased and stored as mentioned above. Asparagus racemosus mother tincture and placebo were prepared into bottle in Department of homoeopathy pharmacy, Bharatesh Homoeopathic Medical College, Hospital and Research Center, Belagavi. The asparagus racemosus 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 asparagus racemosus 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. There were no in double blind randomized controlled studies on the anti hyperlipidemic effect of asparagus racemosus mother tincture. In the abovementioned works, administration of asparagus racemosus mother tincture was safe and effective.

Figure 1. Graphical Abstract



STUDY DESIGN

The double blind randomized controlled 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 double blind randomized controlled study. The study was a double blind randomized controlled study, three months, double blind randomized controlled study which was carried out on 50 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 25 patients in each group.The patients were randomly divided into the treatment (25 patients) treatment group and the placebo (25 patients) groups. Finally, 50 patients successfully completed a double blind randomized controlled study.

INTERVENTIONS

Participants were randomized to 2 intervention groups of 25 patients. The patients in the treatment group were taking asparagus racemosus 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 double blind randomized controlled 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) 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 asparagus racemosus 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

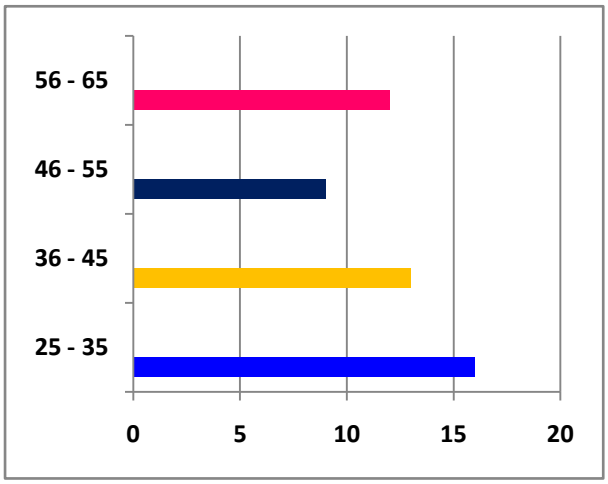
Baseline characteristics were analyzed using independent t-test or $\chi 2$ tests. The significant differences at various time points were assessed by repeated measures of ANOVA. The variables were reported as mean and standard deviation (Mean ± SD). P value less than 0.05 was considered statistically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

Table 01: Demographic data of the patients

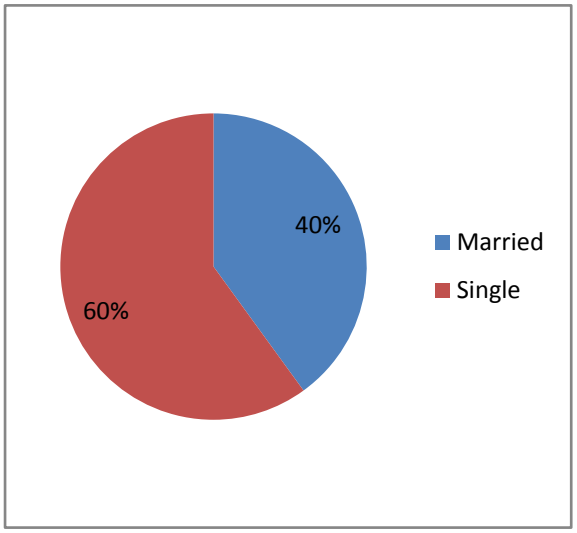
(A) Distribution of cases according to sex

Sex	Total Cases	Percentage (%)
Male	25	50
Female	25	50
Total	50	100

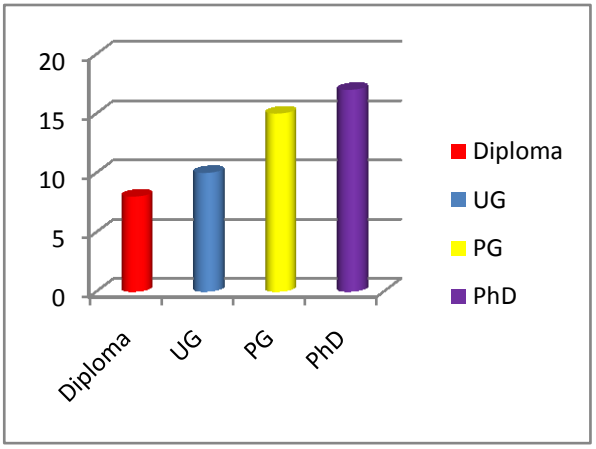
(A) Distribution of cases according to age



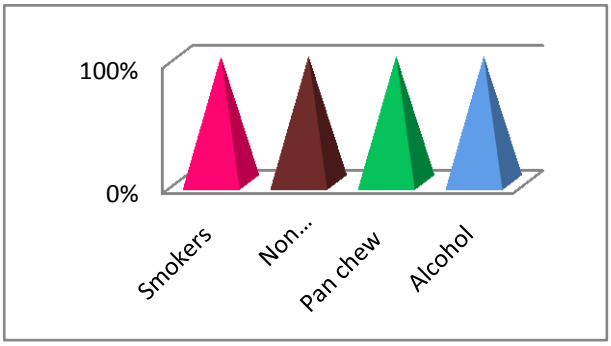
(c) Distribution of cases according to marital status



(d) Distribution of cases according to education levels



(E) Distribution of cases according to habits



(A) Table 2: The measurements of lipid profile between two groups (M±SD)

Variable	Control group	Test group	P Value
Cholesterol			
At base line	212.6 ±4.4	210.3 ±0.8	0.012
After 6 weeks	212.4 ±4.4	179.5 ±1.3	0.001
After 12 weeks	211.6 ±4.3	141.3 ±3.5	0.0001
TG			
At base line	159.4 ±0.9	159.55 ±0.6	0.424
After 6 weeks	159.4 ±0.9	129.8± 4.6	0.001
After 12 weeks	158.6 ±1.1	96.5 ±1.3	0.001
LDL			
At base line	149.7 ± 1.0	149.7±1.1	0.357

After 6 weeks	149.2 ± 0.7	129.9±1.8	0.001
After 12 weeks	148.7 ± 0.8	99.2 ±1.5	0.0001
HDL			
At base line	32.25 ±2.0	32.4 ±2.0	0.31
After 6 weeks	31.9 ± 2.2	41.6 ±3.7	0.001
After 12 weeks	31.5 ± 2.5	52.5 ± 3.2	0.0001

RESULTS

Among 50 type 2 hyperlipidemia patients in age group cases were observed 50% in males and 25 % females. Hyperlipidemia patients in sex group cases were 16 in 25 to 35 years, 13 cases were in 36 to 45 years, 9 cases were in 46 to 55 years, 12 cases were in 56 to 65 years. Marital status of married patients were 40 % married and 60% single (un married), Level of education in diploma were 8, 10 were under graduate, 16 were post graduate, 17 were PhD. In double blind randomized controlled study habits cases were 31 smoking, 7 cases were non smoking, 2 cases were pan chewing and 10 cases were alcohol drinking habits daily (Table 01). In 50 hyperlipidemia patient mean ±SD of cholesterol is 212.6 ±4.4 in control group, 210.3 ±0.8in test group, P_{value} is 0.012 at baseline. After 6 weeks of the cholesterol mean and standard deviation values are 212.4 ±4.4 in control group, 179.5 ±1.3 in test group, p_{value} is 0.001. After 12 weeks mean ±SD values 211.6 ±4.3 in control group, 141.3 ±3.5 in test group, P_{value} is 0.0001. P value is very signification in cholesterol variable. Triglycerides base line mean ±SD values are 159.4 ±0.9 in control group, 210.3 ±0.8 in test group, P_{value} is 0.424. After 6 weeks mean ± SD values were 159.4 ±0.9in control group, 129.8± 4.6 in test group, P_{value} is 0.001. After 12 weeks mean ± SD values were 158.6 ±1.1 in control group, 96.5 ±1.3 in test group, P_{value} is 0.001. In low density lipoprotein base line values were 149.7 ±1.0 in control group, 149.7 ±1.1 in test group, P_{value} is 0.357. After 6 weeks mean ± SD values were 149.2 ± 0.7 in control group, 129.9 ± 1.8 in test group, P_{values} 0.001. After 12 weeks mean ± SD values were 148.7 ± 0.8 in control group, 99.2 ± 1.5 in test group, P_{value} is 0.0001. HDL base line mean ± SD values were 32.25 ±2.0 in control group, 32.45 ± 2.0 in test group, P_{value} 0.31. After 6 weeks mean ± SD values were 31.9 ±2.2 in control group, 41.6 ±3.7 in test group, P_{value} is 0.001. After 12 weeks mean ± SD value is 31.5 ± 2.5 in control group, 52.5 ± 3.2 in test group, P_{value} is 0.0001 (Table 02).

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. Crude extract and purified aqueous fraction of asparagus racemosus have been demonstrated for its antioxidant effect. The activity was tested in rat liver cell mitochondrial membrane damage induced by generated free radicals. The lipid peroxidation induced was evaluated by the formation of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH). The extract exhibited antioxidant effect against oxidative damage by providing protection against lipid peroxidation, protein oxidation and depletion in the levels of protein thiols and antioxidant enzyme, superoxide dismutase. The purified aqueous fraction which consisted of polysaccharides was found to be a potent antioxidant as compared to the crude extract. Purified fraction was more effective against lipid peroxidation whereas the antioxidant effect of the crude extract was more effective in inhibiting protein oxidation.

The lipid lowering effects of *A. racemosus* root extract in hypercholesteremic rats was demonstrated and the investigation revealed that primary reason of antihypercholestrolemic effect was increased excretion of cholesterol, neutral sterols, bile acid and increase in hepatic bile acid content [18,19]. Increased HMG-CoA reductase activity in hypercholesteremic rats upon treatment with asparagus racemosus root powder was powder. Interestingly, normocholesteremic animals under asparagus racemosus treatment, exhibited no significant variations either in excretion of cholesterol, neutral sterols, bile acid, hepatic cholesterol and bile acid content. Significant increase in plasma HDL-C levels with a concurrent decline in the plasma cholesterol level and an improvement in the atherogenic index of hypercholestrolemic test animals clearly indicated the beneficial role of root administration in hypercholesteremic animals. The reduction in the levels of HDL-C is an indicative of

high risk of cardiovascular disease, so improvement in its levels gives cardioprotective activity [20].

CONCLUSION

The results obtained in this study therefore suggest that the hypocholesteremic effect of asparagus racemosus could be mediated through an increased bile acid synthesis for elimination of body cholesterol. The increased hepatic antioxidant activities in asparagus racemosus homoeopathic mother tincture administered people indicate that an oligospirostanoside named 3-0-[α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl]-25(S)-spirosta-3β-oil is obtained in asparagus racemosus 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.

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