EFFECT OF TRUSHNAIDI LOHA ON HYPERLIPIDAEMIA: A CLINICAL STUDY

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ABSTRACT

Hyperlipidaemia is a rise in plasma cholesterol, triglycerides or both. It is a major lifestyle disorder in affluent societies, which has been referred to as the sanatarpanajanya vyadhi (over nutrition) in the classical texts. A herbo-mineral formulation named trushanadiloha that has been advocated by Acharya Yagaratnakara for the management of medoroga / hyperlipidaemia selected for open labeled randomized trial and was carried out on 54 patients suffering from hyperlipidaemia. Of the 54 patients, 50 completed the entire course of treatment. The result of the study on serum cholesterol, serum low density lipoproteins, serum very low density lipoproteins, and triglycerides with trial drug found tube extremely statistically significant (p <0.0001). The response of the drug was extremely statistically significant on moderate improvement of clinical features. (Ch 49, DF 2, p <0.0001). The response of the drug on Body weight, Body weight index, Body surface area was extremely statistically significant (p < 0.0001). Increase in Ponderal index was statistically significant (p > 0.0180). 48 % (24 out of 50 patients) increase of serum high density lipoproteins was observed. The drug decreased total lipid atherogenisity index, which was extremely statistically significant (p < 0.0001). The drug was proved as free radical scavenger and powerful anti-oxidant, anti-hyperglycemic, anti-obese and anti-hyperlipidemic by conducting in-vitro and in-vivo preclinical studies. Keywords: Hyperlipidaemia, Medoroga, Sanatarpanam, Trushanadiloha, High density lipoproteins, Low density lipoproteins, Triglycerides.

INTRODUCTION

Hyperlipidaemia is the condition of abnormally elevated levels of any or all lipids (serum total cholesterol and triglycerides) and/or lipoproteins (low density lipoproteins, total cholesterol and high density lipoproteins ratio and low level of high density lipoproteins) in the blood. Plasma lipids (serum total cholesterol and triglycerides) and lipoproteins have been established to be associated with atherosclerosis and its consequences in different vascular channels in the body. The plasma levels of these lipids and lipoproteins are reflections of various factors like food habits, life style, inherent genetic characteristics, obesity, insulin resistance and presence of other co-morbid conditions such as diabetes mellitus, renal diseases and hyper uricaemia. Changes in lifestyle and food habits are thought to be the likely cause of higher incidence of hyperlipidemia or dislipidemia, coronary heart disease and diabetes mellitus¹. Hyperlipidaemia is a clearly risk factor for coronary artery disease. Of the lipoproteins, it is a low density lipoprotein which is most atherogenic. Very low density lipoprotein is comparatively less atherogenic. High density lipoprotein offers a protective effect and helps in removing cholesterol from the arterial wall. The ratio of total cholesterol / high density lipoprotein is a common way to assess the atherogenisity index. A ratio of more than 4.5 is supposed to be atherogenic². Plasma cholesterol and triglycerides are clinically important because they are major treatable risk factors for cardiovascular disease, whilst severe hypertriglyceridaemia also predisposes to acute pancreatitis³. Research in the field of herbal remedies has added several drug for the management of chronic diseases, but still there are several new avenues for obtaining structurally and functionally newer drugs. The hypertriglyceridaemia, hyperglycemia, hypertension, obesity are the risk disorders and main causative factors for atherosclerotic heart disease. There is no medicine which can cover all these risk factors together, so need of multi-targeted drug is always in demand. Therefore, lipid lowering, anti-obesity, anti-oxidant and anti-hyperglycemic drug taken together will be beneficial for the management of atherosclerosis. From the Ayurvedic point of view hyperlipidemia is a result of sanatarpana (over nutrition). A herbo-mineral formulation named trushanadiloha is selected for clinical trial. It is advocated by Acharya Yagaratnakara for the management of medoroga / hyperlipidaemia, (rasagata sneha vridhdi, rasa raktagata sneha vridhdi, medovriddh, medoroga or medodosha, ama- Medodhatu and asthayi medo dhatu vridhdi etc., are the nomenclatures used by various scholars for hyperlipidemia.) prameha and ati-sthoulya.

MATERIAL AND METHODS

Aim and objectives

To assess the efficacy of trushanadiloha in the management of hyperlipidemia

Criteria for Inclusion

- Patient aged between 25 - 60 years.
- Willingly given informed consent by patient.
- Patient on either sex with Hyperlipidemia.
Exclusion Criteria
- Age below 25 and above 60 years.
- Patient with ongoing therapy with statins or fibrates etc., or having undergone a therapy with the same in the previous three months.
- Pregnant, lactating women or women at a risk of becoming pregnant.
- History of active peptic ulcer in preceding six months or bleeding ulcer at any time in the past.
- Patient having received any investigational drug in the preceding one month or having donated blood in the past three months.
- Patient with severe renal, hepatic disease, coronary heart disease or myocardial infarction as revealed by laboratory investigations or other tests.
- Patient on corticosteroids, androgens, diuretics, cyclosporine, tricyclic anti depressants, valproate, contraceptive pills which contains estrogens.
- Patient is suffering with Cushing’s syndrome, hypothalamic tumors or injury, insulinoma, liver disorders and hepatocellular diseases.
- Patient is suffering with psychological disorders like depression, eating disorders, anorexia nervosa.
- Athletes and body builders having muscular hypertrophy.
- Malignancy.
- Diabetes mellitus
- Hypertension

Place of study
As a part of PhD study under Dr. NTR University of health sciences, Vijayawada, Andhra Pradesh, India; present study was conducted at Govt. Ayurvedic Hospital, Warangal, Govt. Research Department (Ayurveda) Erragadda, Hyderabad, Andhra Pradesh, India during 2010-2012.

Type of the study
The study was conducted as an open label clinical trial with randomized selection of 50 patients of either sex within age groups of 25 to 60 years suffering from hyperlipidemia. Some of the cases were referred cases from other out-patient departments or from local doctors. 54 patients were registered out of them only 50 hyperlipidemic cases were selected for the present study.

Ethical clearance
The Institutional ethical committee of National Ayurvedic Research Institute for vector borne diseases Vijayawada, Andhra Pradesh, India (Institute under Central Council for Research in Ayurveda and Siddha, Department of AYUSH, M/O Health and Family Welfare, Govt. of India) has given Ethical Clearance Certificate (F.No.4-50/2003/NARIVBD/ VJA/ TECH/ 381. Date: 17-07-2010) to conduct the trial

Analytical Study
The analytical study was conducted at Varun herbs, R and D organization Hyderabad and Andhra Pradesh state level drug testing laboratory, Erragadda Hyderabad Andhra Pradesh, India.

Organoleptic tests
- Texture - Fine Powder
- Odour - Aromatic
- Color - Reddish Brown
- Taste - Astringent and salty

Physicochemical Analysis
pH - 2.95, Moisture content - 7.34 %, Total Ash - 41.5 %, Water soluble matter – 53 %, Alcohol soluble matter – 25 %, Acid insoluble ash - 13.5 % and Inorganic/organic contents - Total chloride estimation 2.81 % (in terms of NACL)

Under the observations of TLC (Thin layer Chromatography)
The TLC of sample was done by using solvent system-Toluene: Ethyl acetate with spraying reagent vanillin and sulphuric acid and detected 3 spots with Rf values 0.22 (purple), 0.15 (yellow), 0.18 (Brown). The monograph is not available to trushanadiloha in volumes of Ayurvedic pharmacopoeia of India and in Ayurvedic formulary of India Vol. I and II for reference of analysis till date.

Inductively coupled plasma mass- spectrometer (ICP-MS)
The analytical study was conducted at SGS India Pvt. Ltd. Laboratory, 1/509 A, Rajiv Gandhi Salai (O.M.R.) Opp. Government School, Thoraipakkam, Chennai, Tamil Nadu, India. The sample was examined by ICP-MS to estimate the heavy metals. After examination the observations were shown in the Table 1 and correlated with permissible limit of heavy metals in the dietary contents as per WHO (Table 2).

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Protocol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>AOAC 18th EDN:200667 ICP-MS</td>
<td>14.05 ppm</td>
</tr>
<tr>
<td>Cadmium</td>
<td>AOAC 18th EDN:200667 ICP-MS</td>
<td>0.03 ppm</td>
</tr>
<tr>
<td>Lead</td>
<td>AOAC 18th EDN:200667 ICP-MS</td>
<td>3.78 ppm</td>
</tr>
<tr>
<td>Mercury</td>
<td>AOAC 18th EDN:200667 ICP-MS</td>
<td>3.07 ppm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of the heavy metal</th>
<th>Protocol</th>
<th>Result</th>
<th>Permissible limits recommended by WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>AOAC 18th EDN:2006 by ICPMS</td>
<td>3.78 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Arsenic</td>
<td>AOAC 18th EDN:2006 by ICPMS</td>
<td>14.05 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>Mercury</td>
<td>AOAC 18th EDN:2006 by ICPMS</td>
<td>3.07 ppm</td>
<td>1 ppm</td>
</tr>
<tr>
<td>Cadmium</td>
<td>AOAC 18th EDN:2006 by ICPMS</td>
<td>0.03 ppm</td>
<td>0.3 ppm</td>
</tr>
</tbody>
</table>
One gram of each original sample was calibrated ray Crystallography Indian institute of chemical technology, Hyderabad, Andhra Pradesh India.

Experimental and instrumental details

XRF
One gram of each original sample was taken in an aluminum cup and pressed into a pellet using a hydraulic press (HERZOG, type: TP40/2D) at 15 tons to obtain pellet of moderate thickness. Samples were characterized by using WD-XRF spectrometer (Bruker S4 Pioneer), equipped with a 4 KW. Rh anode X-ray tube with six analyzer crystals [LiF (220), PET, OVO-55, OVO-N, OVO-C and OVO-B]. It has sealed proportional counter for lighter elements and a scintillation counter for heavy element detection. X-ray exposure time and power conditions were adjusted for each element by a pre-calibrated program. The method was measured by FastXVac.34.

The concentration of various elements determined in trushanadihlo by WD-XRF was 19 (Table 3). These are considered to be essential to the life systems. In that 9 are macro-nutrients (Na, Mg, Al, Si, P, S, K, Ca and Ti) and 5 are defined as micro-nutrients (Mn, Fe, Cu, Zn and Ni). The concentration of four heavy elements As, Cd, Pb, and Hg in the drug is shown in the Table 1. Arsenic, one of the four heavy metals which are present in the sample is recently used in the form of arsenic trioxide to treat acute leukemia. The concentration of it in sample is 0.005 % and 14.05 ppm. Mercury is a toxic element, its concentration in sample is 3.07 ppm as determined by ICP-MS. The compound was prepared and analyzed after general and special shodana (means purification of minerals, metals and poisonous herbal drugs in order to remove inherent impurities and poisonous effects) and marana (incineration) according to the textual reference. The heavy metals like Hg, as present in trushanadihlo are just above the permissible limits recommended by WHO (Table 2). The trace elements - chromium works with insulin in the metabolism of sugar to stabilize blood sugar levels and also cleans the arteries by reducing the cholesterol and triglyceride levels. The concentration of Cr detected in the sample is 0.0304 %. Lead is one of the heavy toxic metals that have known to biological functions. The absorption of lead increases in protein and iron deficiency. The concentration of lead in the sample is 3.78 ppm detected by ICP-MS. The bone loss in osteoporosis and osteopenia is prevented by strontium detected in sample which is in the form of oxide is 0.009.

Preliminary Phytochemical Analysis
The drug is positive to Alkaloids, Steroids, proteins, Glycosides, Tannins, Phenolics, Flavonoids and negative to Carbohydrates, Starch.

Acute Oral Toxic Studies
The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the tested dose levels for all the products. Finally acute oral toxicity testing of screened herbomineral formulation did not produce any treatment-related adverse effects up to the dose level of 5000 mg/kg-1 body weight. All protocols for animal experiment were approved by the Institutional Animal Ethical Committee (IAEC bearing No. 439/01/ a / CPCSEA), Shri Vishnu College of pharmacy, Bhimavaram, Andhra Pradesh, India.)

Preparation of drug and dose
All the herbs were procured from davasaz (local herbal shops) located at begumbazar, Hyderabad, Andhra Pradesh, India. The purified and incinerated sample of iron was prepared by author in pharmacy. The drugs Triphala, Trikatu, Pippali and Chitramoolaa Twak were powdered and kept separately; Pippali was fried in cow’s ghee then powdered. Bakuchi seeds purification was done in cow’s urine for more than 7 days by doing bhavana, then dried and powdered, Loha bhasma (incinerated iron) and all ingredients in equal quantity were mixed together thoroughly and kept in a glass container. The compound was preserved in sealed and labeled bottles; powder of 1 g bid with unequal parts of honey and ghee, preferable on empty stomach for 90 days to 50 patients were given.

Sample size and calculation
Sample size calculation was based on the assumption that a sample size of 50 cases would provide a 90 % powder to detect mean change in frequency of growth per fortnight at 5 % level of significance.

Table 3: Percentage concentration of the elements in the drug sample by WD-XRF

<table>
<thead>
<tr>
<th>Na</th>
<th>Mg</th>
<th>Al</th>
<th>Si</th>
<th>P</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.06</td>
<td>0.595</td>
<td>0.346</td>
<td>1.55</td>
<td>0.436</td>
<td>1.830</td>
</tr>
<tr>
<td>K</td>
<td>Ca</td>
<td>Ti</td>
<td>Cr</td>
<td>Mn</td>
<td>Fe</td>
</tr>
<tr>
<td>4.678</td>
<td>1.853</td>
<td>0.0436</td>
<td>0.0304</td>
<td>0.0615</td>
<td>14.040</td>
</tr>
<tr>
<td>Ni</td>
<td>Cu</td>
<td>Zn</td>
<td>As</td>
<td>Cl</td>
<td>Br</td>
</tr>
<tr>
<td>0.0129</td>
<td>0.0424</td>
<td>0.0133</td>
<td>0.005</td>
<td>15.42</td>
<td>0.0131</td>
</tr>
<tr>
<td>Sr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0099</td>
</tr>
</tbody>
</table>

WD-XRF Spectrometer
The analytical study was conducted at Laboratory of X-ray Crystallography Indian institute of chemical technology, Hyderabad, Andhra Pradesh India.

Diagnosis of Hypercholesterolemia, Hyperlipidemia, Hyperlipoproteinemia
Suspected cases of Hypercholesterolemia, Hyperlipidemia, Hyperlipoproteinemia with presenting symptoms of Android distribution of Fat, Polyphagia, Polydipsia, Excessive sweating, Bad odour from body, Excess sleep, Body Fatigue, Loss of libido, Palpitation / Dyspnoea on Exertion, Sudden arrest of expiration were selected and put on laboratory investigations either as outpatient departments case or after hospitalization.
Detailed history of patient covering both demographic and other Ayurvedic parameters was noted on special proforma. To study the state of lipid profile serum cholesterol, serum triglyceride, serum low density lipoproteins and serum very low density lipoproteins were investigated. They were subjected to routine examination of blood, urine and special investigations like serum creatinine, alkaline phosphates (KA units), blood sugar levels, electrocardiogram and thyroid profile. Weight and Height were recorded before and after treatment to estimate the indices of obesity. Based on Ayurvedic and modern disease reviews and research, CCRAS, Department of AYUSH, New Delhi, Govt.of India had prepared a standard protocol and proforma for medoroga (hyperlipidemia). In the present study based on this standard proforma with some minor modifications new proforma was prepared and clinical findings of each patient were noted in terms of score before the treatment on O (i.e. on the date of enrolment) and 90th day.

**Serum Cholesterol**
Normal values: vary with diet and age
Adults = 130 - 200 mg / 100 ml serum/ plasma.
Children = Lower values are found.

**Clinical significance**
High values may be found in diabetes mellitus, hypothyroidism, obstructive jaundice, nephrotic syndrome, biliary cirrhosis, atherosclerosis etc. Low values may be found in hyperthyroidism, malnutrition gusher’s disease and acute hepatitis.

**Serum Triglycerides**
Normal Values
As at there is no general agreement regarding the normal range of serum triglycerides. Fredrik son et al has proposed a range of 90 -190 mg/ 100 ml. to be used depending on age as follows.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Serum Triglycerides (mg / 100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 29</td>
<td>10 - 140.</td>
</tr>
<tr>
<td>30 - 39</td>
<td>10 - 150.</td>
</tr>
<tr>
<td>40 - 49</td>
<td>10 - 160.</td>
</tr>
<tr>
<td>50 - 59</td>
<td>10 - 190.</td>
</tr>
</tbody>
</table>

**Serum High Density Lipoproteins**
Normal range: 30 - 60 mg. / dl. (S.I Units 0.8 - 1.5 mmol / l)

**Clinical significance**
The ratio of serum cholesterol to high density lipoproteins over 5: 1 in males 4.5: 1 in females are considered as increased risk of coronary heart diseases. Increased concentration of high density lipoproteins in serum seems to have inverse correlation with coronary heart diseases.

**Criteria for the categorization of hyperlipoproteinemic types**
Hyperlipoproteinemia was categorized using the following criteria of WHO and Jones, 1973 (Table 5).

**Diagnosis of Obesity**
It was done on the basis of height and weight relationship giving due consideration to the frame of individual also. The subjects were classified to be non-obese, moderately obese, obese and severely obese (Table 6).

**Electrocardiogram diagnosis**
The patients having the clinical symptoms of ischemic heart disease were subjected to E. C. G. study.

**Administration of drug**
For the purpose of trial 50 patients of hyperlipidemia were selected. All the patients were strictly instructed not to use any type of hypcholesterolemic or hypolipidemic drugs during the course of treatment.

**Diet**
Regarding diet, all patients were advised to take their normal diet except intake of excess fried material and red meat. They were permitted to use sunflower / rice bran oil for 20 days and groundnut oil for remaining 10 days in a month. No deliberate attempt was done to impose dietary restriction upon them or to prescribe major dietary management.

**Living conditions**
Majority of the patients were ambulatory and visited on every month at the outpatient department for treatment, assessment, and follow-up. The patients were given instructions to indulge in strenuous exercise (briskly walk for at least 30 minutes per day) during the course of trial. They were allowed to do their normal duties as long as they do not get any difficulty.

**Assessment of Results**
In order to evaluate the effect of rushanadiloha as a hypolipidemic drug and the response in the management of obesity, the assessment of the results has been done from the following parameters:
- Clinical assessment.
- Assessment using obesity and atherogenic indices (anthropometric indices).
- Biochemical assessment for hypercholesterolemia, hyperlipidemia and hyperlipoproteinemia.

**Clinical assessment**
In the clinical assessment, the emphasis was given on 1. Android distribution of Fat 2. Loss of libido 3. Dyspnoea on exertion 4. Excess sleep 5. Polyphagia 6. Polydipsia.
Excessive sweating 8. Palpitation 9. Body Fatigue 10. Sudden arrest of expiration 11. Bad odour from body. As regards to body weight, it was recorded before treatment and on 90th day of treatment on empty stomach with usual dress in kg.

Assessment by Indices
The following indices were used in the assessment of the patients who participated in this trial; Body weight index, Ponderal Index, Obesity Index, Body surface area and Total lipid Atherogenisity. These indices were obtained by the manner shown below.

Body weight index (B.W.I): Ref. value: B.W.I > 1.10 refers obesity.

\[
B.W.I = \text{Body weight / Height in cm. - 100.}
\]

Ponderal Index: The expression used is: \(P.I = \text{Height in cm.} / (\text{weight in kg})^{\frac{1}{3}}\)

Body surface area: Formula of Du Bois is used to determine B.S.A in square meters.

\[
B. S. A. = 0.20247 \times (\text{height in meters})^{0.725} \times (\text{wt in kg})^{0.425}
\]

High risk > 5.

Total lipid atherogenisity = Total cholesterol / high density lipoprotein

Biochemical Assessment
For the assessment of hypercholesterolemic, hypolipidemic and hypolipoproteinemic action of the trial drug, the estimation of total serum cholesterol, serum triglyceride, high density lipoprotein, low density lipoprotein and very low density lipoprotein were repeated after completion of 90 days trial. The data was analyzed to demonstrate the degree of response.

RESULTS
Clinical assessment
Response of Treatment on Clinical features in this study, 5 patients out of 50 had complete improvement (Good), 40 patients had moderate improvement, and 5 patients had poor improvement in clinical features, after 3 months of treatment with trial drug Trushanadiloha. The moderate improvement was extremely statistically significant (chi = 49, df = 2, \(p < 0.0001\)). (Table 8)

Assessment by Anthropometric index
The initial weight was 69.64 ± 10.59 kg. By the treatment with Trushanadiloha up to 3 months, it was reduced to 66.27 ± 10.54 kg. The average reduction was 3.37 kg. This difference is considered to be extremely statistically significant (t = 10.7177, \(p < 0.0001\)). So, the trial drug was proved more effective in reducing body weight (Table 9). The average Body Weight index was 1.17702 ± 0.1284 before treatment. After 3 months treatment it was reduced to 1.12120 ± 0.12014, which was highly significant (t = 4.2187, \(p < 0.0001\)). So, the trial drug was proved more effective in reducing Body surface area (Table 9). The average Ponderal Index was 38.693 ± 2.413 before treatment. After 3 months treatment it increased to 39.340 ± 2.283. The average increase was 0.647, which was statistically significant (t = 2.449, \(p > 0.0180\)). So, the trial drug was effective in increasing Ponderal index (Table 9).

Biochemical assessment for hypercholesterolemia, hyperlipidemia and hyperlipoproteinemia

Serum cholesterol
The average initial serum cholesterol was 217.62 ± 47.33 mgm%. By the treatment with trushanadiloha up to 3 months, it was reduced to 178.22 ± 28.82 mgm% with average reduction of 39.40 mg%. This difference is considered to be extremely statistically significant (t = 8.7207, \(p < 0.0001\)). So, the trial drug was proved more effective in reducing serum cholesterol (Table 9).

Effect on serum high density lipoprotein
The initial mean H.D.L Cholesterol was 39.44 ± 7.03 mgm%. By the treatment with trushanadiloha up to 3 months, it was reduced to 38.78 ± 6.49 mgm% with average reduction of 0.66 mg%. This difference is considered to be not statistically significant (t = 0.2355). So, the trial drug was proved not effective in increasing Serum high density lipoprotein (Table 9).

Effect on serum low density lipoprotein
The initial mean low density lipoprotein was 129.38 ± 40.68 mgm%. By the treatment with trushanadiloha up to 3 months, it was reduced to 102.51 ± 21.73 mgm% with average reduction of 26.87 mg%. This difference is considered to be extremely statistically significant (t = 5.7621, \(p < 0.0001\)). So, the trial drug was proved more effective in decreasing serum low density lipoprotein (Table 9).

Effect on serum very low density lipoprotein
The initial mean very low density lipoprotein was 47.46 ± 24.76 mgm%. By the treatment with trushanadiloha up to 3 months, it was reduced to 35.02 ± 15.87 mgm% with average reduction of 12.44 mg%. This difference is considered to be extremely statistically significant (t = 6.1505, \(p < 0.0001\)). So, the trial drug was proved more effective in reducing Serum very low density lipoprotein (Table 9).

Effect on triglycerides
The initial mean triglycerides were 265.84 ± 180.63 mgm%. By the treatment with trushanadiloha up to 3 months, it was reduced to 182.63 ± 80.13 mgm% with average reduction of 83.21 mgm%, which was considered to be extremely statistically significant (t = 4.2187, \(p < 0.0001\)). So, the trial drug was proved more effective in reducing Serum triglycerides (Table 9).

Assessment by total lipid atherogenisity index
The average initial total lipid atherogenisity Index was 5.637 ± 1.284. By the treatment with Trushanadiloha up to 3 months, it became 4.665 ± 0.916 with average reduction of 0.972, which was considered to be extremely statistically significant (t = 4.3287, \(p < 0.0001\)). So, the
The observations emerging out from the present study indicates that the serum high density lipoprotein levels of hyperlipidemia patients were comparatively lower than the levels of serum cholesterol, serum low density lipoprotein, serum very low density lipoprotein and serum triglycerides. The observations on the clinical trial (in-vivo and in-vitro) of trushanadiloha exhibit a significant beneficial effect in cases of hyperlipidemia in terms of clinical improvement, reduction in body weight, body weight index and body surface area, increase in ponderal index, reduction in high density lipoprotein, serum cholesterol, serum very low density cholesterol, serum triglycerides and reduction in lipid atherogenisity index. In this study 54 patients of hyperlipidemia were registered, out of them 50 proved cases were selected for clinical trial. Total 50 patients were treated with trial drug. Referring to the general observations on the present series of hyperlipidemia, it has been noted that, majority of the patients were male. The ratio of male and female was approximately 2:1. This observations supports finding of previous workers that male sex is more prone to hyperlipidemia. Females are affected less often due to the estrogen hormone as it increases high density lipoprotein levels. Approximately 86 % patients were of age group 20 - 60 years, which may be expected observation, because total cholesterol, triglycerides and low density lipoprotein rise gradually in both men and women through middle age. The incidence of the disease was found in subjects with mixed diet. That is 34 (68 %) patients affected in this trial. In the present study majority of patients were professionals (14 (28 %), house-wives

**DISCUSSION**

The observations emerging out from the present study indicates that the serum high density lipoprotein levels of hyperlipidemia patients were comparatively lower than the levels of serum cholesterol, serum low density lipoprotein, serum very low density lipoprotein and serum triglycerides. The observations on the clinical trial (in-vivo and in-vitro) of trushanadiloha exhibit a significant beneficial effect in cases of hyperlipidemia in terms of clinical improvement, reduction in body weight, body weight index and body surface area, increase in ponderal index, reduction in high density lipoprotein, serum cholesterol, serum very low density cholesterol, serum triglycerides and reduction in lipid atherogenisity index. In this study 54 patients of hyperlipidemia were registered, out of them 50 proved cases were selected for clinical trial. Total 50 patients were treated with trial drug. Referring to the general observations on the present series of hyperlipidemia, it has been noted that, majority of the patients were male. The ratio of male and female was approximately 2:1. This observations supports finding of previous workers that male sex is more prone to hyperlipidemia. Females are affected less often due to the estrogen hormone as it increases high density lipoprotein levels. Approximately 86 % patients were of age group 20 - 60 years, which may be expected observation, because total cholesterol, triglycerides and low density lipoprotein rise gradually in both men and women through middle age. The incidence of the disease was found in subjects with mixed diet. That is 34 (68 %) patients affected in this trial. In the present study majority of patients were professionals (14 (28 %), house-wives
improvement in indices.

Clinical obesity, unnoticed, as (familial hyperlipidemia). Most symptoms observed – Excessive sleep, polyphagia, polydipsia, excessive sweating, body fatigue, bad odour from body, reduction in body weight, reduction in body surface area, increase in ponderal index, significant reduction in serum cholesterol, serum triglycerides, serum low density lipoprotein, serum very low density lipoprotein, minor increase in serum high density lipoprotein (a minor increase of 1 mg/dl in high density lipoprotein produces a 2 - 4 % decrease in the rise of developing Acute myocardial infarction) and reduction in total lipid atherogenity index.

Pre clinical trials

The Acute oral toxicity testing of screened Herbomineral formulation Trushanadiloha did not produce any treatment related adverse effects up to the dose level of 5000 mg / kg body weight. Ethanolic extract of Trushanadiloha exhibit significant free radical scavenging and antioxidant activity. The overall antioxidant activity might be attributed to its phytochemical constituents. The findings of the present study suggest that, this herbomineral drug could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. For anti diabetic testing, the aqueous extract of Herbomineral formulation Trushanadiloha produced significant reduction in blood glucose of normal rats. The observations on Lipid profile of serum of triton induced rats indicates that, increased triglyceride and cholesterol levels were significantly reduced by treatment with dose of 100 to 3000 mg/kg body weight of aqueous extract of trushanadiloha. This dose markedly lowers the levels of serum cholesterol and triglycerides. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids by inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia by their elevated levels of serum cholesterol, triglycerides. It can be concluded that 100 to 3000 mg/kg body weight of aqueous extract of trushanadiloha treatment was effective in reduction of cholesterol, triglycerides, and high density lipoprotein in a dose dependant manner.

Clinical Trial

The response of treatment on clinical features (Table 8) of 5 patients out of 50 had complete improvement, 40 patients had moderate improvement, and 5 patients had poor improvement in clinical features, after 3 months of treatment with trial drug trushanadiloha. The moderate improvement was extremely statistically significant. (chi 49, df 2, p < 0.0001). Regarding response of treatment on body weight, body weight index and body surface area (Table 9) with trial drug after 3 months treatment was extremely statistically significant. So, the trial drug was proved more effective in reducing above parameters. Increase in ponderal index (Table 9) was seen at statistically significant level. So the trial drug was effective in increasing ponderal index. The pattern of fall in serum cholesterol (Table 9) with trial drug, where initial serum cholesterol levels over 217.6 mg% was 39.40 mg% after three months, which was extremely
statistically significant. It was observed during trial that hypocholesterolemic effect was more where initial serum cholesterol levels were high and low with the lower serum cholesterol. The serum high density lipoproteins levels are slightly reduced in 24 patients (48 %) out of 50 patients after three month’s treatment. The average reduction was 0.66 % in 39.44 mg% of initial (Table 9). This decrease was not statistically significant. So, the trial drug was not effective in increasing high density lipoprotein. But at the same time remaining parameters of lipid profile were decreased. It was observed that after withdrawal of the trial drug the serum high density lipoprotein was increased and reached to initial levels. A minor increase of 1 mg/dl in high density lipoprotein produces a 2 to 4 % decrease in the risk of developing acute myocardial infarction. In this trial this type of high density lipoprotein increase was observed in remaining 26 patients after three months. Mean reduction of 26.87 mg% was observed in initial of 129.38 mg% in low density lipoprotein (Table 9), when the patients were treated by trial drug. This decrease is considered to be extremely statistically significant. In trial group 12.44 mg% reduction was observed in 47.46 mg % of initial in very low density lipoprotein (Table 9). Reduction was extremely statistically significant. Reduced level of serum very low density lipoprotein and serum low density lipoproteins are considered good for reducing atherosclerosis. Reduction in serum triglycerides (Table 9) in trial group was observed 83.21 mg% in initial of 265.84 mg%, which was considered to be extremely statistically significant. Simultaneous reduction in serum cholesterol as well as triglycerides has an important significance in the management of coronary heart disease, because increase in both parameters is more pathogenic for ischemic heart disease. It is proposed by casdoerph (1971) that when the cholesterol and triglyceride levels in the blood are reduced by the use of hypocholesterolemic agents there is backward flow of these substances from the arterial wall to the blood leading to regression in the lesion11. In this study reduction in serum cholesterol and serum triglycerides by trial drug was observed extremely statistically significant. When this effect was measured in terms of total lipid atherogenesity index (Table 9) it was extremely statistically significant with trial drug. So, the trial drug proved more effective in reducing or checking the process of atherosclerosis. With all the above information it is felt more appropriate by author to mention some of the important observations made by author during this course of study.

- In some patients after having seen the results the anticipated/ required weight could not be obtained. The reason for the same may be that duration of the treatment should be prolonged. If this is advocated, anticipated results may be obtained.
- Apart from the hypolipidemic activity, this drug was proved more useful in increasing chaya and prabha (colour and complexion) in the patients undergoing treatment with this drug.
- Most of the patients were coming out with the symptom that appetite was suppressed and their body became lighter (Laghutwa) after starting treatment of 10 days.

- In some cases the hemoglobin levels are found raised, the reason being the ingredient loha bhasma.
- Though this drug is proved to be safe, non- toxic in hyperlipidemia and its allied conditions, a little care is advised to be taken in the bleeding disorders and for in pitta prakruti associated with hyperlipidemia.
- Finally it is concluded with observations that this preparation gave more significant results with normal diet.

With all above observations and results it can be proposed that the preparation trashanadiloha is more significantly effective to meet the challenges of the present day to combat hyperlipidemia / medoroga and obesity.

**CONCLUSION**

The concept of medoroga (asthayi medodathuvirdhi) described in Ayurvedic text is similar to the modern concept of hyperlipidemia. It is a metabolic disorder (dhatwagnipaka vikara janya vyadhidi) involving all three agnis viz., jataragni, bhumagni and mainly dhatwagni. Hyperlipidemia mostly manifests at the age of 20 - 60 years i.e., middle age, old age and post-menopausal women. Male sex is affected more often than females. Disease was more prevalent in people taking mixed diet. Excessive intake of carbohydrates, oily food was observed in many of the patients. Professionals, housewives and business persons were affected more. Persons mainly with addiction of alcohol and non-additives were also suffering with this disease. Persons who were severely obese were more prone to this disease than obese. Patients having kapha prakruti, medosara, heredity and vihtiated kapha dosa have predominantly higher incidence of this disease. Patient having pravara ahara shakti suffers less. It is disease of madyamavastha (30 – 60 years). Android distribution of fat, loss of libido, dyspnoea on exertion, excessive sleep, polyphagia, excessive sweating, body fatigue, bad odour from body etc are the main findings of this disorder. Majority of patients were hyperlipoproteinemic type IIa (familial hypercholesterolemia) and type IV (familial hypertriglyceridaemia). Guru, sheeta, snigdha and madhura ahara sevana (high caloric intake) were noted in many patients. Sedentary lifestyles and divaswapna (day time sleep) were observed in most of the cases. Efficacy of the drug is proved to be moderate in reducing the bodily symptoms. Long-term evaluation of the objective parameters (triglycerides, total cholesterol, high density lipoprotein, low density lipoprotein and very low density lipoprotein) may be beneficial furthermore. The trial drug trashanadiloha is proved as powerful free radical scavenger and in vitro anti-oxidant (best rasayana), in vivo anti-obese (ati-sthouyla bara), anti-hyperglycemic (mehagnam) and anti-hyperlipidemic (medoghnam by aagnivvardhanam). The trial drug trashanadiloha is effective in reduction of Body weight, Body weight index, Body surface area and increase in Ponderal index, decrease in all abnormal parameters of lipid profile. Mild increase in serum high density lipoprotein is observed. Reduction in total atherogenesity index is observed. So, the trial drug is a drug of choice for hyperlipidemic patients and prevents the risk of coronary artery disease.
by reducing the process of atherosclerosis. In follow-up studies it was learned that the raising of lipid profile was not observed in subjects who were maintained on diet restriction advised at initial with good lifestyle. Results of the present study become a background for further research. It is a good remedy and proved to be the drug of choice for patients of hyperlipidemia. A mass level clinical trial is recommended in a comparative fashion.

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REFERENCES

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