Research Article

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EFFECT OF TRUSHNADI LOHA ON HYPER LIPIDAEMIA: A CLINICAL STUDY

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ABSTRACT

Hyperlipidemia is a rise in plasma cholesterol, triglycerides or both. It is a major lifestyle disorders in affluent societies, which has been referred to as the santarpanajanya vyadhi (over nutrition) in the classical texts. A herbomineral formulation named trushanadiloha that has been advocated by Acharya Yogaratnakara for the management of medoroga / hyperlipidemia selected for open labeled randomized trial and was carried out on 54 patients suffering from hyperlipidemia. 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: Hyperlipidemia, Medoroga, Santarpanam, Trushanadiloha, High density lipoproteins, Low density lipoproteins, Triglycerides.

INTRODUCTION

Hyperlipidemia 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 mellitus[1]. Hyperlipidemia 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 atherogenic[2]. Plasma cholesterol and triglycerides are clinically important because they are major treatable risk factors for cardio-vascular disease, whilst severe hypertriglyceridaemia also predisposes to acute pancreatitis[3]. Research in the field of herbal remedies have 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 santarpana (over nutrition). A herbo-mineral formulation named trushanadiloha is selected for clinical trial. It is advocated by Acharya Yogaratnakara for the management of medoroga / hyperlipidemia, (rasagata sneha vriddhi, rasa raktagata sneha vriddhi, medovriddhi, medoroga or medododsha, ama- Medodhatu and asthayi medo dhatu vriddhi 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 herbals, 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 R_f 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 1: Concentrations of four heavy elements present in trushanadiloha by ICP-MS

Constituent	Protocol	Value
Arsenic	AOAC 18th EDN:200667 ICP-MS	14.05 ppm
Cadmium	AOAC 18th EDN:200667 ICP-MS	0.03 ppm
Lead	AOAC 18th EDN:200667 ICP-MS	3.78 ppm
Mercury	AOAC 18th EDN:200667 ICP-MS	3.07ppm

Table 2: Concentrations of four heavy elements present in trushanadiloha

Name of the heavy metal	Protocol	Result	Permissible limits recommended by WHO
Lead	AOAC 18th EDTN : 2006 by ICPMS	3.78 ppm	10 ppm
Arsenic	AOAC 18th EDTN : 2006 by ICPMS	14.05 ppm	10 ppm
Mercury	AOAC 18th EDTN : 2006 by ICPMS	3.07 ppm	1 ppm
Cadmium	AOAC 18th EDTN : 2006 by ICPMS	0.03 ppm	0.3 ppm

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

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

WD–XRF Spectrometer

The analytical study was conducted at Laboratory of X-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 Fast-Vac.34.

The concentration of various elements determined in trushanadiloha 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, it's 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 trushanadiloa 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[4]. 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[5-7].

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[8,9]. 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, Pippalimoola and Chitramoola 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[10]. 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.

Diagnosis of Hypercholesterolemia, Hyperlipidemia, Hyperlipoproteinemia

Suspected cases of Hypercholesterolemia, Hyperlipidemia, Hyperlipoproteinaemia with presenting symptoms of Android distribution of Fat, Polyphagia, Polydipsia, Excessive sweating, Bad odor 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 out-patient 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, electro cardiogram 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. be used depending on age as follows[11].

Table 4: Age related elevation of triglycerides in plasma

Age(Years)	Serum Triglycerides (mg / 100 ml)
0 - 29	10 - 140.
30 - 39	10 - 150
40 - 49	10 - 160
50 - 59	10 - 190

Serum High Density Lipoproteins

Normal range: 30 - 60 mg. / dl. (S.I Units 0.8 - 1.5 mmol / I)

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[11].

Criteria for the categorization of hyperlipoproteinemic types

Hyperlipoproteinaemia was categorized using the following criteria[11] 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)[11].
The frame was decided on bi-acromian measurement (Between the lateral extensions of the acromian spines of scapula, highest points of shoulders), as follows[11] (Table 7).
Diagnosis of diabetes: In this study diabetes mellitus has been considered as that of fasting sugar level of 110 mg % or above.
Diagnosis of Hypertension: In this study hypertension was classified using the criteria shown below.
Systolic blood pressure: 140 mm of Hg or above- probable.
Diastolic blood pressure: 90 mm of Hg or above - probable.

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 hypocholesterolemic 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 trushanadiloha 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 7.

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. = Body weight / Height in cm. - 100.

Ponderal Index: The expression used is: P.I = Height in c.ms / (weight in kg)$^{1/3}$

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

B. S. A. = 0.20247*(height in meters) $^{0.725}$ *(wt in kg). High risk > 5.

Total lipid atherogenisity = Total cholesterol / high density lipoprotein[11].

Biochemical Assessment

For the assessment of hypocholesterolemic, 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 ± 0.19309 before treatment. After 3 months treatment it was reduced to 1.12120 ± 0.19419. The average reduction was 0.056, which was highly significant (t 12.3362, p < 0.0001). So, the trial drug was proved more effective in reducing body weight index (Table 9). The average Body surface Area was 1.172284 ± 0.1517 square meters before treatment. After 3 months treatment it reduced to 1.68104 ± 0.14571. The average reduction was 0.491, which was highly significant (t 10.5434, 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 to178.22 ± 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 1.2014, p > 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

trial drug was proved more effective in reducing total lipid atherogenisity index (Table 9).

Table 5: Categorization of types of hyperlipoproteinemia

Lipoprotein pattern	Major elevations in plasma				
	Lipoprotein			Lipid	
	HDL. mg. / dl.	LDL mg. / dl	VLDL. mg. / dl	Serum Cholesterol. mg. / 100 ml.	Serum Triglycerides. mg. / 100 ml.
Type I	15	15 -30	150 -400	200 – 500	2000
Type II a	Normal	Increased	Increased	Increased	Normal
Type II b	Normal	Increased	Increased	Increased	140-400
Type III	Normal	150	Increased	Increased	200-1000
Type IV	Normal	150	Increased	200-400	200-500
Type V	40	130	Increased	200-800	200-4000

Table 6: Criteria for diagnosis of obesity

Non obese	Below optimal ideal weight
Moderately obese	10 % above optimal ideal weight
Obese	15 % above optimal ideal weight
Severely obese	20 % above optimal ideal weight

Table 7: Types of frame

Short frame	38 cm. and above
Medium frame	39 cm.
Large frame	44 cm. and above.

Table 8: Improvement in clinical features

Response	Observed value	Expected value	Proportion	Stats	
Good	5	16.67	- 70.01 %	chi-square	49
Moderate	40	16.67	+ 139.95 %	df	2
Poor	5	16.67	- 70.01 %	p	< 0.0001

Table 9: Effect of drug on anthropometric index and biochemical parameters

Parameter	n	Mean and SD		S. E. Diff	SEM		t- value	p- value
		BT	AT		BT	AT		
Body weight (kg)	50	69.64 ± 10.59	66.27 ± 10.54	0.318	1.50	1.51	10.7171	< 0.0001
Body weight index	50	1.17702 ± 0.1930	1.12120 ± 0.1941	0.005	0.0273	0.0277	12.3362	< 0.0001
Body surface area in square meters	50	1.172284 ± 0.1517	1.6810 ± 0.1457	0.004	0.0214	0.0208	10.5434	< 0.0001
Ponderal index	50	38.693 ± 2.413	39.340 ± 2.283	0.004	0.0214	0.0208	2.449	equals 0.0180
Serum cholesterol in mgm %	50	217.62 ± 47.33	178.22 ± 28.82	4.518	6.69	4.08	8.7207	< 0.0001
Serum low density lipoprotein in mg %	50	129.38 ± 40.68	102.51 ± 21.73	4.544	5.75	3.10	5.7621	< 0.0001
Serum very low density lipoprotein in mgm %	50	47.46 ± 24.76	35.02 ± 15.87	2.090	3.50	2.27	6.1505	< 0.0001
Serum high density lipoprotein in mg %	50	39.44 ± 7.03	38.78 ± 6.49	0.612	0.99	0.93	1.2014	equals 0.2355
Triglycerides in mgm %	50	265.84 ± 180.63	182.63 ± 80.13	20.357	25.55	11.45	4.2187	< 0.0001
Total lipid atherogenisity index	50	5.637 ± 1.284	4.665 ± 0.916	0.225	0.181	0.130	4.3287	< 0.0001

BT: Before Treatment; AT: After Treatment

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

(14 (28 %) and business (10 (20 %) with less physical activity. Alcohol is a secondary cause for hyperlipidemia. In the present study 16 (32 %) were alcoholics, 30 (60 %) are non-alcoholics. Obesity was a major disease which is found associated in the present study. Its incidence is higher. Overweight and obese people are at high risk for hyperlipidemia. Obesity reduces high density lipoprotein levels and increase triglycerides levels. Among 50 patients, 36 (72 %) had obesity. Regarding prakruti, maximum patients were kaphaja 30 (60 %) and then vatakaphaja 6 (12 %) and pittakaphaja 7 (14 %), this indicates that persons with kaphaja constitution are predisposing to hyperlipidemia. Majority of patients had signs and symptoms of predominance kapha. This may be due to excessive vitiation of kapha. This incidence was in 34 (68 %) patients. Medosara patients were more in this study. These patients have predominance of meda in the body, so they may develop sthoulya (obesity) much more easily if they indulge in overeating and sedentary type of living. Patients were shown to have their digestive and assimilative power considerably reduced. While 16 (32 %) had it at the lowest level. This reduction of dhatwagnipaka gives some direction to the samprapthi of the disease in which the person eats excessive and irregularly due to increased samana vata in the amasaya due to obstruction by ama; the improper nourishment of other dhatu and much nourishment of medodhatu (lipids/fat) takes place. This improper eating reduces the digestive and assimilative power and transforms the food in to madhurasayuktha amarasa at the level of amasaya and dhatu, as a result of which saama rasotpathi / medotpatti occurs (production of extra lipids). This excessive accumulation of samarasa / medodhatu in the srotus produces medoroga / hyperlipidemia. Incidence of various degree of obesity was seen in this trial. This confirmed relation of obesity with hyperlipidemia. Among 50 patients, 11 (22 %) had obesity and 25 (50 %) were severely obese. Body weight index of most of the patients were more than 1.10 (refers obesity). Average body surface area was 1.701 square meters (high risk if > 5). Average ponderal index was 39.01. Regarding signs and symptoms observed – Android distribution of fat, excessive sleep, polyphasia, excessive sweating, body fatigue, bad odour from body were the main cardinal symptoms observed, other signs and symptoms was loss of libido, dyspnoea on exertion, polydipsia. Not observed symptoms are palpitation and sudden arrest of expiration. Most of the patient's are of hyperlipoproteinemic type II (familial hypercholesterolemia and familial defective Apo B 100) and IV (familial hypertriglyceridaemia). Type I (familial lipoprotein lipase deficiency) patients were unnoticed, as it is rare. In order to evaluate effect of trushanadiloha as a hypolipidemic drug, response in management of hyperlipidemia and response in reducing obesity, the assessment of results has been done by clinical assessment, by using obesity and atherogenic indices. Results were analyzed statistically by Paired t-test and test from the observations made before and after the clinical trial. The clinical trial (*in vivo*) of trushanadiloha exhibited a significant beneficial effect in cases of hyperlipidemia / medoroga in terms of clinical improvement in Android distributation of fat, loss of libido, dyspnoea on exertion, 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 atherogenisity 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 antidiabetic 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 lesion[11]. 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 atherogenisity 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 trushanadiloha is more significantly effective to meet the challenges of the present day to combat hyperlipidemia / medoroga and obesity.

CONCLUSION

The concept of medoroga (asthayi medodathuvridhi) described in Ayurvedic text is similar to the modern concept of hyperlipidemia. It is a metabolic disorder (dhatwagnipaka vikara janya vyadhi) involving all three agnies viz., jataragni, bhutagni 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, house-wives and business persons were affected more. Persons mainly with addiction of alcohol and non- addictives 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 vitiated 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 distributation 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 trushanadiloha is proved as powerful free radical scavenger and *in vitro* anti-oxidant (best rasayana), *in vivo* anti-obese (ati-sthoulya hara), anti-hyperglycemic (mehagnam) and anti-hyperlipidemic (medoghnam by agnivivardhanam). The trial drug trushanadiloha 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 atherogenisity 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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