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An experimental evaluation of bark of *Chirbilva* (*Holoptelea integrifolia* Planch.) for Hypercholesterolemia in Albino Rats

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ABSTRACT

Hypercholesterolemia is an emerging problem affecting population now a days and responsible for CVD, HTN, Diabetes. Not following *Dincharya, Ritucharya*, smoking, consuming alcohol, lack of exercise, stress, intake of fast food are one of common cause for hypercholesterolemia. Hypercholesterolemia can be compared in *Ayurveda* as *Medodusti* which is due to intake of excessive *Sleshmavradhaka Ahara* (heavy food) leads to *Agnimandhya* (diminished digestive fire) and in turn *Medodusti* that is abnormal accumulation of *Meda* (Cholesterol). *Chirbilva* in Sushruta Samhita mentioned as *Kaphamedovishoshaka*. Hence to trace out its hypercholesteremic effect present study is taken.

Key words: *Hypercholesterolemia, Medodusti, Chirbilva, Holoptelea integrifolia*

INTRODUCTION

Global Health observatory data W.H.O risk of heart disease and stoke due to raised cholesterol levels. Globally ischemic heart disease is due to high cholesterol.[1]

Overall, raised cholesterol is estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million disability adjusted life years (DALYS), or 2.0% of total DALYS. According to Journal of Clinical Lipidology April 2020 Rates of atherosclerotic cardiovascular disease is increasing in India as compared to western countries also found in younger age with hypercholesterolemia.

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CVD is an important cause of mortality and morbidity in India too. According to data of Registrar General of India, age-adjusted CVD is more in southern and eastern India. Studies reported the various factors. In real sense fast moving life-style and getting away from nature have invited number of physiological problems in general human being. Industrialization, heavy work load bringing the stress, changed dietary habits, lack of exercise, good consumption of beverages, fast food and lower intake of fruit and vegetables etc. have added fuel with physiological problems especially when high total serum cholesterol related problems are considered. World Health Organization has set the goal to reduce the global death rate by 2% a year up to 2015.[2] The goal is attributed with lowering of high total serum cholesterol as this is directly related with cardiovascular diseases. Various combination of statins are suggested as safe and potent medication for the problem throughout globe, but adverse effects are also reported with use as Australian Adverse Drug Reactions Advisory Committee had received 22 reports of paraesthesia associated with simvastatin.[3] Symptoms most frequently involved the face, scalp, tongue, and limbs and ranged from hypoaesthetic to hyperaesthetic sensations, although 4 cases of more

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serious neurological damage had also been reported. To avoid such side-effects, the whole world is looking towards alternative medicine and Ayurveda comes ahead amongst different traditional system of medicines with long lists of medicine for the purpose. The problem In *Ayurvedic* classical texts, a condition described by name of *Medo Dhatu Dusti* very much resembles hypercholesterolemia and this is resulted from imbalance of energy consumption and intake. Number of tissues in any human body is rich in *Sneha* (oil) and they are *Medodhatu*, *Vasa* and *Majjadhatu*. They have *Snehatva* as common feature but they differ in their site and function.[4]

Meda's is present mainly in *Udara*, but some are also present in *Mamsa* and *Asthi*. *Medas* present in small *Asthi* is called as *Sarakta Medas* and those present in large *Asthi*, is termed as *Majja*. Pure *Meda* present in *Mamsa* is known as *Vasa.*[5] It is *Medodhatu* which is having significant role in developing many metabolic disorders like *Medoroga* etc. There are two types of *Medodhatu* by name of *Poshaka* and *Poshya*. *Poshaka Medodhatu* is mobile in nature which is circulated in the whole body along with the *Rasarakta Dhatu*. Its purpose is to provide nutrition to *Poshya Medodhatu* and recent researches have proved that cholesterol is circulated with blood, means *Gatiyukta*. *Poshya Medo Dhatu* is having immobile nature, which is stored in *Medodharakala*. The site of *Medodharakala* is *Udara*, *Anuasthi, Sphika*, *Stana* and *Gala*. Such conditions are result of derangement of metabolism of *Medas*. Excessive intake of *Slesma Vardhaka Ahara* produces *Agnimandya*. This *Agnimandya* brings *Ama Dosa* resulting in *Ama Annarasa* which is circulated in body. This vitiates *Dosa, Dhatu, Srotasa, Bhutagni* and *Dhatwagni*. Excessive vitiation occurs in *Medodhatwagni* which brings more production of *Sama Medasa*. This *Sama Medasa* circulates in body which brings *Posaka* and *Posaka Dhatus* in *Sama* condition. Due to this *Medo Dhatu Dusti* occurs. *Medodushti* is abnormal accumulation of *Meda Dhatu* in body. It includes several numbers of other *Medovikaras*, which are collectively known as *Medoroga*. Various formulations and plants are suggested for *Medoroga* viz., *Musta, Haridra, Daruharidra, Vacha, Agnimantha, Agaru, Ashoka, Triphala, Trikatu, Chirabilva, Shimsapa* etc. Reference regarding *Chiravilva* is found in *Sutrasthana 38/8-9* of *Sushruta Samhita* as *Kaphamedovishoshana*. Less references are found regarding hypocholesterolemic effect of *Chirbilva* (*Holoptlea integrifolia*). So, present work is taken for study.

Materials and Methods

Pharmacological Study[6-10]

Animals Procurement and Standard Setup for Experiment

1. Male *Wister Strain Albino rats* weighing 150-250g were used for the study.
2. The animals were obtained from the animal house attached to the A.L.N Rao Memorial Ayurvedic Medical College, with Reg. No. -191/ CPCSEA, IAEC Approval no: A.E.D.G.03/11.
3. They were maintained on "Amrut" brand animal pellet feed of Pranav Agro Industries and plain tap water given ad libitum.
4. Animals were exposed to natural day and night cycles with ideal laboratory conditions in terms of ambient temperature (22 ± 3ºC) and humidity (50-60%).
5. The experiments were carried out after obtaining permission from "Institutional Animal Ethics Committee".

Pilot Study

Maximum dose of cholesterol powder in rat is around 25g/kg/day. Average weight of rat is around 200 gm. Hence, dose of cholesterol comes as:25×200/1000 = 5mg

A pilot study was conducted to fix the dose of the rat. For that 5mg of cholesterol powder was mixed with 5ml of vanaspati ghee by making it in lukewarm state, the 5ml of saturated solution was administered with the help of gastric tube, but on the next day the rat was not survived because of excess dose of vanaspati ghee. Therefore, dose of ghee was reduced to 2ml and was mixed with same 5mg of cholesterol powder in

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lukewarm state, it was observed that this particular dose was suitable for rat and easily digestible.

Autopsy Finding

On autopsy, for 5mg cholesterol in 5 ml vanaspati ghee, it was found that ghee regurgitated back and entered the airways of rat and to lungs, due to these, rat by choking.

Dose Fixation and Schedule

As, per FDA approved *The Jounal of Korean Oriental Medicine* formula for conversion of Human dose to rat dose is:

Rat dose (mg/kg) = HED (mg/kg) × Conversion factor

Whereas, Conversion factor for rat is 6.17 (As per reference for specified weight of rats)

Conversion of the dose obtained above to dose in mg/kg/day by multiplying with suitable conversion factor based on the average weight of the animal.

Dose of Kashaya for human being = 2pala = 2×48 =96ml/60kg body weight

So, HED = 1.6ml/kg body weight

This can be calculated as per formula that is:

Animal dose = 1.6 × 6.17 = 9.872/kg body weight

As, the average weight of rat taken is in grams so, 9.872 × 210/ 1000 = 2.07ml/210 gm body weight of rat

2ml of freshly prepared *Kashaya* was given for experiment.

Dose fixation of standard drug:

Human dose of Atorvastatin is 10-80 mg once in a day. As per earlier studies both 20mg and 40mg human dose was proved effective in reduction of serum lipids in rats and there was no significant difference between them. Hence the dose selected was 20mg, conversion of human dose to rat dose based on formula.

Rat dose = HED × Conversion factor for rat that is 6.17

As, dose of Atorvostatin for human is 20 mg.

Average weight of an individual is 60kg

So, according to formula,

20/ 60 = 1/3 mg is per kg bodyweight for human

Therefore, for rat

1/3 x 6.17 = 2.05 mg is per kg bodyweight

For 210 gm rat,

2.05 mg x 0.210 = 0.43 mg

20 mg of standard was dissolved in 25 ml of distilled water.

0.5ml of Atorvostatin was administered in morning hours to the standard group.

Preparation of *Kashaya*

Fehling method was applied for removal of bark, then it was cut into small pieces and was allowed to dry in shade. When bark of both the drugs got dried then they are pounded well and make to coarse powder form by passing through the sieve no. 2000/355, According to W.H.O. guidelines. Then powders of both the bark were stored in air tight container in dark.

Test Drug:

To prepare freshly *Twaka Kashaya* of drug *Chirbilva* (*Holoptelea integrifolia* Planch.) as mentioned in classics (*Sarangadhara*) i.e., 1 *Pala* (48gms) of powdered drug was taken with 16 parts of water. It was reduced to 1/8th part. The mentioned methods were adopted to prepare *Kashaya* of bark of drugs *Chirbilva* (*Holoptelea integrifolia* Planch.) Roxb.). Fresh *Kashaya* was prepared under *Mandagni* (low flame) as per told in *Sharangdhara* every day to give to rats. The dose given was 2ml as obtained by dose conversion formula. The drug was administered in morning hours to both trial groups.

Experimental study design and protocol

Sample: 18 Albino rats of either sex will be randomly selected.

Inclusion: Criteria Healthy, active rats each weighing in between 150-200 g.

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Exclusion Criteria: Pregnant, diseased rats weighing below 150 g or above 200 g and rats under trial for other experiments will be excluded.

Grouping

Groups	No. of Albino rats	Drugs	Forms	Dose / 200 g Body Weight	Purpose
Control Group	6	Distilled water		2ml	To serve as prophylactic control
Standard Group	6	Atorvastatin 20mg	Liquid	0.4ml	As positive Control
Trial Drug I	6	Stem bark of *Chirabilva*	*Kashaya*	2ml	To bring about *Medohara* effect

Initial body weights of all the animals were taken and the animals were fed on the hyperlipidemic diet except control (Group-1) where they were kept on pellet feed and water. Rats of groups 2 and 3 were given with hypercholesterolemic diet. In Group-2 standard drug Atorvastatin 20mg diluted in 25ml of water and 0.5ml of saturated solution was administered (in morning hrs) whereas Groups-3 received *Chirabilva Twak Kshaya* respectively for 30days. The hypercholesterolemic diet included hydrogenated vegetable oil (Vanaspati Ghee and Cholesterol extra pure powder) made into suspension form. The suspension was administered in the dose of 0.5 ml/210 g bodyweight of rats daily for 30 days (at evening hours) to all the rats. On the 30th day final weight of all the rats will be taken and on the 31st day after overnight fasting, rats were anaesthetized by giving mild dose of Ketamine subcutaneously. Then, the blood was collected by Retro-orbital puncture and was sent to laboratory for biocicalestimations[8-10]

Duration: 31days

Statistical Test: Anova followed by Post hoc. Scheffe test was done.

Observation

1) Subjective Criteria: Weight (B.T. & A.T.) are taken
2) Objective Criteria: HDL, LDL, VLDL, Total Cholestrol, Triglycerides

Collection of Blood

According to guidelines for Collection of Blood from Laboratory Animals following procedure is opted for the collection of blood: Under general anesthesia the rats were grasped so that its back rested on the palm with its head toward thumb. The thumb was placed just lateral to the animal's trachea so that the jugular vein on the same side as the eye from which occluded blood was collected. The fur on the animals head was drawn into the palm, this caused the animal's eye to proptose (bulge) slightly. A 50 µL sterile micro hematocrit tube was directed into the medial canthus (junction of eyelids closest to the animal's nose) of the eye rotating slightly as the tube was directed to a point directly behind the globe. Sufficient pressure was applied to cut through the fibrous layer that surrounded the sinus. Blood flowed through the tube once the sinus had been penetrated. After blood collection, the tube was removed and the eyelids were closed and a dry cotton pad was applied over the eye with gentle pressure to prevent retro-orbital hemorrhage. Blood was not collected from the same eye more than 2 times. An antibiotic ophthalmic ointment was applied following bleeding.

Mode of Drug Administration:

Oral route was selected for administration of drug to respective group of animals by using syringe with an attached gastric tube.

Criteria for assessment of results:

Blood cholesterol levels were checked before and after treatment. Blood was drawn from the rats by retro-orbital method. These values were subjected to statistical analysis to evaluate the hypercholesterolemic activity.

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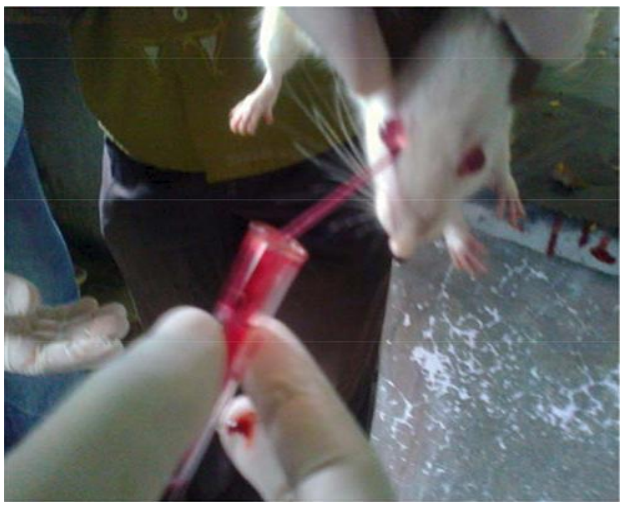
RESULTS

Animal Experiment

Animals (Rats) in Cage

Feeding to Rat



Collection of Blood by Retro-orbital Puncture

Bodyweight Comparison:

Group	Bodyweight (Before Treatment) Mean + SD	Bodyweight (After Treatment) Mean + SD
Control	210.00 + 14.49	236.66 + 9.83
Standard	216.66 + 10.80	235.83 + 11.58
Trial I	211.66 + 14.71	246.66 + 9.83

Mean of Change in Bodyweight

Groups	Mean + SD
Control	26.66 + 6.83
Standard	18.33 + 4.08
Trial I	35.00 + 6.32

Mean and Standard Deviation of Total Cholesterol (mg/dl) Before and After Treatment

Groups	Before Treatment	After Treatment
Control	53.60 + 4.48	55.13 + 4.57
Standard	53.81 + 2.69	41.36 + 2.66
Trial I	52.85 + 3.33	43.90 + 2.65

Mean and Standard Deviation of Triglycerides (mg/dl) Before and After Treatment

Groups	Before Treatment	After Treatment
Control	68.03 + 4.75	69.56 + 4.59
Standard	74.88 + 7.76	51.35 + 6.79
Trial I	71.66 + 8.85	52.27 + 8.58

Mean and Standard Deviation of HDL (mg/dl) Before and After Treatment

Groups	Before Treatment	After Treatment
Control	21.13 + 1.85	20.26 + 1.88
Standard	23.51 + 2.51	24.23 + 2.57

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Trial I	23.52 + 2.11	24.09 + 2.17

Mean and Standard Deviation of LDL (mg/dl) Before and After Treatment

Groups	Before Treatment	After Treatment
Control	18.86 + 3.72	20.98 + 4.04
Standard	15.31 + 1.55	6.69 + 1.59
Trial I	15.00 + 0.75	9.18 + 1.74

Mean and Standard Deviation of VLDL (mg/dl) Before and After Treatment

Groups	Before Treatment	After Treatment
Control	13.60 + 0.95	13.88 + 0.89
Standard	14.97 + 1.55	10.27 + 1.35
Trial I	12.87 + 1.83	10.45 + 1.71

Discussion

When bodyweight before and after treatment was compared, it was seen that increase in weight was maximum with Trial I being 35 gm while it was 19.17 gm, 34gm and 26.66 gm respectively.

When total cholesterol was attained, it was increased by 1.53 gm/dl for control group while for standard it got reduced, 12.45 gm/dl. For Trial drugs, quantity was reduced by 8.95 gm/dl.

Total Triglycerides were observed increasing in control group slightly by 1.53

mg/dl while it was reduced by 23.53 mg/dl, 19.39 mg/dl respectively for standard, Trial drug I

HDL was noted decreased by 0.87 mg/dl while it was marked increased by 0.72mg/dl and 0.57 mg/dl in sequence for standard, Trial drugs I.

Conclusion

Mean of bodyweight changes before treatment and after treatment was 26.66, 18.33 and 35 respectively for control, standard and trial I groups. Mean of changes in total cholesterol before and after treatment in mg/dl for control, standard and trial I were respectively 1.53 (increased), 12.45 (reduced) and 8.95 (reduced). Mean of changes in triglycerides before and after treatment in mg/dl for control, standard and trial I were respectively 1.53 (increased), 23.53 (reduced) and 19.39 (reduced). Mean of changes in HDL before and after treatment in mg/dl for control, Standard and trial I were respectively 0.87 (reduced), 0.72 (increased) and 0.57 (increased). Mean of changes in LDL before and after treatment in mg/dl for control, Standard and trial I were respectively 2.12 (increased), 8.62 (reduced) and 5.82 (reduced). Mean of changes in VLDL before and after treatment in mg/dl for control, Standard and trial I were respectively 0.28 (increased), 4.70 (reduced) and 2.42 (reduced). *Holoptelea* bark was having better in action in case of HDL, LDL and VLDL. Statistical analysis of total cholesterol by means of ANOVA revealed Bark as significant after treatment.

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