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EXPERIMENTAL STUDY ON THE ACTION OF KATURASA PRADHANA AHARA ON LIPID PROFILE

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ABSTRACT

Hyperlipidemia is one of the burning problems globally which will hamper different systems in the body. It leads to several complications like Hypertension, Coronary Heart Disease, Diabetic mellitus, Osteoarthritis and even death. Ayurveda explain Hyperlipidaemia in the context of *Ash-tounindideeyamadhyayam* (a chapter on eight types of undesirable body constitution)as *Sthoulya*ta where there will be abnormal increase in the *Medodhatu* (Lipid entities).The Bio-medical parameter to check the amount of *Sneha* (oily) substances or *Medas* from the body can be the SerumLipidprofile Since, *Medas* and Lipids both have almost same oily nature. *Katurasa* (Pungent taste) has action on *Sthoulya*, the study is designated to evaluate the effect of *Katurasa A hara* (Pungent taste food) on *Medas* through the Serum Lipid Profile. The Experimental study was conducted by taking 5 groups of Winster albino rats. In each group 6 rats were there. The animals were fed with normal laboratory food, Standard drug, Test drug-*Moolaka* (Radish) etc: according to their groups for a period of 28 consecutive days and took their blood samples for checking parameters such as Lipid Profile, Liver function test and Kidney function test. Finally the animals were scarified by dissecting their Organs and send them for Histopathological examinations.

Key words: *Sthoulya*, *Medas*, Lipids, *Moolakagl*

INTRODUCTION

Hyperlipidaemia is one of the burning problems which will hamper the different systems in the body. It is the term used to denote raised serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, triglycerides, or both Total Cholesterol and Triglycerol (combined hyperlipidaemia). Dyslipidaemia is a wider term that also includes low levels of high-density lipoprotein cholesterol. It is not only a condition of deposition of fat in Areolar tissues but a condition in which the utilization of the collected fat is hampered, the treatment of which is highly difficult. It often leads to Obesity. Many types of hyperlipidaemia carry an increased risk of cardiovascular diseases.[1]

AtiSthoulya[2] (Over weight) that falls under the category of *Santharpanajanya Vyadhi* (nutritional disorder), mentioned in *Ashtanindateeyamadhyaya* (a chapter on eight types of undesirable body constitution) is a nutritional disorder due to sedentary lifestyle , improved socio-economic conditions, lack of physical activity , increased intake of food and Heredity.Ayurveda described the preventive as well as the treatment aspects of the disease *Sthoulya*. The preventive aspects is dealt under *Dinacharya*[3] (Daily regimen), *Ritucharya*[4]

(seasonal regimen) and *Sadvritta*[5] (personal code of conduct) whereas the treatment aspect considered under the individual diseases. CharakaAcharya mentioned the causes, signs, symptoms, complications, prognosis and management of *AtiSthoulya*. Sushruta Acharya considered *Sthoola* as *Sadaatura* (always prone to diseases) because *Sthoulya* needs regular and continuous treatment and prevention of *Nidana* (causes) is the best way of its management. In *Sthoulya* there is excess accumulation of *Medo dhatu*[6] (Lipid entities). The word *Meda*is derived from the word '*Mid'-dhatu*[7]. It means *Snigdha* that is; it gives *Snigdhata* (unctuousness) to the body. Fats, Cholesterol, Triglycerides and Lipoproteins are considered as the lipids, the presence of which provides *Snigdhata* to the body. Hence, these substances can be considered as *Medodhatu*. But their excess accumulation, leads to Hyperlipidemia and Obesity. Hence these diseases and *Sthoulya*can be considered as the same disorder due to *MedodhatuVikruti* (abnormal lipids). Any abnormal increase in these *Sneha* substances (oily substances) is a serious warning of health issues. The parameter to check the amount of lipids from the blood Serum is Serum Lipid profile Test. Hence, the *Medo- Vrudhi* (increase in lipids) can also be assessed through the *Vrudhi* of these lipids using the same parameter. There are Six individual *Rasas*[8] (tastes). Among them the *Katu rasa*[9] (pungent taste) has action against *Sthoulya* (over weight), *Alasaka* (abdominal discomforts), *Udardda* (a type of skin disorder), *Kushta* (skin diseases), *Kandu* (itching) and soon. It has various *Karma* (action) like *Lekhana* (scraping), *Sodhana* (cleansing), *Roukshya* (drying or provide roughness), *Theekshna* (sharpness), *Deepana* (stimulate digestive power), *Pachana* (digestion) and due to the presence of *Panchamahabhootas*[10] (five gross elements) like *Vayu* (air) and *Agni* (fire).

Aim of the study:

The study was designed with the objective to evaluate the role of *Katurasapradhanaāhara* on *Sthoulya*.

An *Aharadravya* (a food material) having *Katurasapradhana* (predominant in pungent taste) was selected, for the purpose of analysing its action on *Stoulyata* through Serum Lipid profile. *Moolaka*[11] (Radish-*Raphanussativus*)[12], a major *Aharadravya*, having *Rooksha (rough or dry), Ushna (hot potency), Laghu (light) Guna* was selected as it is more Palatable than any other *KaturasaSkandaDravya*[13]and easily available .Its action on the *Medas*was evaluated through the Serum Lipid profile and the effect of *Katurasa*on *Sthoulya* had evaluated.

Materials and Method

The whole study is divided in two parts as

1. Literary study
2. Experimental Study

Literary study

For the literary study all the available Ayurvedic texts and dessertations, till date were thoroughly reviewed along with modern literature. Review on *Sthoulya*, Obesity, Hyperlipidaemia, *Rasa, Katurasa*, its *Guna*and *Karma*, Taste perception, Pungent taste, *Medodhatu*, Lipids, Lipid metabolism had done thoroughly. The Drug review[11, 12] about *Moolaka* from all possible *Nighantu* (dictionary) also had done. The same drugs mentioned in several modern textbooks related with medicinal plants where the detailed description about the structure, habitat, uses, chemical constituents and therapeutic uses has been mentioned were also referred.

Experimental study

The Test drug *MoolakaSwarasa* was taken every day from tender *Moolaka* as per classical method of preparation using Khalwa

yantra for Crushing and a clean white cloth for squeezing. About 100gram of *Moolaka Swarasa* was taken and squeezed it to get 40ml every day.

Dose Selection: The dose selection was done on the basis of body surface area ratio using the table ofPaget and Barnes (1964 cited by Ghosh ; 1984).

Dose for Animals: Human dose x 0.018 x 5 / Kg wt

Dose for rats: Human dose x 0.018 x 5 x wt of the rat / 1000 g

Route of Drug Administration: The test drugs were administered according to the body weight of the animals byoral route with the help of gastric catheter.

Animals: Wister albino rats weighing between 160–250 g and wereused for experimental study with the following conditions.The animals were obtained from the animal house attached to the Pharmacology Laboratory of SDM Centre for Research in Ayurveda and Allied Sciences. They were exposed to natural day and night cycles with ideal laboratory conditionin terms of ambient temperature, humidity. They were fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water and libitum. The experiments were carried out after obtaining the permission of the Institute's Ethics committee.

Animal selection criteria:

Inclusion criteria: Not less than eight week old healthy albino rats of either sexes weighing about 160-250gwere selected in randomly.

Exclusion criteria: Diseased rats,rats under trail for other experiments,pregnant rats and rats below 160 g and more than 250 g

Animal Grouping: The Wistear albino rats of either sex were grouped into five with 8 animals in each group.

Group 1:Normal control

Group 2: Only Cholesterol (positive control)

Group3: Cholesterol diet and Test drug (*Moolaka* + cholesterol)

Group 4: Cholesterol and Standard Drug (Atorva statin)

Group 5: Normal diet and Test Drug (Only *Moolaka*)

Experimental protocol: The first group or Normal control group rats were administered with normal tap water at a dose of 10ml / Kg with normal diet and water *ad libitum*.The Second or positive control group rats were administerd with hyper lipidaemicdiet that contain 40% cholesterol suspension in hydrogenated vegetable oil [Vanaspati ghee-(RM) Ruchi Soya Industries Ltd.,Survey no:2 (p),3(p),4(p), KIADB industrial area]. The suspension was administerded at a dose of 1ml/100g rat, daily for 28 consecutive days, orally, in the morning and evening sessions.The third group was given Test drug in morning session and the cholesterol suspension in the morning and evening sessions. The fourth group or the Standard group was administered with Atorvastatin 5mg/Kg along with hyper lipidemic diet. The fifth group rats were given the test drug *Moolaka* Swarasa ,following the Animal dosage formula ,at morning hours for 28 consecutive days along with the normal diet.

On 28th day after over night fasting ,the animals were weighed and sacrificed with ether overdose after collecting the blood from retro-orbital plexus. Liver,Kidney and Heart wereexcised out , cleaned, weighed and transferred to 10% formalin solution to tissue and sent for histopathological investigations.

Parameters studied:

Serum Bio-Chemical Parameters: For estimation of bio-chemical parameters, Serum was seperated from collected blood and requisite quantity of serum was fed to the auto

analyser which was automatically drawn into the instrument for estimating different parameters. Bio-chemical parameters likeSerum Cholesterol,Serum Triglycerides, Serum HDL- Cholesterol,Serum LDL-Cholesterol were estimated.

Observations and Results

Cholesterol (mg/dl) of all groups

Roll no:	Normal control	Cholestrol control	Cholesterol and Moolaka	Cholesterol and Standard	Only Moolaka
1	45.0	123	80	51	68
2	65.0	79	122	51	54
3	36.0	81	60	54	81
4	58.0	91	65	50	71
5	59.0	129	74	52	71
6	53.0	83	96	43	71
7	40.0			53	92
8	63.0			47	77

Triglycerides (mg/dl) of all groups

Roll no:	Normal control	Cholestrol control	Cholesterol and Moolaka	Cholesterol and Standard	Only Moolaka
1	85.0	105.0	80	321	95
2	82.0	68.0	122	187	79
3	69.0	51.0	60	212	108
4	101.0	40.0	65	292	92
5	108.0	91.0	74	180	124
6	92.0	201.0	96	200	126
7	34.0			115	100
8	104.0			98	139

HDL (mg/dl) of all groups

Roll no:	Normal control	Cholestrol con-trol	Cholesterol and Moolaka	Cholesterol and Standard	Only Moolaka
1	23.0	24	46	19	26
2	34.0	17	41	25	18.7
3	13.0	20	43	29	33.6
4	34.0	20	38	23	25
5	30.0	18	31	20	29.5
6	27.0	22	44	22	29.2
7	39.0			20	38.7
8	58.0			21	27.9

LDL (mg/dl) of all groups

Roll no:	Normal control	Cholestrol control	Cholesterol and Moolaka	Cholesterol and Standard	Only Moolaka
1	13.0	23	9.1	10	15
2	17.0	6.9	12	15	8.78
3	20.0	16.3	8.9	14	12.81
4	20.0	19.2	15.7	10	11.79

5	18.0	19.2	26.8	7	10.52
6	14.0	12.3	13.9	9	15.32
7	10.0			9	16.90
8	18.0			15	11.33

Statistical Analysis

The data were analyzed by One way ANOVA followed by the Dunnett multiple comparison t- test using the Post hoc test, P<0.05were considered as statistically significant, using Graph pad InstatSoftware.The data have been presented as Mean±SEM.

1. Changes in Lipid profile

Effect of *Katurasa* of *Moolaka* on Serum Total Cholesterol level:

Group	Serum Total Cholesterol (mg/dl)Mean±SEM	% change
NormalControl	53.71±4.18	-
Positive control	97.66±9.14**	81.82 % ↑@
Cholesterol + Moolaka	75.0 ±6.29*	23.20 % ↓#
Cholesterol+ Atorvastatin	50.12±1.26**	48.67 % ↓#
Only Moolaka	68.66±3.16**	29.69 % ↓#

Data in Mean ±SEM,

** =P<0.01

* = P < 0.05

@ = compared with normal control

=compared with cholesterol control

The data related to the effect of *Moolaka* on cholesterol level on 28th day of Hyperlipidemic diet administration has been presented in the table

The data shows that due to the hyperlipidemicdiet, there was an increase in serum cholesterol level in Cholesterol control group when compared to the normal control group. The observed increase was found to be statistically very significant.

The data shows there was a decrease in serum cholesterol level in Cholesterol+ Atorvastatin group and in Only *Moolaka* group when compared to the Cholesterol control group. The observed decrease was found to be statistically very significant.

The data shows there was a decrease in serum cholesterol level in Cholesterol+ *Moolaka* group when compared to the Cholesterol control group. The observed decrease was found to be statistically significant.

Effect of Katurasa of *Moolaka* on Serum Triglyceride level

Group	Triglycerides(mg/dl)Mean±SEM	% change
Normal Control	100.5±3.59	-
Positive control	205.62 ± 19.57**	104.59 %↑ @
Cholesterol + Moolaka	82.83 ±9.36**	59.7%↓ #
Cholesterol + Atorvastatin	156 ±20.63	24.13 % ↓ #
Only Moolaka	107. 87 ± 7.16 **	47.53 %↓#

Data in Mean ±SEM,

** =P<0.01

* = P < 0.05

@ = compared with normal control

=compared with cholesterol control

The data related to the effect of test drug on cholesterol level on 28th day of Hyperlipidemic diet administration has been presented in the table.

The data shows that due to the hyperlipidemicdiet, there was an increase in serum Triglycerides level in Cholesterol control group when compared to the Normal control group.

The observed increase was found to be statistically very significant.

The data shows there was decrease in serum Triglycerides in Cholesterol+ Atorvastatin, Cholesterol +*Moolaka* group and in only *Moolaka* group, when compared to the Cholesterol control group. The observed decrease was found to be statistically very significant in Cholesterol +*Moolaka* group and in only *Moolaka* groups.

The observed decrease was found to be statistically not significant in Cholesterol+ Atorvastatingroup.Comparison of the values from normal control group with only *Moolaka* group showed a marginal and statistically non-significant increase in the latter group.

Effect of *Katurasa* of *Moolaka* on Serum HDLlevel

Group	HDL- Cholesterol (mg/dl)	Mean ± SEM % change
NormalControl	39.66 ±1.35	-
Positive control	20.16 ± 1.04**	49.16 % ↓@
Cholesterol +Moolaka	40.5 ±2.20**	100.89 %↑#
Cholesterol + Atorvastatin	28.54 ±1.47**	41.56 % ↑#
Only Moolaka	28.57 ± 2.09**	41.71 % ↑#

Data in Mean ±SEM,

** =P<0.01

* = P < 0.05

@ = compared with normal control

=compared with cholesterol control

The data related to the effect of test drug on cholesterol level on 28th day of Hyperlipidemic diet administration has been presented in the table.

The data shows that due to the hyperlipidemicdiet, there was a decrease in the serum HDL-Cholesterol level in Cholesterol control group when compared to the Normal control group. The observed decrease was found to be statistically very significant.

The data shows there was an increase in the serum HDL-cholesterol in Cholesterol+Atorvastatin group, Cholesterol+*Moolaka* group and in only *Moolaka* group,when compared to the Cholesterol control group. The observed increase was found to be statistically very significant in all the three groups. The only *Moolaka* group exhibited moderately higher level of serum HDL- cholesterol in comparison to the normal control. The difference was found to be statistically significant.

Effect of katurasa of *Moolaka* on Serum LDL level

Group	LDLCholesterol (mg/dl)Mean ± SEM	% Change
NormalControl	13. 83 ± 1.37	-
Positive control	16.15 ± 2.35	16.77%↑@
Cholesterol +Moolaka	14 .4 ± 2.7	10.83 %↓ #
Cholesterol + Atorvastatin	11.12 ± 1.09	31.14 %↓#
Only Moolaka	12.8 ± 0.96	20.74 %↓#

Data in Mean ±SEM,

** =P<0.01

* = P < 0.05

@ = compared with normal control

=compared with cholesterol control

The data related to the effect of test drug on cholesterol level on 28th day of Hyperlipidemic diet administration has been presented in the table: 34.

The data shows that due to the hyperlipidemic diet, there was an increase in the serum LDL-Cholesterol level in Cholesterol control group when compared to the Normal control group.

The observed increase was found to be statistically non significant.

The data shows there was a decrease in the serum LDL- Cholesterol in Cholesterol+Atorvastatin group, Cholesterol + *Moolaka* group and in only *Moolaka* group, when compared to the Cholesterol control group. The observed decrease was found to be statistically non-significant in all the three groups .

The only *Moolaka* group exhibited marginally lower level of serum LDL-cholesterol in comparison to the normal control. The difference was found to be statistically non-significant.

DISCUSSION

Effect of *Katurasa* of *Moolaka* on LipidProfile

Administration of hyperlipidemic diet lead to significant elevation in serum total cholesterol, serum triglyceride level, moderate non-significant elevation in LDL_cholesterol and significant decrease in HDL- cholesterol. These hyperlipidemic diet induced changes were significantly reversed by the administration of *Moolaka* – thus providing evidence for its efficacy in reversing the hyperlipidemia induced changes.

The above information shows that *Moolaka* has very good potential a dietary intervention for the treatment of hyperlipidemic conditions. It would be interesting to analyze the probable mechanisms involved in this effect. This can be done by focusing on the mechanisms of action of Atorvastatin. This drug has multiple modes of actions like inhibition of the rate limiting enzyme in the cholesterol biosynthesis pathway- HMG- Co A. This leads to decrease in the intracellular cholesterol storage and increased utilization of extracellular cholesterol. It also increases the activity of the enzyme CycloOxygenase -2 involved in the generation of inflammatory mediators which are involved in atherogenesis. It also inhibits the formation of cytokines involved in chemotaxis of inflammatory cells. It is to be ascertained whether the active principles present in the Moolaka share these mechanisms (Henry N.Ginsberg. 2006). The above mechanisms may be responsible for the lowering of cholesterol level.

Moolakaswarasa (juice of Radish) also produced significant decrease in the serum Triglyceride level. Hence its probable mode of modulatory effect on VLDL metabolism requires discussion. It may be through inhibition of hepatic secretion of VLDL (example nicotinic acid) and decreasing the FFA release from adipose tissue through inhibition of lipolysis (Vajinath S Kamanna and Moti LKashyap 2008). The third mechanism involves activation of what are known as PPRAα (peroxisomeproliferator-activated receptor-α). Activation of this factor influences the expression of genes involved in lipid metabolism. This type of activity is seen in fibrates, which activate this factor in both liver and muscle. The result is increased oxidation of fatty acids in liver and as a corollary decrease in the formation of TriAcylglycerol and VLDL. Further there can be enhanced clearance of Chylomicron. All these ads up to provide significant hypolipidemic effect. The test plant active principles are required to be assessed for this type of activity (M. John Chapman 2006).

Moolaka reduces the Total Cholesterol level significantly due to the presence of various factors such as *Rasa*, *Guna*, *Veerya* etc: The lowering levels of Total Cholesterol, Triglycerides and LDL level in blood serum indicates the following possibilities:

1. The chemical constituents present in the *Moolaka* might have increases the activities of the enzyme Lipase.
2. They might also down grade the Cholesterol synthesis.

3. They might helps in increasing the peripheral utilization of Lipids.
4. They might have increases the utilization of Cholesterol for Bile acid synthesis.

Inhibition of the activity of the enzyme lipase- resulting in decreased release of the fatty acids and decreased availability of NADPH which is required for fatty acids and Cholesterol synthesis. Other mechanisms are the increase in the activity of bile acid pump diverting more cholesterol to this pathway and increased peripheral utilization of the lipids.

The high fiber content and low Glycemic index of *Moolaka*, increases the regular bowel movements, and increases the efficiency of the metabolism. Studies proved that the *Moolaka* contain Vitamin-C, that can boost our immune system, regulate metabolism, contributes to the formation of collagen, which is an essential protein that strengthens blood vessel walls and reduces the chances of Artherosclerosis and various other heart diseases.

The presence of *Ushna (hot)* and *Teekshna* (sharp) *Guna*, imparts *Pachana (stimulate digestive power), Sodhana (purifying), Bhedana* (breaking or destroying) and *Bhinnati Sonitasanghata karma (reduces the resistance to blood flow)* and finally eliminate the unwanted *Medas* through the *Srotas* (channels) keeping the values in normal level.

The drug increases the HDL level. High density lipids play an important role in transport of cholesterol from the cells and tissues back to the Liver and prevent excess Cholesterol deposition. Increase of HDL after intake of *Moolaka* can be attributed to the improved state of *Medodhatwagni* due to its *Ushna* and *Teeksha Guna* and *Katu rasa*.

HDL-cholesterol plays important role in reverse cholesterol transport which is considered as a protective mechanism against atherosclerosis process. During this process HDL plays an important role by collecting the excess cholesterol stored in peripheral tissues such as lipid rich foam cell and transport it back to liver for excretion. Statins have been reported to increase the synthesis of apolipoprotein-A-I and HDL- neogenesis in the liver (Shizuya Yamashita et al 2010). It plays major role in the formation of cholesterol ester transfer protein (CEPT) which is secreted in the liver and has a role in promoting the exchange of Cholesteryl esters (CE) from HDL for Triglycerides in lipoprotein containing apoB (LDL and VLDL). Enhanced CEPT activity leads to lowering of cholesterol levels in HDLs and its catabolism. Because of this important role in maintaining the circulation level of lipoproteins its inhibition has marked effect on the lipid profile. It is to be seen whether *MoolakaSwarasa* has influence on the activity of this protein. Considering the very good hypolipidemic effect observed in the test drug it would be useful to assess the drug for the above type of mechanisms.

Modulation of the absorption of cholesterol from the intestine is the other mechanism which may be involved. The absorption inhibition can be by either simple adsorption as exemplified by Cholestyramine or by inhibiting the activity of the cholesterol transporter protein like NPC1L1 (Niemann-Pick type C-1 like 1). This type of activity is exhibited by new generation of drugs like Ezetimibe. Resins bind to bile acids leading to decreased re-absorption. This releases feedback inhibitory effect on the formation of bile salts diverting more of cholesterol for their synthesis and lowering of plasma cholesterol level (Phan et al 2012). Effect on Hyperlipidemic diet induced degenerative changes. This was done by noting the effect of test drug and reference standard on Hyperlipidemic diet induced changes in ponderal, biochemical and histopathological changes. The liver, heart, kidney weight were not affected to significant extent

by the Hyperlipidemic diet indicating low magnitude tissue degeneration or compensatory hypertrophy.

CONCLUSION

Feeding of high cholesterol diet leads to marked increase in the lipid profile components by 28th day. Liver sections showed moderate degenerative changes in all the rats in high cholesterol control group, mild to moderate changes were observed in kidneys. These changes were significantly reversed by both *Moolakaswarasa* and reference standard. This provides further evidence that *Katu rasa Ahara* has lipid metabolism modulation activity. Regular intake of *Katurasa Ahara* will cause reduction in *Medodhatu*.

Limitations of the study

- The study was based on Serum Lipid profile only. The body weight, BMI, Fat deposits are not measured in the study.
- The Ayurvedic methods of diagnosis of *Sthoulya* such as Subjective features of the disease, *Rogiparikshavidhi*, *Pramanapariksha* etc: are not concerned for the study.
- Not only the *Rasa* but due to the *Prabhava* also a drug can act. In the present study which part of the *dravya* that is which "*Rasa Panchaka"* was acting exactly was not cleared.

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