

An Experimental Study on Hypolipidemic effect of some selected Rūksha Guna drugs

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ABSTRACT : *Āyurveda* as well as Philosophies accepted the *Guna* as the basic entity of the *Srishti*. *Gunās* can be classified under various categories like *Ādhyātmika Guna*, *Gurvādi Guna*, *Parādi Guna*, *Vishishta Guna*. For the treatment purpose *Gurvādi Gunas* are widely used. Among them the *Snigdha Guna* and *Rūksha Guna* are widely used in the *Samhitās*. This study has been carried out to establish the *Rūksha* property drugs on animals as a hypolipidemic effect on induced hyperlipidemia animals. The drugs selected were having *Rūksha* property by *Rasa panchaka*. The drugs were *Vachā* (*Acorus calamus* Linn), *Kushtha* (*Saussurea lappa* C.B. Clarke), *Haridra* (*Curcuma longa* Linn), *Daruharidrā* (*Berberis aristata* DC), *Chitraka* (*Plumbago zeylanica*), *Karanja* (*Pongamia pinnata* Pierre). All the drugs are having *Lekhana* property and *Srotosodhaka* karma due to *Rūksha* property. Based on this promise the test drug (*Rūksha Guna*) had been studied on various experimental models such as body weight, weight of liver, heart and kidney, food intake and faecal out put, water intake, total faecal fat content etc. The selected drugs are the representative of highest magnitude of *Rūksha* property which are able to influence *Dīpana* and *Pāchana* property and the test drug was administered with the simultaneous administration of hyperlipidemia inducing diet, but the biochemical values are found under control.

Key words : Hyperlipidemia, *Rūksha Guna*, *Dīpana*, *Pāchana*, Experimental models.

INTRODUCTION

The *Āyurvedic* term “*Bheshaja Pariksha*” can be considered as equivalent to the term drug examination and evaluation. In *Āyurveda*, the drugs which are not scientifically explored have been totally condemned, for example *Charaka* says, “A drug that is not understood perfectly is comparable to poison, fire or the thunderbolt, while the drug which is properly understood is comparable to ambrosia. (Cha.Su.1/124).”

In this clinical science, all concepts are having their practical utility. One of the concepts is *Guna*, which has multifold meanings according to its use, like in social, cultural, philosophical and literary field². The concepts of *Āyurveda* are expressed with *Gunās*³. *Gunās* can be classified under various categories like *Ādhyātmika Guna*, *Gurvādi Guna*, *Parādi Guna*, *Vishishta Guna*. For the treatment purpose *Gurvādi gunas* are widely used. The functional property of *Snigdha* is *Sneha*, *Mrudutā* (softness), *Ādratā* (malleability)^{4,5}. It stimulates *Kapha* and normalizes *Vāta* and also increases *Mala*. Similarly the functional property of *Rūksha* is dryness. It increases *Vāta* and normalizes *Kapha*, decreases *Bala*, *Varna* (normal colour). In the general treatment two principles are

adopted i.e. *Santarpana* & *Apatarpana*. The increased elements are treated by opposite *Guna*⁶. So if *Rūksha Guna* is increased, then it is to be managed by *Snigdha Guna* and vice-versa. So diseases can be treated by applying the two *Gunās* and drugs for the required patient can be selected by applying these *Gunās*⁷.

The aim of the present research work is to observe the effect of *Rūksha Guna* on *Snigdhatā* of animals by providing some selected *Rūksha Guna* drugs after increasing the *Snigdha Guna* in the animals. It was thought worthwhile to supplement the concept with experimental studies in laboratory animals. There are chances that certain factors like status of *Agni* (metabolic activity) and changes in organs with biochemical parameters may be assessed more specifically with objective manner in animals.

Aims and Objectives:

The present study was undertaken with the following aims and objectives¹⁷:

1. To assess the effect of *Rūksha Guna* through various experiments in the animals.
2. Pharmacological evaluation of test drug formulation to assess its effect on lipid profile of albino rats for possible use as hypolipidaemic agent.
3. To assess the action of test drug on *Dīpana Pāchana* activity in experimental animals.

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MATERIALS AND METHODS

Procurement of test drug : The test formulation "Medicinal group A" contains following drugs viz. *Vachâ*, *Kushtha*, *Haridrâ*¹⁰, *Dâruharidrâ*, *Chîtraka*⁸, and *Karanja*^{7, 11, 12} in equal amount. All the drugs were procured from Pharmacy of I.P.G.T. & R.A., Gujarat Ayurved University, finely powdered and processed with *Karanja tvak* decoction and tablets of 500mg each were prepared by adopting standard procedure.

Animals : Wister strain albino rats of either sex weighing between 170-290g were selected for the study from the animal house attached to the institute. They were housed at $22 \pm 2^\circ\text{C}$ with constant humidity 50 - 60%, on a 12th natural day and night cycles. They were fed with diet Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water *ad libitum*. The experiments were carried out in accordance with the directions of the Institutional Animal Ethics Committee (IAEC).

Dose selection & schedule : The dose of the test drug was calculated by extrapolating the human dose to animals based on the body surface area ratio by referring to the standard table of Paget & Barnes, (1969)⁹. The test drug was suspended in tap water (360mg/ml) and administered orally at a volume of 0.5 ml/100g body weight.

Statistical analysis : The data generated during the study was subjected to students 't' test for unpaired data to assess the statistical significance. A 'p' value less than 0.05 were considered as statistically significant.

Experimental procedure : The experiment was carried out by the method designed by Ravishankar B. *et al.* with slight modifications^{13, 14, 15, 16}. It is carried out in two phases viz. Phase I and Phase II.

Phase - I : Preliminary study :

The selected rats of either sex were assigned to 3 groups. The first group receiving tap water served as normal control group, second as cholesterol control group to which only hyperlipidaemic diet (The hydrogenated vegetable oil and cholesterol extrapure powder made in to suspension (20%) in coconut oil in the dose of 0.5 ml/100 g body weight) was administered and the third group was administered with test drug along with hyperlipidaemic diet. Initial weights of selected animals were taken and they

were placed in individual metabolic cages used which had special arrangements for preventing admixture of food with the faecal material. Also faecal material could be collected as the urine was drained out with the specific arrangements.

Phase - II : Therapeutic study :

This phase of the study was started on 6th day and continued till 16th day. At this phase also the food consumption, faecal output and water intake were assessed as mentioned in preliminary study and hyperlipidaemic diet was continued (given at evening hours). The test formulation was administered (morning hours) from 6th day and continued up to 15th day. On the 16th day after overnight fasting, the rats were sacrificed by stunning and severing of jugular vessels, blood was collected and sent for biochemical investigations. From the sacrificed animals, liver, kidney and heart were dissected out and weight was noted and transferred to (including aorta) fixing solution (10% formalin). The tissues were processed for histo-pathological examinations following standard procedure²³ to note down cytoarchitectural disturbances if any. Other parameters studied were changes in body weight, weight of liver, heart and kidney, food intake and faecal out put, water intake and total faecal fat content and also serum biochemical parameters like serum total cholesterol²¹, serum triglyceride²¹, serum LDL cholesterol²¹, Blood sugar¹⁸, serum urea¹⁹, serum creatinine²², and serum alkaline phosphatase²⁰ activity.

OBSERVATIONS AND RESULTS

The results obtained in the present study are given in the form of consolidated statement in the following table. (Table No. 1)

Table No. 2 shows the effect Medicinal group A on food consumption, food conversion ratio and faecal output in Wistar rats. The data presented by Mean \pm SEM where 6 animals in each group. A 'p' value less than 0.05 is considered as statistically significant.

Table No. 3 shows the effect Medicinal group A on various biochemical parameters of Wistar rats. The data presented by Mean \pm SEM where 6 animals in each group. A 'p' value less than 0.05 is considered as statistically significant. A 'p' Value less than 0.001 is considered as statistically highly significant.

TABLE NO. 1 : CONSOLIDATED STATEMENT OF THE OBSERVATION AND RESULTS :

Parameters	Cholesterol control*	Medicinal group A**
1. Body weight	—	NSI
2. Liver weight	NSE	NSE
3. Heart weight	NSE	NSE
4. Kidney weight	SI	NSE
5. Biochemical parameters		
a. Blood sugar	SI	NSE
b. Serum triglyceride	SI	NSE
c. Total cholesterol	SI	NSE
d. Serum creatinine	SI	NSE
e. Serum uric acid	SI	NSE
f. Alkaline phosphatase	SI	NSE
g. Serum total protein	NSE	NSE
6. Total lipid content in faecal matter	—	NSE
7. Food conversion ratio	NSE	NSE
8. Food consumption	NSE	NSE
9. Faecal output	NSE	NSE
10. Water intake	—	NSE

*- In comparison to normal control group; ** - In comparison to cholesterol control group
NSE - No significant effect; SI- Significant increase

TABLE NO. 2 : EFFECT OF TEST DRUG MEDICINAL GROUP A ON FOOD CONSUMPTION, FOOD CONVERSION RATIO AND FAECAL OUTPUT :

Parameters	Control	Cholesterol control	Test drug
Food consumption (g)			
Preliminary phase	17.86 ± 00.66	13.46 ± 02.38	14.31 ± 01.95
Therapeutic phase	16.40 ± 00.85	14.41 ± 02.01	15.59 ± 02.32
Faecal output (g)			
Preliminary phase	02.81 ± 00.14	02.52 ± 00.51	02.95 ± 00.49
Therapeutic phase	02.31 ± 00.10	02.10 ± 00.38*↓	02.38 ± 00.47
Food conversion ratio			
Preliminary phase	06.41 ± 00.31	06.08 ± 01.03	05.41 ± 01.19
Therapeutic phase	08.41 ± 00.52	07.99 ± 01.41*↓	07.92 ± 01.38

↓: Decrease, * p < 0.05, Data : Mean ± SEM

TABLE NO. 3 : THE EFFECT OF TEST DRUG MEDICINAL GROUP A ON BIOCHEMICAL PARAMETERS :

Parameters	Control	Cholesterol control	Test drug
S. Triglycerides(mg/dl)	64.66 ± 04.77	107.50 ± 09.91** ↑	100.08 ± 04.39 ↓
S. Cholesterol (mg/dl)	64.16 ± 06.56	88.67 ± 06.35* ↑	79.80 ± 06.83 ↓
S. Creatinine(mg/dl)	00.66 ± 00.03	00.87 ± 00.04 ***↑	00.82 ± 00.03 ↓
S. Uric acid (mg/dl)	01.28 ± 00.06	02.12 ± 00.17*** ↑	02.28 ± 00.32 ↑
ALP activity(IU/L)	149.83 ± 12.59	245.00 ± 20.23** ↑	232.80 ± 33.48 ↓

↓: Decrease, ↑: Increase, * p < 0.05, ** p < 0.01, *** p < 0.001

DISCUSSION

Experimental models have been selected based on the presumption that the Agni is the main factor for the conversion of Snigdha to Rūksha. For assessment of role of Rūksha Guna the Dīpana - Pāchana activities were considered appropriate. For this purpose three experimental models were selected viz. food

consumption ratio, water intake and faecal output. These three models were suggested earlier by Dwivedi R. R., Dixit U. D. and Ravishankar B. to assess the effect of test drug on Agni and the same has been incorporated in this present study to assess the functions of Pitta in general as well as on Agni too. As Agni is related to Dīpana and Pāchana effect of body, so the

study was done with the relation to Dīpana and Pāchana. This protocol is in use for more than over a decade and is considered as a good model for assessing Dīpana and Pāchana effects there by helpful in assessing the status of Agni^{13,14,15,16}.

Effect on body weight : A moderate increase in the body weight was observed in test drug treated group in comparison to cholesterol control group but it is not up to significant level due to variation in data. The test drugs are Rūksha by Guna and Lekhana and Pāchana in nature. It also indirectly indicate the increase in the Pāchana effect on the food consumed (Table-2). In normal conditions, Pāchana property increases the food assimilation, leading to increase in body weight, but in hyperlipidaemic conditions the Pāchana drugs will act on excessive fat, the net result is comparative decrease in the rate of body weight gain. Thus in the present study a 19.62% higher body weight gain rate was observed in test drug administered group in comparison to cholesterol control.

Enhanced food intake is considered to indicate increased Dīpana property. Decreased faecal out put without concomitant increase in the food conversion ratio (Table -2) is considered to indicate increase in the Pāchana property. Dīpana property is mainly concerned with food breakdown and digestion. If there is decrease in food digestion, there may be reflex inhibition in food intake. Pāchana property is concerned with assimilation of digested food into body constituents. Any change in it will be reflected by the changes in food conversion ratio and body weight.

Analysis of the data shows increase (08.94%) in food intake at therapeutic phase in the test drug administered group in comparison to cholesterol control group but the observed increase is not statistically significant. That means the test Drugs have a good Dīpana property by which it could increase the food intake property.

Faecal output : The perusal of the data generated shows that test drug administration decreased the faecal output up to 19.32% in comparison to cholesterol control group, but it was not sufficient to reach statistically significant level in both preliminary phase and therapeutic phase in comparison to cholesterol control group. Decreased faecal output with concomitant increase in the food conversion ratio is considered to indicate increase in the Pāchana property.

This type of activity may be indicative of the Rūksha Guna present in the test drugs. The increase in food intake without affecting the food conversion ratio

and decreasing faecal output (Table-2) is suggestive of the increased Dīpana. But since there was an increase in the weight gain pattern in hyperlipidaemic rats, the drugs were not able to act on metabolism to cause comparative body weight reduction with respect to normal rats.

Effect on total lipid content in faecal matter : A marginal to moderate 2.00% decrease in total lipid content in faecal matter was observed in test drug treated group at drug free preliminary phase and therapeutic phase respectively in comparison to control. It clearly shows the drug has moderate action on fat metabolism and it expels the fat through faeces showing an action of Srotoshodhana and Sramsana activity with the assimilation activity of Pāchana property.

Effect on water intake : During preliminary phase a moderate and statistically non significant decrease in water intake was observed in test drug treated group in comparison to control group. Decrease in water consumption may be related to increase in the Snigdha property in the body.

Effect on organ weight : Analysis of the data shows increase in both absolute and (8.87%) relative liver weight in the test drug administered group in comparison to cholesterol control group, but the observed increase is not statistically significant. The test drug did not affect both absolute and relative weight of the heart and kidney to significant extent in comparison to cholesterol control group. The only inference which can be drawn on the basis of the available data is that Rūksha Guna has influence on organ's weights. As Snigdha property diet (Cholesterol provoking diet i.e. Cholesterol and Ghee) administered simultaneously with the Rūksha Guna test drug and the Snigdha Guna developed in the organs would have been counteracted by the test drug.

Biochemical parameters : The next set of parameters that was taken into consideration is the effect of Test Drug administration on the Serum Lipid Profile. Administration of cholesterol along with coconut oil and hydrogenated oil lead to significant hyperlipidemic condition as reflected in the form of significant elevation in total cholesterol and triglyceride level in these rats in comparison to normal control. Test drug with sufficient magnitude of Rūkshatā are expected to reverse this hyperlipidemic condition. If this presumption is correlated with findings of this study then this would be an ideal protocol to ascertain Rūkshatā in a drug. Analysis of the result indicates that the test drug produced weak to moderate decrease in serum triglyceride (Table-3) and serum cholesterol (Table-3) levels. With the simultaneous

administration of hyperlipidemic diet with test drug, it has been observed that the biochemical parameters were under control with decreasing result, which is not statistically significant. It shows the Test Drug tablet has a good magnitude of Rūkshatâ as a property.

The other important changes occurring during Hyperlipidemic state are increased blood sugar level, increased serum creatinine level (Table-3), increased alkaline phosphatase activity and increased serum uric acid level (Table-3). These changes can be attributed to increased Snigdhatâ of the diet. In the present study as observed in the case of serum lipid level, marginal antagonism of elevation in most of these parameters was observed in test drug administered group. That is marginal reversal of the increase in the activity or level of the above parameters except serum uric acid. This can be taken to represent presence of Rūksha Guna in the test drug.

Effect on serum creatinine : In hyperlipidemic rats moderate elevation in serum creatinine level is observed in comparison to normal rats. This increase is indicative of disturbance in the kidney functioning. This observation along with the fact that hyperlipidemia leads to increase in kidney weight would suggest that higher level of Snigdhatâ in the body is detrimental to the kidney function. In treated group a marginal decrease on serum creatinine level (05.74%) was observed in comparison to cholesterol control group. This may be considered to represent presence of Rūkshatâ in it.

Effect on Alkaline phosphatase : The test formulation exhibited weak attenuation of Alkaline phosphatase elevation by combating the Snigdhatâ, indicating Rūkshatâ property in it (Table-3).

Effect on Serum uric acid : Effect of test drug on serum uric acid shows a marginal increase (07.54%) in test formulation treated group in comparison to cholesterol control group. The raise can be attributed to two reasons- as already mentioned it is indicative of increased metabolism of uric acid and the second is it reflects the status of kidney functioning. It can be suggested that increased Snigdhatâ influences purine metabolism and impairs kidney function. The changes in the weight of the kidney, increased serum creatinine level and the increased serum uric acid level are all indicative of disturbance in the kidney function too, of which are marginally reversed by the test drug indicating presence of Rūkshatâ in it which can counteract the Snigdhatâ property, which may be for improper kidney function.

Effect on total protein : The proteins are the chief solids of the plasma, an increase in total protein is called

hyperproteinaemia and it may occur due to high level of plasma globulins. The serum total protein level is increased during inflammation due to the increased protein synthesis. In test formulation treated group an apparent marginal decrease was observed in comparison to cholesterol control group. The decrease was found to be statistically non-significant. This indicates measurement of serum protein level is not likely to provide information about the Snigdhatâ or Rūkshatâ attributes in a test formulation.

Histopathological study :

Mild micro fatty changes were observed in the liver sections obtained from rats fed hyperlipidemic diet. In test drug administered group these changes were absent. Kidney sections obtained from different groups exhibited normal cytoarchitecture. There were no differences in the microscopic profile. The heart shows administration of hyperlipidemic diet leading to mild to moderate myocarditis as could be seen in cholesterol and coconut oil fed group. These changes were found to be attenuated in test drug administered groups.

CONCLUSION

The selected drugs are the representative of highest magnitude of Rūksha property which are able to influence Dîpana and Pâchana activity. The test Drug was administered with the simultaneous administration of hyperlipidemia induced diet, but the Biochemical values are in under control. It is indicative of Rūksha Guna which can counteract the Snigdhatâ of body. The test drugs were able to maintain the normal tissue profile of the organs though the hyperlipidemia induced diet was administered.

Though the test drug conceptually, is supposed to have highest magnitude of Rūksha property, it was not found sufficient to antagonize the probable highest level of Snigdhatâ achieved through administration of hyperlipidemic diet. Nevertheless, tendency towards reversal of Snigdhatâ is observed in many biochemical parameters. Based on this premise it can be suggested that it may at least, moderately counteract to Vaikrita Sneha in hyperlipidemia state.

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हिन्दी सारांश

रूक्ष गुण युक्त द्रव्यों का हाइपोलिपिडेमिक प्रभाव पर एक प्रयोगात्मक अध्ययन

संग्राम मिश्रा, आर. आर. द्विवेदी, बी. रविशंकर एवं बी. के. अशोक

गुण सृष्टि का मूल अस्तित्व है। आयुर्वेद के अतिरिक्त अन्य दर्शन भी इसको स्वीकार करते हैं। प्रयोग के अनुसार गुण के अनेक अर्थ हैं जैसे, समाज, संस्कृति, दर्शन, साहित्य में अलग अलग संदर्भ हैं। चिकित्सा के उद्देश्य से गुर्वादि गुणों का अधिकाधिक प्रयोग है। इनमें से स्निग्ध और रूक्ष गुण युक्त औषधि का संहिता में सर्वाधिक प्रयोग है। यह अध्ययन, स्निग्ध गुण युक्त औषध प्रेरित हाइपरलिपिडेमिक चूहों पर हाइपोलिपिडेमिक प्रभाव करता है, यह स्थापित करने के लिये किया गया। रस पंचक के अनुसार रूक्ष गुण रखने वाले औषध लिये गये। यथा - वचा, कुष्ठ, हरिद्रा, दारुहरिद्रा, चित्रक, करंज सभी औषध रूक्ष गुण के कारण लंघनकारक और स्रोतोशोधक कर्म करते हैं। यह परीक्षण विभिन्न प्रायोगिक मानकों पर आधारित है, जैसे शरीर का आकार, यकृत का वजन, हृदय और वृक्क, लिया गया भोजन और बाहर निकाला मल, पिया गया पानी, मल में वसा की मात्रा आदि। सभी आवश्यक रूधिर परीक्षण, जीवरासायनीक परीक्षण भी किए गए। परिणाम में प्राप्त हुआ कि लिया गया औषध रूक्ष गुण रखने वाले औषधियों का प्रतिनिधित्व करता है और इसमें दीपन पाचन के गुण भी हैं। परीक्षित औषध के साथ साथ अत्याधिक वसायुक्त भोजन भी दिया था, इसके बाद भी जैविक रासायनिक मात्रा नियंत्रित रही और वृद्धि नहीं पायी गयी। यह रूक्ष गुणों की कार्मुकता का स्नेहाधिक्य में प्रभाव दर्शाता है।

