

Validation of *Medodhara Kalain* Context of its Correlation with Visceral Fats - An Experimental Study

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Abstract:

Background: The seven *Kalas* are the interface between *Dhatu* and *Aashaya*. *Kala* can be understood as different membranes in the body. The third *Kala* is called '*Medodhara*'. *Meda* is chiefly present in the abdomen of all animals, as well as in *Anu- asthi*. Variable amount of fat is present in human body. Fat is more abundant in fatty persons but it is markedly seen in abdomen, thigh and chest regions. Fat plays numerous roles; metabolic communication and control are linked to it. Adipose tissue is associated with *Medodhara Kala* which is present in abdomen. Once the *Medodhara Kala* or membrane associated with it can be demonstrated experimentally, its structural and functional attributes which are deficient in *Samhita* can be elaborated. The similes are used in regards of other *Kalas* but in case of *Medodhara Kala* it is missing. This experimental study may help to generate a simile related to *Medodhara Kala* in context to structural and functional correlation with visceral fats.

Objectives: Correlation of *Medodhara Kala* with components of visceral fats and evoking a simile in context to the existing correlation through its embryological, structural, functional, histological study and biochemical parameters in High fat diet animal model.

Methodology: This experimental study will be conducted in 8 weeks old 18 Wistar male Rats weighing 250-260 gm. Rats will be divided into three groups: Normal diet (n = 6), HFD with saline (n = 6) and HFD with therapeutic dose 70 mg of *MedoharGuggul* (n = 6). The groups will be compared on biochemical, lipid profile, histopathology and immunohistochemistry parameters.

Expected Results: The variations observed in Lipid profile, histopathology and immunohistochemistry in terms of Mean, SD, SE and other relevant statistical tests will be compared and analysed to reach the study goals.

Conclusion: The embryological, structural and functional aspects of components of visceral fat will be studied in present study to find out the correlation of *Medodhara Kala* with it.

Keywords: *Kala*, *Medodhara*, visceral fat, *MedoharGuggul*, fat

INTRODUCTION:

Sushrutacharya stated that the knowledge of the anatomical structure of the body is of great value, as it helps the surgeons and physicians to comprehend the etiopathogenesis at subtle level and management can be done accordingly.[1] Sushruta has described the '*Sapta- Kala*' in *Sharir-sthana* which is an evidence of minute research.[2]

The seven *Kalas* are the interface between *Dhatu* and *Aashaya*. These *Kala* are extensively supplied with "*Snayus*" bathed in '*Jarayu*' and encased in '*Shleshma*'. [3]

Kala can be understood in three ways:

- 1) Membrane (fibrous, serous or mucous)
- 2) Parts of the body
- 3) Qualities

In *Rachana-sharir Kala* is membrane, as described by *Astang-sangraha*. The '*Kleda*' which lies between *Dhatu* and *Aashaya* undergoes *Pachana* due to its *Agni* producing *Kala*. Which is like *Kashtha- sara* i.e. core of a wood piece and it is covered by *Shleshma*, *Snayu* and *Jarayu*. The words *Snayu*, *Shleshma* and *Jarayu* may be understood as fibrous, mucous and serous respectively. [4] *Kalas* can be correlated with membrane as compared to part of the body and qualities. Other two are also important to understand different features of *Kala* like its properties or functions.

While describing *Kala*, *Dalhanacharya* wisely used '*Avyaktata*'. [5] This *Avyaktata* is due to '*Antarnirgudta*' and is not due to *Sukshamta* (subtle). Here '*Antarnirgudta*' means *Kala* is placed inside the human body and cannot be seen from outside in living beings but can be assessed by its functions. *Kala* is visible by naked eyes i.e. '*Indriyagrahya*' and it can be counted in cadaver after dissection.

The third *Kala* is called '*Medodhara*'. *Meda* is chiefly present in the abdomen of all animals, as well as in *Anu- asthi*. The fat present in large bones is '*Majja*'. Inside large bones Marrow is found; whereas similar substance seen inside small bones should be named as *Meda* mixed with blood. The fatty substance found in pure muscular tissue (*Mamsa*), is included in *Vasa*. [6]

Variable amount of fat is present in human body. Fat is more abundant in fatty persons but it is markedly seen in abdomen, thigh and chest regions. [7] *Charak* proclaims that increase in quantity of *Meda* in body results in movable *Spheek* (buttock), *Udar* (abdomen) and *Sthana* (breast). [8]

Adipose Tissue:

The adipocytes are mostly present in it. This loose connective tissue also contains the stromal vascular fraction (SVF) of cells. SVF includes fibroblasts, preadipocytes, vascular endothelial cells and a number of immune cells like adipose tissue macrophages. The main role of adipose tissue is to store energy in body lipids, though it also insulates and

cushion the body. It is classified in two types viz. white adipose tissue (WAT) & brown adipose tissue (BAT). WAT stores energy and BAT generates body heat. The adipose gene partly controls the formation of adipose tissue. [9]

WAT is deposited in subcutaneous tissue throughout the body in variable amount. There are many structures in vicinity of abdomen which also contains a large amount of fat. Mesentery is a structure that suspends the small intestine from posterior abdominal wall contains more fat. [10] Greater omentum is attached to greater curvature of stomach like an apron which is often laden with fat. [11] The *Vapavahanam* mentioned in *Panchdash-koshtanga* may be compared to greater omentum. The retroperitoneal fat is also more abundant around the kidney.

Superficial fascia: It is one of general coating beneath the skin predominantly formed of loose areolar tissue containing altering amount of fat. Fat is more seen in the anterior abdominal wall below umbilicus, gluteal region, front of thighs, lumbar region, post deltoid region, mammary gland and the cervicothoracic region. Fat distribution is more even and abundant in females than in males. [12]

Panniculus adiposus is a subcutaneous layer of fat. In female body fat is in the superficial fascia of upper thigh, lower abdomen whereas in males it is inside the abdominal cavity. In general women have more amount of fat and even more layer than in men. [13]

NEED AND PURPOSE OF STUDY:

Kala described in Ayurved are comparable with membranes in the body and have certain functions in it. *Medodhara Kala* is said to be store house of fat and may have some role in its maintenance. Adipose tissue performs a numerous functions; it provides a vital nexus of metabolic communication and control, a cushion against trauma and the cold an arbitrator of thermoregulation, and a controller of reproduction and satiety. Fat is also linked with emotional issues, imparting various psychosocial imprints. Over deposition of fat in the body leads to major lifestyle disorders i.e. Obesity and many other conditions like DM, HT etc. associated with it. Obesity and associated conditions like DM; HT etc. are global health issues.

Once the *Medodhara Kala* or membrane associated with it can be demonstrated experimentally, its structural and functional attributes which are deficient in Samhita can be elaborated. The similes are used in regards of other *Kalas* but in case of *Medodhara Kala* it is missing. This experimental study may help to generate a simile related to *Medodhara Kala* in context to structural and functional correlation with visceral fats.

OBJECTIVES:

1. To study the embryological development of components of visceral fat & its associated membrane.
2. To study the structural variations in components of visceral fat viz. mesentery, omentum, perirenal fat & its associated membrane in High fat diet induced animal model through histology.

3. To study the functional aspect of components of visceral fat viz. mesentery, omentum, perirenal fat & its associated membrane in High fat diet induced animal model through biochemical parameters.

4. To assess the type of *Medodhara Kala* according to Ayurved& contemporary science i.e. fibrous, serous or mucous.

5. To study the effect of *MedoharGuggul* on fat accumulation in components of visceral fat and other biochemical parameters in HFD induced animal model.

METHODS:

Type of study: Experimental

Study design: Randomized control trial

Sample size: 18 Wistar Rat weighing 250-260 gm, 8 weeks old

Grouping: 3 groups

Rats will be divided into three groups: [14]

1. Normal diet (ND; n = 6)
2. HFD (Research Diet, Kim et) with saline (n = 6)
3. HFD with therapeutic dose 70 mg of *MedoharGuggul* (n = 6)

Table 1: Grouping of animals

Group	Diet	Treatment	Dose mg/kg oral
I Normal control	Normal diet	Distilled water	0.5 ml
II Experimental	HFD	Saline	0.5 ml
III Experimental	HFD	<i>MedoharGuggul</i>	70 mg

Anupan: For treatment group III distilled water will be added with drug

Duration of Drug dose - 56 Days (8 weeks) [15]

Route - Oral (feeding syringe)

Sample size:

18 Wistar Rat weighing 250-260 gm, 8 weeks old

Inclusion and exclusion criteria

Inclusion criteria - Wistar Rats weight around 250-260 gm

Exclusion criteria- Wistar Rat of less than 250 gm & more than 260 gm weight

Randomisation:

Randomization will be done by Lottery method.

Blinding:

No blinding

Outcome measures:

Biochemical investigations

1. Glucose
2. AST (Aspartate Aminotransferase/SGOT)
3. ALT (Alanine transaminase/SGPT)
4. Serum Creatinine
5. Alkaline phosphatase

Analysis of serum markers: The light anesthesia will be given and blood samples (BS) will be drawn from the aorta of wistar rat. It will be kept up on ice for 30 min prior centrifugation at 13,000 rpm at 4 °C for 10 min and then BS will be stored at -80 °C. Serum levels mentioned in biochemical investigations will be measured with an automatic analyzer in Central Research Laboratory of DMIMS.

Objective parameters: Lipid profile

1. Total cholesterol
2. Blood LDL
3. HDL
4. TG
5. VLDL

Histopathology: Deep anaesthesia will be given to wistar rats with sodium thiopental and then perfuse with cold phosphate-buffered saline (PBS), followed by overnight fixation with 10 % (w/v) paraformaldehyde. Haematoxylin and Eosin (H&E): Liver tissue will be fixed in paraffin and sectioned serially at 4 µm and then stained with H & E. [16]

Immunohistochemistry / sacrifices of animal – Livers, omentum, perirenal fat and mesentery will be dissected and fixed in the solution as mentioned above for 24 hours at 4 °C. Fixed tissues under study will be frozen and stored at -80 °C. Stained sections will be then analyzed by microscope.

Statistical methods:

Values obtained in the present study will be stated as the mean \pm standard error of mean (SEM). Statistical comparisons will be performed using Student's unpaired t-tests and analysis of variance (ANOVA).

Experimental animals:

24 Male Wistar Rats; 8 weeks old weighing 250-260 gms

Housing and husbandry:

All the animals will be accommodated in stainless steel cages having internal barriers so that each rat will get individual housing. 6 wistar rats will be kept in a SS cage and each rat will have a tag number. They will be kept in standard environmentally controlled, clean-air room with temp. $24 \pm 5^\circ\text{C}$, regular illumination (12 hrs dark/12 hrs light cycles), an approximate humidity of $60 \pm 4\%$ and rodent chow and water will be available ad libitum during the investigation period. [17]

Animal care and monitoring:

Every experimental protocol relating to the use of animals will be followed in accord with the CPCSEA guidelines after the approval of institutional animal ethical committee. Care will be taken to avoid any suffering or distress of animals. Animals will be daily monitored for 56 days/end of study.

Interpretation/ scientific implications:

- The *Medodhara Kala* or membrane associated with it will be validated structurally, functionally or both and the ambiguity on this topic will be resolved.
- If the effectiveness of *MedoharGuggul* will be established on membrane or visceral fat and other standard parameters (Lipid profile) in rats then it may generate collateral evidence for lipolytic action of said drug.

Experimental procedures:

The experimental procedures will begin since the preparation of animal experiment. All the experimental material will be made available prior beginning of procedure. The sampling, housing and husbandry of animals will be followed as stated previously. The details of diet are as follows.

Diet:

A) **Normal Diet:** Kim et al. formula will be followed for composition of diets to experimental animals (g/kg diet). [18]. The control wistar rats will be given normal diet (carbohydrates 65%, proteins 20.3%, fat 5%, fibre 5%, salt mixture 3.7% and vitamins mixture 1%).

B) The ingredients of **high fat diet** will be carbohydrates 24%, fat 46%, proteins 20.3%, mixture of salts 3.7% fibre 5% and vitamin mixture 1%. Normal diet and high fat diet constituents will be purchased from recognize store and HFD will be preserved at 4°C until used.

Drug procurement: Readymade study drug (*MedoharGuggul*) will be procured from GMP certified Pharmacy as per doses requirement.

Dose: Animal dose will be calculated by the "Paget & Barns" formula.

Conversion formula: Human Dose = 1.5 gm/ Day

Total clinical dose (Human dose) x conversion factor (0.018) per 100 gm of rat

Dose calculation for 1.5 gm of human dose = 67.5 mg (70 mg Approx)

The diet and drug doses will be administered in animals as per specified in methods and all the animals will be monitored till the end of study. The outcome measures i.e. Biochemical investigations, Objective parameters, Histopathology and Immunohistochemistry / sacrifices of animal will be compared to fulfil the study objectives.

EXPECTED RESULTS:

The variations observed in Lipid profile, histopathology and immunohistochemistry in terms of Mean, SD, SE and other relevant statistical tests will be compared and analysed to reach the study goals.

DISCUSSION:

In present study the embryological development of components of visceral fat & its associated membrane will be studied to find out the relationship of visceral fat and other body fat. The histology study of visceral fat components viz. mesentery, omentum, perirenal fat & its associated membrane in high fat diet induced wistar rats will be done to verify the structural variations among them. The biochemical parameters will be studied to assess the functional aspect of components of visceral fat viz. mesentery, omentum, perirenal fat & its associated membrane in High fat diet induced animal model. The classification of *Medodhara Kala* will be made clearer through assessment of its type according to Ayurved & contemporary science i.e. fibrous, serous or mucous. The accumulation or reduction of visceral fat in relation to abdomen will be assessed by observing the effect of *MedoharGuggul* on fat accumulation in components of visceral fat and other biochemical parameters in HFD induced animals. The gender is one of significant factor for fat accumulation in the body. To avoid it male wistar rats are preferred in this study. There are so many factors associated with fat accumulation and obesity in case of human being so the results obtained in animal model may vary in human. The fat tissue is variable and spread all over the body but *Medodhara Kala* is said to be present in abdomen of all animals. Keeping in mind this fact visceral fat is preferred for comparison in present study. Thus in present study the correlation of *Medodhara Kala* with its Structural, functional or both aspects with components of visceral fat will be made which may evoke a simile based on it. Few studies related to obesity were reviewed[19-23].

CONCLUSION:

The embryological, structural and functional aspects of components of visceral fat will be studied in present study to find out the correlation of *Medodhara Kala* with it. The present study may help to evoke a simile on its structural, functional or both the aspects.

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