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ANATOMICAL COMPARISON OF RAKTADHARA KALA WITH THE HELP OF ANIMAL MODEL, A PILOT STUDY.

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ABSTRACT :

Saptakala are explained in *Sustuta samhita sharir sthana ,adhyaya* 4 and 5 with regards formation and nomenclature of *Kala* respectively. In *Kalpasthan, Visa cikitsa, Acharya Sushruta* has described about *visaveg* passing from one *Kala* to the other *Kala* and its importance. *Raktadhara Kala* is second in order and as the name indicates it is in relation with *Raktadhatu*. In present pilot animal experiment, aqueous extract of an organic irritant i.e. *Gunja* (Abrus precatorius) is administered with dose of 120 mg/kg body weight in albino mice. Both controlled and experimental animals were sacrificed on 7th day and visceral organs were sent for histo-pathological examination. Compared to other organs, liver and spleen have shown marked hemorrhagic and necrosis changes.The drug vitiates *Raktadhatu* due to its *usna, tiksna guna*. This *Raktadhatu* in turn vitiates *Raktadhara Kala*.

Key Words: *Raktadhara Kala., Gunja, visavega, Raktadhatu,* Histopathological slides.

INTRODUCTION: It seems wonder to know that in ancient *ayurved* compendiums like *Susruta samhita, Charaka samhita* etc, the intricate structural details of human body have been explained in detail about *Kala Sharir* i.e these are the membranous structures which may be fibrous, serous or mucous which are lining, supporting and separating which are in the form of septa, fascia, sheath or capsule. These sheath / capsule like structure covers the viscera of abdomen or thorax and perform the functions like secretion, protection and absorption etc.

In *Garbha Sharir* itself, the method / steps of formation of these different *kala* have been explained[1]. The *ashaya* / the hollow structures of the body are formed by *dhatu*. The *kleda* part is present in between these two gets modified due to action of *dhatvagni* and takes the shape of a membrane[2]. Hence become the limiting membrane in between *dhatu* and *ashaya* [3].

Even though the definition and the meaning of *kala* have been explained in a clear cut manner, little confusion / controversy seems to persist in the explanation of individual *kala*. Eg: In the explanation of *Shukradhara Kala.* It is said that it is present in all parts of the body as *sarpi* is present in the milk / sweetness in all parts of sugar cane.[4] While explaining to the students and treating the disease related with *Shukradhara Kala*, it becomes difficult to consider the location (*sthana*).

In the same manner confusion seems in relation with *Raktadhara Kala*. Grossly we can understand that *Raktadhara Kala* means these are the structures present in relation with *Raktadhatu.* In *Sushruta Samhita*, it has been explained that in the sequence, *Raktadhara Kala* is second one and explained that, the *Raktadhara Kala* is present inside *mamsa dhatu*[5] and also in particularly among highly vascular organs

like *Yakrut* and *Pliha*. Among all these, the common structure is blood vessels. Hence it can be assumed that *Raktadhara Kala* means it is endothelial lining of blood vessels irrespective of artery / vein. Even though the location of *Raktadhara Kala* is explained with this much clarity, it is high time to still clarify the location of *Raktadhara Kala* precisely and provide the evidence of animal experiment.

AIMS AND OBJECTIVES:

Aim: To decide the location of *Raktadhara Kala* with the help of animal model.

Objectives: 1.To fix up the limitations of *Raktadhara Kala*.

2. To compare location of *Raktadhara Kala* on the basis of findings of animal study.

MATERIALS AND METHODS:

Materials: For Literary study *Ayurved* and Modern Texts, papers published and other web materials are used.

For Animal study: Animals: Albino mice For the present study Animal Ethical Committee clearance has been obtained from IAEC, B.V.V. Sangha's Hanagal Kumareshwar College of Pharmacy, Bagalkot, Karnataka, (Ref : HSKCP/IAEC, Clear/2013-14/Ayu) dated:12/07/2014.

Following protocol has been used for animal study.

1	Centre where animal study conducted	BVVS, HSK College of Pharmacy, Bagalkot, Karnataka.
2	Animal species	Albino mice
3	Strain	Swiss Albino
4	Source of animal	BVVS, HSK College of Pharmacy Animal house, Bagalkot, Karnataka.
5	Wt of mouse	25-35 gms average
6	No of animals	02 (One control and another experimental)
7	Sex of animals	Both male
8	Diet of animals	Rodent pallets
9	Water	Community tap water ad libitum
10	Temp	18-30^0 C
11	Humidity	40-60%
12	Light cycle	12 Hrs light, 12 Hrs Dark
13	Vehicle used	Water
14	Period of Acclamatization	15 days
15	Period of fasting	Overnight
16	Drug	Gunja seeds (Abrus precatorius) Aqueous extract
17	Dosing	120 mg/kg/day
18	Route of administration	Oral

Drug: Aqueous extract of *gunja* (Abrus precatorius) which is organic irritant[6] is used in the form of its aqueous extract. For which 250 gms of dried *gunja* seeds are taken, pulverized, dissolved in 1000 ml of water, boiled till its boiling point. Then kept overnight and filtered using filter paper no 1 followed by concentration at 60^{0}c in incubator. After which it is stored in freezer. At the time of requirement, the feeding solution is prepared by adding with Tween 18 and the feeding solution is

prepared in such a way that its quantity per animal should below 1ml.

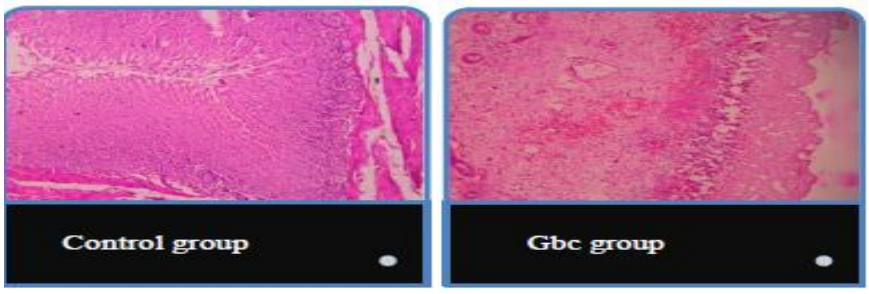
Methodology: Literary study has been undertaken from *Brihattrayi*, *Laghuttrayi* and other classical texts, published papers and other web materials.

Method of collection of data: Since it is pilot study, one animal is taken as experimental (Gbc) and the other is control. The experimental animal is fed with aqueous extract of *gunja beeja* (Abrus precatorius) in the dose of 120 mg/kg body wt for 6 days. On 7th day the animal was sacrificed to obtain the viscera like stomach, liver, spleen, aorta and kidney. which has been preserved and sent for histo - pathological studies and they were analysed.

OBSERVATIONS: During the study the behavioural changes in the animal has been observed. The concept of *visa vega* has been taken into consideration[7], which are caused due to presence of *kala*. In *Susruta samhita* it is explained that after ingestion of *visa*, seven *visa vegas* are seen corresponding to crossing of *saptakala* by *visa*. These *visavega Laksana* ranges from *sunangata* till death in seventh *vega*. Different *lakshanas* are seen depending on *vegantara*. Correspondingly the behavioural and other changes in the animal were observed as follows,

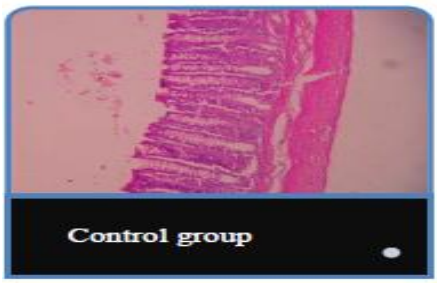
Changes	Observations
Skin	Blanching, Cyanosis Erythema, Itching
Fur	Falling of fur, Piloerection, Discoloration
Eyes	Ex opthalamus, Redness, Ptosis, Lacrimation, Pupil constricted / dilated
Behavioral pattern	Restlessness, Grooming, Lying flat on belly, Lying flat on side, Lying flat on back, Sleeping.
Salivation	Viscid Watery
Respiration	Depression, Stimulation failure
Motor activity	Muscle relaxation analgesia, arching and rolling
CNS	Defecation, Urination, Squatting, Ataxic gait, Tremors, Timidity, Writhing, Paresis of hind limbs, Paresis of forepaws, Twitches, convulsions.

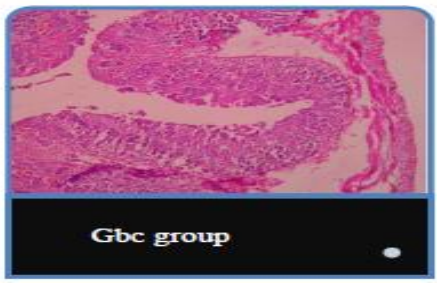
HISTOPATHOLOGY OF STOMACH



High desquamation, severe edema and congestion and moderate mucosal hemorrhage of Stomach Tissue

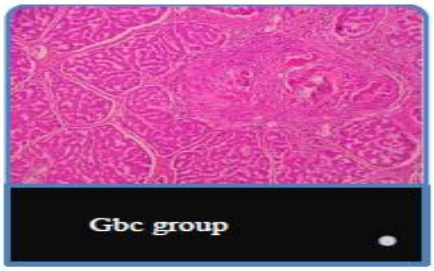
HISTOPATHOLOGY OF INTESTINE





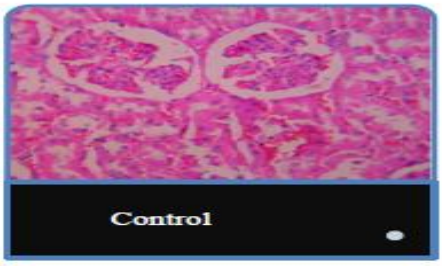
Moderate mucosal congestion, desquamation, nutrophilic infiltration and cellular degeneration of Intestinal Tissue

HISTOPATHOLOGY OF LIVER



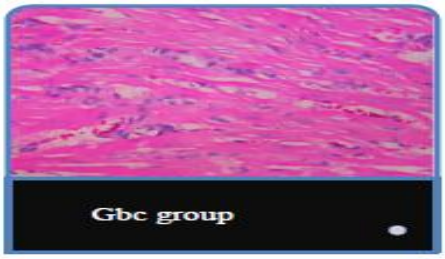
Severe Central Vein & sinusoidal congestion, inflamation, central granulomas, cirrhosis, perivenular fibrosis of Liver Tissue.

HISTOPATHOLOGY OF KIDNEY



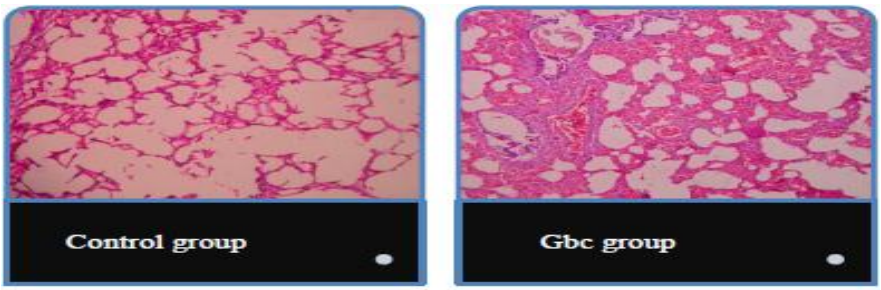
Severe peritubular inflamation,tubular & glomerular congestion of Kidney Tissues.

HISTOPATHOLOGY OF EPITHELIUM OF AORTA



Severe congestion, oedema and mild inflammation of Aorta Epithelium.

HISTOPATHOLOGY OF SPLEEN



Severe Inflammation with marked congestion and focal necrosis of SpleenicTissue.

RESULTS AND DISCUSSIONS: In the Pilot study after careful examination of the histo pathological slides of viscera it has been observed that, due to the toxic effects of *Gunja* (Abrus precatorius) especially on stomach, liver, spleen, aorta etc, the changes like haemorrhage and necrosis are seen in moderate to severe degree. But the degree of same changes is less in other viscera like intestines and kidney.

Extensive endothelial damage is seen in aorta. It is observed that the haemorrhagic changes like congestion, haemorrhage and necrosis is less in small intestine and still less in large intestine. It is evident that the hemorrhagic changes like mild to moderate haemorrhages, necrosis are seen in only blood vessels or in the organs which are highly vascular in liver and spleen, which are the main seats of *Raktadhatu* [8].

Here for the study the drug used is *Gunja*, which has *usna virya, tikshna guna* [9] it vitiates *pitta dosha* and this vitiated *pitta dosha* in turn vitiates *Raktadhatu*. As both of these are in *asrayasrayi bhava* with each other, [10] we can conclude that the *Raktadhara Kala* is vitiated through vitiation of *Raktadhatu*. The vitiated / pathological changes are seen majorly in liver and spleen, may be due to the presence of *Raktadhara Kala*. In this present study, the process of pathological and anatomical changes in these tissues can be understood with the help of *vishavega* concept.

CONCLUSION: With the help of concept of *vishavega* the *sthana* of *Raktadhara Kala* can be assumed as *Yakrut* , *Pliha* and *sira*.

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