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EXPERIMENTAL DETERMINATION OF ORIGIN (MOOLASTHANA) OF RAKTAVAHA STROTAS IN ALBINO RAT

Vaikos Chandrashekhar Dattatraya*, Kamthe Amol Baban
Govt Ayurved College, Nanded, Maharashtra, India

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ABSTRACTS
Ayurveda, the science of life, believes in the existence of the things, which are beyond the perception of most intellectual human approach. Strotas [fine network/meshwork of large or small channels (excluding blood vessels and nerves) with micro or macroscopic spaces meant for various types of secretion and excretion of useful and waste product] and its origin is such part of sharir (i.e. body) which is beyond the capacity of human being to prove it practically. They are found in cell-tissue-organ-system and thus occupy the whole body. Origin of Strotas is difficult to show in human being hence, Albino Rat, is selected to prove its existence. Out of 14 strotas (as described by carak), an attempt is made to verify and determine the Origin of Raktavaha Strotas only in Albino Rat. Raktavaha strotas are those micro-macro hollow channels where Rakta Dhatu i.e. blood and its content, are formed, conducted, metabolized and are transformed in to newer substances to meet the need of life.

KEYWORDS: Strotas, Manjishtha (*Rubia cordifolia linn*) & Kulattha (*Dolicos biflorus linn)*, strotas Raktavaha strotas

***Author for Correspondence**
Assistant Professor, Dept of Sharir Rachna, Govt Ayurved College, Nanded, Maharashtra

INTRODUCTION

The term, Strotas is derived from "Sroo" meaning 'Sravana' means to secrete/oozing/percolate/exudates/infiltrate. The term strotas means network/meshwork of channels containing microscopic or macroscopic spaces where cellular functions like digestion/transformation, absorption, assimilation, excretion, etc. takes place and through which some gaseous and aqueous substances like hormone, enzyme etc. secrete/ooze/percolate/exudates/infiltrate. They are big or small, perceptible or imperceptible[1]. The 'Chakrapanidatta's comment on this definition is, this exudation or oozing is pertaining to the permission of percolation of Rasa (i.e. nutrient fluid that nourishes the tissue) and excretion of waste product (Mala) from the cells & tissues[2].

The anatomy and physiology of strotas includes color, size, and shape. The color of strotas depends upon the nutrient material that it contents. They (strotas) are either round, large, small, or microscopic in size and possess the shape of long and slender tendril like network of fibers specially designed for performing the function like the act of secretion/infiltration/percolation[3]. The mesh (network of open space/opening/apertures) of network is small, microscopic and the structure that forms the network is long like tendril of herb or shrub. Through these open spaces the act of percolation/secretion/infiltration ('SRAVAN') occurs. 'Strotas' is found throughout the body therefore the whole human body is full of Strotas. Blood has such strotas whose origin lies in liver, spleen, bone marrow, kidney where the above-mentioned functions take place.[3] hence, it is necessary to confirm the origin of Raktavaha strotas by animal experiment using available methods of standard to understand the pathogenesis of disease.

Strotas are quite different from blood vessel and a nerve[4]

The Origin/moolasthana of Raktavaha strotas

The Origin (Moolasthana) of strotas is 1) Liver (Yakrit) 2) Spleen (Pleeha)3) Bone marrow (Sarakta Meda) 4) Kidney (Vrikka)

MATERIALS AND METHODS

Aim & Object

To determine the of Origin or Seat (Moolasthana) of Raktavaha strotas and to establish its correlation with Rakta Dhatu or blood and its contents in albino rats in the light of modern science using modern parameter.

Designing of Experiment

As per Ayurvedic text, the prime origin (Moolasthana) of strotas is Liver, spleen, bone marrow (saraktameda) and kidney. It is essential to design an experiment to find out the relationship between Raktadhatu (blood and its contents) and the origin (Moolasthana) of Raktavaha strotas. If Raktadhatu Dushti is done (artificial disorder of blood), it is supposed to affect the origin of strotas i.e. liver, spleen, bone marrow and kidney and thereby causing structural abnormality or derangement or functional disorder of these organ. Considering this concept of strotas, an animal experiment was designed to establish the above-mentioned correlation in 30 Albino rat. Albino Rat was taken in 3 groups of 10 each group A, group B, and group C.

Group A was given Raktaprasadak dravya i.e. Manjishtha (*Rubia cordifolia*)[5] is a Raktaprasadak dravya which eliminates and nullifies all types of impurity, toxicity, contamination, and harmful effect of unwanted material from blood and restores its health [6]. It is anti-inflammatory, radio protective[7] and hepatoprotective [8]

Group-B was given Raktadushtikar dravya Kulattha (*Dolicos biflorus*) [9] which will produce toxicity, impurity or contamination in blood which cause the disease (dushti) in the origin (Moolasthana) of Strotas i.e. Liver, spleen, bone marrow and kidney. Kulattha is dietary substance that makes the Raktadushti means one which gives toxicity, impurity to blood and deteriorates the its health

Thus, these Raktaprasadak and Raktadushtikar dravya (drug) directly affect the origin of Raktavaha strotas i.e. liver, spleen, bone marrow, and kidney.

Group C contains 10 Albino rat and was given normal mesh diet

Manjishtha (*Rubia cordifolia linn*) & Kulattha (*Dolicos biflorus li*nn)

Fine powder of Root (Manjishtha) & seed of (Kulattha) is used, as nutrients. The nutrients are prepared in the form of fine powder and mixed with normal mesh diet in the dose of 20gm/day, orally for 60 day in morning hour.

Experimental Animals

Wister albino rats are procured from National Institute of Virology (NIV) Pune. Number of animals are 30 (thirty), in which there are 18 (eighteen) females and 12 (twelve) males in the age group of 2 (two) to 3 (three) months.

Rats are brought from National Institute of Virology Pune, at least seven days prior to the commencement of experiment, to acclimatize the animals to laboratory conditions.

Care for 1) Housing 2) physical environment - temp (20^0 -30^0) & 3) humidity between 50-55%, 4) nutrition (20gm mash diet & water as libitum) was taken.

Biochemical Kits

Biochemical estimation of ALT (SGPT), AST (SGOT), Serum Alkaline Phosphatase (SAP), serum total protein (STP) and blood urea nitrogen (BUN) are done using kits of Merck Pvt. Ltd. Co.

METHODS

After acclimatization of Rat to laboratory conditions, they are subjected to blood sampling for zero day (baseline) reading.

Fifteen rats are taken randomly from thirty, in which 9 (nine) are females and 6 (six) are males.

Parameters

Ayurveda explains the physical characteristics of normal (shuddh) and pathological (dushta) Raktadhatu, using these references, and considering the probable origin of Raktavaha strotas following parameters are selected

Body Weight

Rats are weighed weekly using electronic monopan balance. Weight of each group is maintained throughout the experiment.

Average body weight of rats in a week is calculated by weighing the rats of each group.

Organ Color and Weight

Rats are sacrificed and color of the Liver, Kidney, and Spleen are noted, weight in gram percent of total body weight was noted as follows.

Organ weight factor = (Organ weight in gm x 100) / Body weight in gm

Hematology

The blood in two separate EDTA tubes for hematology (with anticoagulant) is collected from the tail of rat and for serology (without anticoagulant) directly from heart for laboratory Investigation.

The following hematological investigations are done as per standard methods described by Sastri (1979) and Schalm et.al. (1975)

Sr. no.	Parameters	Method of Estimation
1.	Hemoglobin	Acid hematin method
2.	Total erythrocyte count	Neubaurs chamber
3.	Total Leukocyte count	Neubaurs chamber
4.	Blood clotting time	Capillary tube method

Biochemical Study

All biochemical estimations of individual samples are analyzed for alanine aminotransferase (ALT), aspertate aminotransferase (AST), alkaline phosphatase (ALP), and serum total protein (STP), blood urea nitrogen (BUN) level. Methodology and set of reagents in respect of each parameter are used as per the recommendation of the manufacturer.

Histological Study

Examination of Hepatocytes in liver, Cortex and Medulla, and epithelial linings of kidney, lymphoid follicles, and Red pulp and white pulp observed in spleen and the the adipose cells, Large polymorphonuclear megakaryocytic of Bone Marrow are done to ensure the structural change.

The pieces of suitable thickness of liver, kidney, spleen, and bone marrow were collected from sacrificed rat. The collected tissue sample of individual rat was fixed and preserved in 10 percent neutral buffer formalin and was sent for histological study and the findings obtained is discussed in Result.

Statistical Analysis

The data of body weight, organ weight, hematological and biochemical are statistically analyzed by Completely Randomized Design (CRD) to know the statistical difference between means of various values at two intervals in each group as per the method described by Snedecor and Cochran (1967).[10]

RESULTS

The study includes body weight, organ weight, and Color, hematological & biochemical changes, and histological changes. The observations obtained are statistically analyzed and are presented in the tables given below:

There was no significant difference in the percent weight of spleen, bone marrow and kidney but the liver showed some significant changes.

Organ color

Liver

Color of liver in all groups appeared reddish brown. The color of liver appeared more reddish in-group A but color are same in group C and group B.

Spleen and Kidney

Appeared reddish brown in all the groups, did not show any color variation.

Bone marrow

The color of bone marrow of group A is Reddish tinge. Bone marrow of Group B and group C did not show any color difference.

Blood

Serum of group A showed reddish tinge. While group B and C serum, did not show any color change.

Hematology

Hemoglobin (gm/dl) (**Table** 1)

Mean values of hemoglobin did not differ significantly between group C and A. The mean values of hemoglobin differ significantly between group C and B. In addition, mean value of hemoglobin differ significantly when compared between group A & B. The mean value of hemoglobin of group B are numerically higher than group A & C.

Clotting Time (CT) (Sec.), Total Erythrocyte Count(TEC), & Total Leukocyte Count (TLC)

No significant changes is observed in CT, TEC & TLC

Biochemical Test

Alanine Aminotransferase (U/L) (Table 2)

Mean values of ALT did not differ significantly when compared between Group A and Group C at 60^{th} day. Mean values of ALT of Group B are significantly higher when compared with group A and C at 60^{th} day.

Aspertate Aminotransferase (AST)(U/L) (Table 3)

Mean values of AST are significantly higher in rats of group A and B. when compared with mean value of group C at 60^{th} day. The mean values of AST are significantly higher in-group B than A. The mean value of AST in-group B was numerically greater than group A and C.

Serum Alkaline Phosphate (SAP) (U/L (Table 4)

Mean value of SAP in-group C was slightly higher at 60^{th} day than 0^{th} day but the difference is not significant. Mean value of SAP in-group A are lower at 60^{th} day than 0^{th} day but did not differ significantly. Mean value of SAP in-group B was higher at 60^{th} day than 0^{th} day and there was significant difference.

Serum Total Protein (STP) (U/L) (Table no 5)

The mean values of STP between group C and A did not differ significantly at 60^{th} day. Mean values of STP was differ significantly between group B & C. Mean values of STP did not differ significantly when compared between group A and B. The mean STP values of group B are numerically lower than group A & C.

Blood Urea Nitrogen (BUN) (mg/dl) (Table 6)

Mean values of BUN did not differ significantly in group A, group B and group C between 0^{th} and 60^{th} day in each group.

Histological Studies

a) Liver b) Kidney c) Spleen d) Bone Marrow (image 4, 5, 6, and 7)

The histological examinations of organs of experimental animals studied during an experimental trial did not reveal any appreciable structural alteration attributable to treatment. The organs of control group animals (Rats) also remained unaltered when exposed to their histology. However, liver of Group C and Group A each, showed minimal focal fatty change. The similar type of change is seen in the liver of two rats of Group B.

DISCUSSION

Organ Color

Color of liver, spleen, and kidney of all the groups did not show any remarkable difference only more reddish color of liver was observed in-group A. Reddish tinge to bone marrow and blood serum was observed in-group A fed with Manjishtha. This indicates that, Manjishtha is responsible for color that is more reddish to liver, blood, and bone marrow.

Organ Weight

a) Liver

Data on percent weight of liver in the present study indicated that there are significant lower values of group A then B & C.

b) Spleen and kidney

Percent weight of spleen and kidney in all groups did not show significant variation but the mean percent weight of spleen of group B is numerically higher than group C and A hence, Raktadushtikar dravya, Kulattha might be responsible for increase in weight.

Hematological Changes

i) Hemoglobin

Increase in hemoglobin concentration in - group B indicates the disease condition or inflammatory condition of Rat & decrease in -Group A suggest anti-inflammatory effect (table 1)

ii) Total erythrocyte count (TEC) Total Leucocytes count (TLC) –there is no much significant effect on TEC, TLC, and Clotting time.

Bio-Chemicals Changes

i) Serum Alanine aminotransferase (ALT) (table 2)

Higher value of ALT above normal indicates hepatocellular damage while lower value of group A indicates better functioning of liver. Raktadushtikar dravya i.e. Kuttha is responsible for hepatocellular damage (i.e. dushti of Yakrit) and Raktaprasadak dravya i.e. Manjishtha is responsible for better functioning of liver (Yakrit).

ii) Serum Aspertate aminotransferase (AST) As per table3

Higher values of AST indicate soft tissue damage (observed in - group B). AST is seen in liver, heart, skeletal muscle, and kidney. Hence, Kulattha is responsible for soft tissue damage.

iii) Serum Alkaline phosphatase (SAP) As per table 4

Increased value of SAP in serum, indicate the defective excretion of SAP through bile, hence indicates the liver dysfunction leading to lower the cholesterol [11] and lower SAP value indicate better functioning of liver. From this, we can say that Kulattha is responsible for liver dysfunction and 'Manjishtha' is responsible for better functioning of liver.

iv) Serum total protein (STP) As per table 5

Reduction in mean values of STP is sign of Hypoproteinaemia which is caused due to Kulattha, indicates the liver diseases concerned with biosynthesis of the proteins.

Normal level of serum total protein in-group C and A indicate the normal functioning of liver in protein biosynthesis.

The decreased serum total protein level indicates the decreased viscosity of blood. Hence, Kulattha is responsible for Raktadushti by causing Hypoproteinaemia and liver dysfunction.

v) Blood Urea Nitrogen (BUN) As per table 6

The mean value of blood urea nitrogen (BUN) of all the groups did not differ significantly. This indicates the unchanged function of kidney in control group, group fed with Manjishtha and group fed with 'Kulattha'.

Hence, Manjishtha and Kulattha have no action on renal parenchyma.

Histological Changes

Hepatocytes in liver, Cortex, Medulla, and epithelial linings of kidney, lymphoid follicles, and Red pulp and white pulp in spleen and the adipose cells, Large polymorphonuclear megakaryocytes of Bone Marrow remain unchanged as shown in image 4,5,6, and 7.

The continuous use (for 60 days) of Manjishtha in-group A and Kulattha in Group B did not show recognizable histo-architectural alteration. It might take longer time for histoarchitectural alteration.

The rat kept on normal diet remained histologically unchanged.

CONCLUSION

The hematological and biochemical investigation clearly indicates that, Raktadushtikar (Kulattha) and Raktaprasadak dravya (Manjishtha) mainly affect the liver and to some extent spleen, rather than bone marrow and kidney. There is no change on histological structure perhaps it may need longer study.

The lower values of ALT & SAP in group A shows improvement in liver function and thus protects the liver.[12]

Hence, the origin or Moolasthana of Raktavaha strotas as described in text is mainly liver and spleen.

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Table 1: Comparative Effect on Hemoglobin (Hb%) Group A, B, and C

Groups	Mean	$S.D._1$	Mean2	$S.D._2$	S.E.	t	P
Cand A	11.26	0.84	10.89	0.9956	0.4265	0.867	> 0.05
Cand B	11.26	0.84	12.78	1.263	0.484	3.14	< 0.05
B and A	12.78	1.263	10.89	0.9956	0.5263	.593	< 0.05

Table 2: Comparative Effect on Serum Alanine Aminotransferase Level

Groups	Mean 1	$S.D._1$	Mean2	$S.D._2$	S.E.	t	P
Cand A	49.0	15.57	41.0	9.043	5.693	1.405	> 0.05
Band C	105.3	10.56	49.0	15.57	5.949	9.464	< 0.001
Band A	105.3	10.56	41.0	9.043	4.395	14.63	< 0.001

Table 3: Comparative Effect on Aspertate Aminotransferase (Ast) Level

Groups	mean 1	$S.D._1$	Mean2	$S.D._2$	S.E.	t	P
Cand A	131.0	16.599	156.7	14.57	6.98	3.682	< 0.01
Cand B	131.0	16.599	194.1	24.57	9.37	6.73	< .001
Band A 194.1	194.1	24.57	15.677	14.5	9.03	4.14	<0.01

Table 4: Difference In Serum Alkaline Phosphatase (Sap) Level

Group	Day	Mean1	$S.D._1$	Mean2	$S.D._2$	S.E.	t	P
C	0 to 60th	251.08	62.85	267.0	35.97	22.47	0.712	>0.05
A	0 to 60th	251.08	62.85	224.1	56.47	25.71	1.049	>0.05
B	0 to 60th	251.08	62.85	353.1	27.90	21.50	4.745	<0.001

Table 5: Comparative Effect on Mean Serum Total Protein(stp) Level

Groups	mean 1	$S.D._1$	Mean2	$S.D._2$	S.E.	t	P
C and A	7.83	0.83	7.34	0.74	0.3502	1.379	> 0.05
C and B	7.83	0.83	6.82	0.69	0.34	2.891	< 0.01
A and B	7.34	0.74	6.84	0.69	0.3196	1.564	> 0.05

Table 6: Difference in Blood Urea Nitrogen (Bun) Level

Group	Day	$mean_1$	$S.D._1$	$mean_2$	$S.D._2$	S.E.	t	P
C	0 to 60^{th}	50.74	8.45	57.24	5.33	3.158	2.058	> 0.05
A	0 to 60^{th}	50.74	8.45	57.19	5.32	3.281	1.966	> 0.05
B	0 to 60^{th}	50.74	8.45	53.84	10.98	4.38	0.707	> 0.05

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