

# **Assessment of Anatomical and Histological changes in Splenomegaly due to Liver Cirrhosis by use of Sharpunkha – An experimental study.**

## **Abstract -**

Splenomegaly is the disease commonly affecting normal anatomy and histology of spleen. It remains undetectable and worsens as the time passes and person suffers from stressful life. It leads as a consequence of many diseases. One of them important disease is Liver cirrhosis. In Ayurveda also relation of liver and spleen is depicted emphatically. Yakrita and Pleeha are described as Moolsthana of Raktvaha Strotasa and site of Raktadhara Kala and which are the important aspects of Ayurvedic Rachana Sharir. The facts regarding liver cirrhosis and splenomegaly are very much serious which has provoked us to think about to detail study of splenomegaly due to liver cirrhosis. There is no absolute treatment of splenomegaly till date. In Bhavprakasha, there is reference regarding Sharpunkha (Tephrosia Purpurea) that it is “Pleehashatru” showing that it might acts on the spleen and cures splenic disorders. But there is evidence base gap regarding efficacy of Sharpunkha on Splenomegaly. Kshara or Churna of Sharpunkha Panchanga are described as its useful preparations. There is no work done till date showing action of Sharpunkha Panchanga Kshara or Churna on Splenomegaly and there is deficit of study showing alteration in anatomy and histology of spleen affecting due to splenomegaly by using Sharpunkha. So it is needed to study effect of both Kshara and Churna separately and compare them so that we can use Panchanga Kshara or Panchanga Churna with more specificity. It can be a new modality in the field of Ayurvedic science to study effect of Sharpunkha Panchanga Kshara and Panchanga Churna on Splenomegaly showing changes in anatomy and histology of spleen affecting due to splenomegaly. Also with the help of that “Pleehghan Prabahva” and classical assert as “Pleehashatru” of Sharpunkha can be proven.

**Keywords** – Histology, Pleehashatru, Raktavaha Strotasa, Raktadhara Kala,

## **Background:**

Spleen is the largest lymphoid organ of the body. it is wedged shape organ having tetrahedral shape. It lies mainly in left hypochondrium and partly in the epigastrium. Its consistency is soft. It is dark purple in color. It is 1 inch or 2.5 cm thick, 3 inches or 7.5 cm broad, 5 inches or 12.5 cm long. It is 7 ounces in weight. It is related to 9<sup>th</sup> to 11<sup>th</sup> ribs. Normally, it is not going to palpate. It lies obliquely with respect to long axis of tenth rib. It makes an angle of 45 degree with the horizontal plane and directing downward, forward and laterally. It has anterior end and posterior end. It consists of three borders viz. superior border, inferior border and intermediate border. There are two surfaces as diaphragmatic surface and visceral surface. Histologically, spleen consist of Capsule which is fibroelastic tissue that supports the it. There are also fine retinaculum coarse and trabeculae. It also consist of white pulp having lymphatic nodules. These nodules are arranged around the Malpighian corpuscle. Also there is red pulp which is formed by the collection of cells in the retinacular interstices. These cells are present in between sinusoids. Population of cells is formed by, all types of blood cells, all types of lymphocytes, fixed and free macrophages

As it is largest lymphoreticular organ, it is involved secondarily in broad range of systemic disorders which causes its dysfunction in the form of splenomegaly. Splenomegaly is the enlargement of spleen in which its cellularity and vascularity of it is increased. Causes of splenomegaly are various infections, disorders of immunoregulations, altered splenic blood flow, lymphoid-haematogeneous malignancies, diseases with abnormal erythrocytes. Many of the causes are exaggerated forms of normal splenic function. The degree of splenomegaly varies with disease entity<sup>(1)</sup> Among the various causes, widely occurring cause for splenomegaly is liver cirrhosis in which portal hypertension occurs that leads to obstruction to blood flow. Cirrhosis is end result of various liver diseases. Annually near about 2 million deaths occurs due to liver diseases worldwide. Out of these 1 million deaths occur due to complications of cirrhosis. Globally, cirrhosis is at present the eleventh most common cause of death.<sup>(2)</sup> Prevalence of splenomegaly due to hepatic diseases is 12-46%.<sup>(3)</sup> Common symptoms of splenomegaly are pain in left hypochondric region of abdomen which usually referred to left shoulder, vague abdominal discomfort. In splenomegaly due to portal hypertension allied symptoms are varices, ascites etc. Likewise other symptoms are generally associated with cause of the disease.<sup>(4)</sup>

It is vital to manage various liver abnormalities by targeting splenomegaly as there is several pathophysiological associations of spleen with liver disease progression. In the past decades, splenectomy has been utilized to ameliorate the fatal complications of cirrhosis-associated portal hypertension. An alternative to splenectomy, however, is required for many patients with precluding conditions such as thrombocytopenia<sup>(5)</sup> There is no absolute treatment of splenomegaly till date. Treatment of it is based upon the cause for splenomegaly. Splenectomy is advised in advance cases which is costlier procedure. It also shows various complications such as severe infection, hernia at the operated site, pancreatitis. Non selective beta blockers are used in the treatment of portal hypertension due to liver cirrhosis<sup>(6)</sup> As per research paper, Nitroflubriprofen and Flubriprofen are used to treat portal hypertension causing splenomegaly.<sup>(7)</sup> They may lead to hyperglycemia and increases the risk of heart block in patients with cardiac problem. They also causes renal failure, gastrointestinal ulceration, and refractory ascites. Thus there are limitations for using these in management of disease.<sup>(8)</sup>

In Ayurveda Pleeha that is spleen is described as Moolsthana of Raktvaha Strotasa.<sup>(9, 10)</sup> It is also considered as Kosthanga by Vagbhata. (Astanga Hridayakara and Astanga Sangrahaakara)<sup>(11, 12)</sup> There are variety of diseases described in Ayurveda which affect the Pleeha. There are various herbal drugs which have action on Pleeha Vikar. One among them is Sharpunkha (Tephrosia Purpurea). In Sanskrit meaning of Sharpunkha is as Shara means having arrow like structure and Punkha means wings. Edges of its leaves become arrow like if ends are pulled and held. It is usually about 1-3 feet. It is perennial and have spreading branches. Leaves are 3-6 inches long asymmetrical and assymetrical in nature. Leaflets are usually 19-21 in number, having trichome below and glabrous above. These are lanceolate. Purple color flowers are originating from raceme which is generally 3-6 inches long. Greenish seeds are present on the pods which are about 1-2 inches long. There is abundant potassium and nitrogen into its seeds. Rotinoid and Rutin are also its constituents. Seeds consists of oil. As per Ayurveda, it is Katu, Tikta Rasatmaka. Its Virya is Ushna. It has Katu Vipaaka. It is Laghu, Ruksha, Tikshna Gunatmaka. It's Prabhava is Pleehaghna. It is Kaphavatashamak. Its Prayojya Anga are Mool or whole plant. It is used in various folk medicines for prevention and cure of many diseases. Whole plant and its Mool (roots) are useful in various Ayurvedic medicines. It is rich in polyphenol and flavanoid

content. It shows many effects such as antiulcer effect, antimicrobial, hepatoprotective effects etc. It is commonly found plant in India. It is self generating plant. Root of Sharpunkha shows anti ulcer effect, anticarcinogenic, anti lipid per oxidative effect, anti microbial against acne inducing bacteria, anti inflammatory and analgesic effect. Whole plant shows anti leshiminal activity, antiepileptic, anxiolytic activity, antidiarrhoeal activity. Arial part shows hepatoprotective activity, anti cholestic activity, immune modulatory activity. Flower shows antiviral activity. Thus these are the various uses of Sharpunkha.

Bhavprakasha has described Sharpunkha as “Pleehashatru”. That means it is used for diseases of spleen. He has also mentioned that Sharpunkha is useful in Yakrit Vikar, PleehaVikar, Gulma, Vrana and VishaVikar. He has said Tikta and Kashya Rasa of Sharpunkha. According to him Sharpunkha is Kasashwasjwarhara. And it is Laghu Gunatmaka.<sup>(13)</sup> Gadanigraha has also mentioned that Kalka of Sharpunkha given with Takra according to Agnibala of patient is useful in treatment of PleehaVikar. According to Rajmartanda, mastication of roots of Sharpunkha cures the Splenomegaly of even firm consistency.

From that, it can be said that Sharpunkha might have action on spleen. And it can be used in spleen diseases. In Shabdikalpdrum Nirukti of “Pleehashatru” is given as Pleehaghna that one which conquers diseases of Pleeha that is spleen.<sup>(14)</sup> Sanskrit Word “Pleehajit” is also generally used to denote conquering property and its meaning is given in Dravyagunkosha as one which reduces enlargement of spleen.<sup>(15)</sup> More specifically Bhavprakasha has quoted Pleeha (Roga) as Vyadhi in which enlargement of Pleeha occurs. This implicates Pleeha (Roga) is nothing but Splenomegaly.<sup>(16)</sup> considering these statements, it can be said that Sharpunkha might reduces the enlargement of spleen by showing action on it and can help in management of splenomegaly. This enlightens us to do this study that might show effect of Sharpunkha Churna and Kshara on splenomegaly.

### **Aim and objectives:**

#### **Aim**

Study of anatomical and histological changes in spleen in experimental model of splenomegaly due to liver cirrhosis by using Sharpunkha Panchanga Kshara and Sharpunkha Panchanga Churna

#### **Objectives:**

- 1) To study normal anatomy and histology of spleen and liver in experimental model of splenomegaly due to liver cirrhosis
- 2) To asses LFT and CBC in experimental model pre and post induction of splenomegaly due to liver cirrhosis and after administration of Sharpunkha Panchanga Churn and Kshara in experimental model
- 3) To compare LFT and CBC in splenomegaly induced due to liver cirrhosis experimental model after administration Sharpunkha Panchanga Churna and Sharpunkha Panchanga Kshara individually
- 4) To study anatomy and histology of spleen and liver in splenomegaly induced due to liver cirrhosis experimental model after induction.

- 5) To study anatomy and histology of spleen and liver in splenomegaly induced due to liver cirrhosis experimental model after administration of Sharpunkha Panchanga Churna and Sharpunkha Panchanga Kshara individually.
- 6) To compare anatomy and histology of spleen and liver in splenomegaly induced due to liver cirrhosis experimental model after administration of Sharpunkha Panchanga Churna and Kshara individually

## **Material and Methods:**

This study will be conducted under following headings.

- A. Pharmacuetical study
- B. Analytical study
- C. Experimental study

### **A .Pharmaceutical study :**

Preparation three different batches of *Sharpunkha Panchanga Kshara and Churna* will be done in order to establish its pharmacurtical standardization. For this following steps will be followed

- Procurement of Raw materials
- Authentication of Raw materials
- Preparation of Churna.<sup>(17)</sup> and extraction of Kshar<sup>(18)</sup>

Procedure given in Sharangdhar Samhita will be used for preparation of Churna. As per the procedure given in Rasatarangini, Kshara will be extracted.

**B. ANALYTICAL STUDY:** It will be carried out to describe Organoleptic characters, Physico-chemical parameters for *Kshar and Churna*

### **C. EXPERIMENTAL STUDY**

For experimental study, rats of weight about 180-200 gm of both the sexes will be procured from animal house. Animals will be acclimatize for 14 days at the beginning of study. Feeding of them will be carried out, with food supplied from animal house, along with distilled water ad libitum.

Treatment protocol

Five groups will be taken as control group (Group I), disease control group(Group II), standard control group(Group III), study group A treated with Sharpunkha Panchang Kshara (Group IV), study group B treated with Sharpunkha Panchang Churna (GroupV)containing 6 animals each.

Before start of treatment, blood samples of all 30 animals will be collected for investigating LFTand CBC. USG will also be carried out of all animals. As per the previous research paper, Splenomegaly will be induced in 4 groups namely disease control group, standard control group, study group A, study group B by administering thioacetamide (TAA), intraperitoneally at most 20 weeks for dose of 200mg/kg, three times a week.

After induction, blood sample from all the groups will be collected for investigating blood parameters (LFT and CBC). USG will be carried out to determine the splenomegaly.

Later on Standard drug, Sharpunkha Kshara and Sharpunkha Churna will be administered to animals of Group III, Group IV, GroupV in proper dose and duration.

Blood will be collected on 29<sup>th</sup> day of treatment.

At the end USG of all animals will be done again and animals will be sacrificed to study changes in histology of Spleen, Liver, heart, lung and GIT.

Table 1. Grouping of animals is given as follows

**Grouping:**

Groups	Intervention	Drug	Dose	No. of animals and species	Duration	Route
Group I (Control group)	No medicine	-	-	6 rats		-
Group II (disease control group)	Hepatotoxic drug producing splenomegaly	Thioacetamide <sup>(19)</sup>	200mg/kg	6 rats	20wks	intraperitoneal
Group III (standard control)	Standard drug	flubriprofen <sup>(20)</sup>	30 mg/kg	6 rats	28 days	Orally
Group IV (study group A)	Kshara	Sharpunkha Panchang Kshara	103.33mg/kg	6rats	28 days	Orally
Group V (study group b)	Churna	Sharpunkha Panchang Churna <sup>(21)</sup>	500mg/kg	6 rats	28 days	Orally

**Study design** – experimental

**Study type** – experimental animal study

**Table 2. Sample size:**

Animals	Wistar Albino rats
Sex	50% males and 50% females (nulliparous) in each group will be taken.
Weight (Average)	180-220 gm.
Number of animals in each group	06 rats
Number of groups	05

**Study Duration** – 6 months

**Study centre** –

We will going to conduct study at the following concerned centers

- Animal house of Datta Meghe Institute of Medical Sciences, (deemed to be university), Sawangi, Wardha
- Mahatma Gandhi Ayurved college, Hospital and Research Institute, Salod(H) Wardha.
- Histopathological lab of JNMC, Sawangi ,Wardha
- As per the necessity the study will be conducted at other renowned institute.

### **Screening Parameters**

All the animals will be screened for

- 1) Blood investigations – LFT and CBC
- 2) USG of abdomen
- 3) Histology of spleen, liver.

### **Analysis plan (Statistical test):**

After applying appropriate tests statistical analysis will be carried out.

### **Results:**

Results will be taken out with the help of statistical test observations, Blood investigations and histopathology reports.

### **Discussion:**

Splenomegaly is the most common disease occurring as complication of liver cirrhosis. Specifically anatomical and histological changes occurs in spleen. Sharpunkha (*Tephrosia purpurea*) is a Dravya that is said to be act on Pleeha (spleen). This study will help to validate the action of Sharpunkha on spleen pertaining to restoration of normal anatomy and histology of it. Pleeha being stated as Moolsthana of Raktvaha Strotasa and site of Raktadhara Kala, further studies can be conducted to evaluate role of Sharpunkha in disorders of Raktvaha Strotasa and the diseases like sickle cell anemia and hepatic disorders where splenomegaly occurs secondary to them. And also it will prove the classical assert as “Pleehashatru” regarding Sharpunkha. Related studies on spleen<sup>22-25</sup> and liver<sup>26-29</sup> disorders were reviewed.

### **Conclusion:**

At the end of study, conclusion will be made on the basis of results of Statistical Analysis and histopathology reports.

## NOTE:

The study highlights the efficacy of " Ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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