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TO EVALUATE HEPATOPROTECTIVE ACTIVITY OF PENTAS LANCEOLATA IN PARACETAMOL INDUCED LIVER CIRRHOSIS IN WISTER RAT.

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ABSTRACT

Liver cirrhosis represents the final stage of various chronic liver diseases, with fibrosis serving as its precursor. Activation of hepatic stellate cells (HSCs) is a crucial event in the development of fibrosis. While synthetic drugs exist to alleviate liver cirrhosis, they often come with adverse effects, notably hepatotoxicity. Paracetamol, a widely used analgesic and antipyretic drug, can induce liver toxicity, particularly at high doses. One of its metabolites, N-acetyl-p-benzoquinone imine (NAPQI), depletes liver glutathione levels and directly damages hepatic cells. This study explores the hepatoprotective potential of Pentas lanceolata against paracetamol-induced liver toxicity. The solvent extract of Pentas lanceolata leaves, belonging to the Rubiaceae family, was obtained through Soxhlet extraction. Phytochemical screening of the plant extract revealed the presence of various metabolites, including alkaloids, carbohydrates, flavonoids, quinines, and phenols. In vitro experiments involved administering the extract to rats, with Silymarin serving as a standard drug for comparison. Biochemical markers such as ALT, AST, ALP, albumin, and total bilirubin were evaluated in the liver cells of the rat model. Histopathological examination confirmed the hepatoprotective activity of Pentas lanceolata against paracetamol-induced toxicity.

***Keywords*:** Liver Cirrhosis, Hepatoprotective Activity, Pentas Lanceolata

INTRODUCTION

Liver: The liver is located in the upper right-hand region of the abdominal cavity, positioned above the stomach, right kidney, and intestines, and beneath the diaphragm. It is a dark reddish-brown organ weighing approximately 3 pounds and has a conical shape. Blood is supplied to the liver from two distinct sources, which include: [1, 2]

- The hepatic artery supplies oxygenated blood.
- The hepatic portal vein brings nutrient-rich blood in.

At any given time, the liver retains approximately one pint, equivalent to 13 percent, of the body's blood supply. Structurally, the liver is divided into two lobes, each composed of eight segments containing around 1,000 lobules (small lobes) each. Small ducts, or tubes, connect these lobules to larger ducts, forming a network known as the common hepatic ducts. The common hepatic duct is a tube responsible for connecting the liver to the bile duct. Bile, produced by liver cells, is transported through the common hepatic duct to the gallbladder and subsequently to the duodenum, which is the initial segment of the small intestine [3, 4].

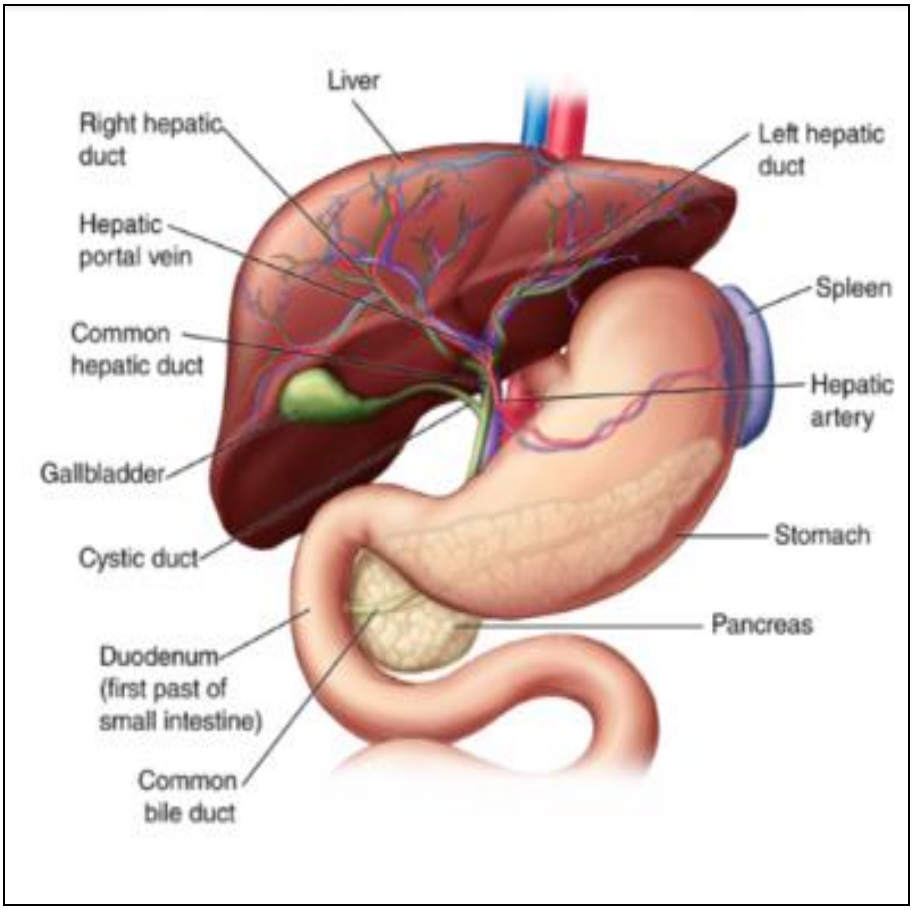


Figure 1. Anatomy of liver

Functions of the liver: [5] The liver is a vital organ responsible for numerous crucial functions in the body. It regulates chemical levels in the blood, produces bile to aid in digestion, and metabolizes drugs, among other functions. Some key functions of the liver include:

- Production of bile to aid in the breakdown of fats during digestion.
- Production of proteins for blood plasma.
- Production of cholesterol and specialized proteins to transport fats throughout the body.
- Conversion of excess glucose into glycogen for storage, which can later be converted back into glucose for energy.
- Regulation of blood levels of amino acids, the building blocks of proteins.
- Processing of hemoglobin to utilize its iron content.
- Conversion of toxic ammonia into urea, which is excreted in urine.
- Clearance of drugs and harmful substances from the blood.
- Regulation of blood clotting.
- Production of immune factors to resist infections and removal of bacteria from the bloodstream.
- Clearance of bilirubin, a by-product of red blood cell breakdown, which, when accumulated, causes jaundice.

The liver releases waste products from these functions either into bile or into the bloodstream. Waste products carried in bile are removed from the body through the digestive system in feces, while those carried in the bloodstream are sifted by the kidneys and expelled in urine.

Liver Cirrhosis: The term "cirrhosis" may have originated from the Greek word "kirrhos," which means "brownish," referring to the orange-yellow color often associated with an unhealthy liver. Although the clinical condition was recognized earlier, it was René Laennec who officially coined the term "cirrhosis" in his 1819 work, where he also introduced the stethoscope.

Cirrhosis is a consequence of ongoing liver disease characterized by the replacement of liver tissue with fibrous scar tissue and regenerative nodules. This process involves the regeneration of damaged tissue, resulting in the progressive loss of liver function.

Liver Cirrhosis is defined as a diffuse process of fibrosis and nodule formation. Extensive fibrosis and disturbed normal lobular and vascular architecture result in progressive portal hypertension and liver dysfunction [6-8].

Types of Liver Cirrhosis:

Alcoholic cirrhosis [9]: Alcoholic liver cirrhosis is the most advanced form of liver disease that's related to drinking alcohol. The disease is part of a progression. It may start with fatty liver disease, then progress to alcoholic hepatitis, and then to alcoholic cirrhosis.

- Also called portal or nutritional
- Usually associated with alcohol abuse.
- First change from excessive alcohol intake is fat accumulation in liver cells - With continued abuse, scar formation occurs.

A. **Post necrotic cirrhosis [10]:** Post necrotic Cirrhosis characterized by necrosis involving whole lobules, with the collapse of the reticular framework to form large scars; it may follow viral or toxic necrosis, or develop as a result of dietary deficiencies.

- Complication of viral, toxic, or idiopathic hepatitis
- Bands of scar tissue form

B. **Primary biliary cirrhosis [11]:** Primary biliary cholangitis, previously called primary biliary cirrhosis, is a chronic disease in which the bile ducts in your liver are slowly destroyed. Bile is a fluid made in your liver. It aids with digestion and helps you absorb certain vitamins.

- Associated with chronic biliary obstruction
- Diffuse fibrosis of liver with jaundice

C. **Cardiac cirrhosis [12]:** Cardiac cirrhosis is a term used to include the spectrum of hepatic disorders that occur secondary to hepatic congestion due to cardiac dysfunction, especially the right heart chambers.

- From long-standing severe right-sided heart failure.

D. Stages of Cirrhosis [13, 14]:

- **Stage 1:** fully compensated cirrhosis, absence of varices; 1-year mortality rate ~1.5%; 1-year progression rate to stage 2 ~6.2% or to stage 3 or 4 ~4.2%;
- **Stage 2:** compensated cirrhosis, presence of esophageal varices; 1-year mortality rate is 2%; transition to decompensation (stage 3 or 4) happens in 12.2% patients per year.
- **Stage 3**: bleeding of the GI tract, related to portal hypertension (esophageal varices), without another decompensating event; 1-year mortal it rate is 10%; 21% of patients develop other decompensating events (mostly ascites) per year;
- **Stage 4**: ascites, jaundice or encephalopathy; 1-year mortality rate increases to 21%; rate of transition to stage 5 is 10% per year;
- **Stage 5**: more than one complication, usually refractory ascites, intermittent encephalopathy, acute kidney injury, advanced liver dysfunction; 1-year mortality in this stage is at least 27%, increasing with the severity of decompensation to 57%.

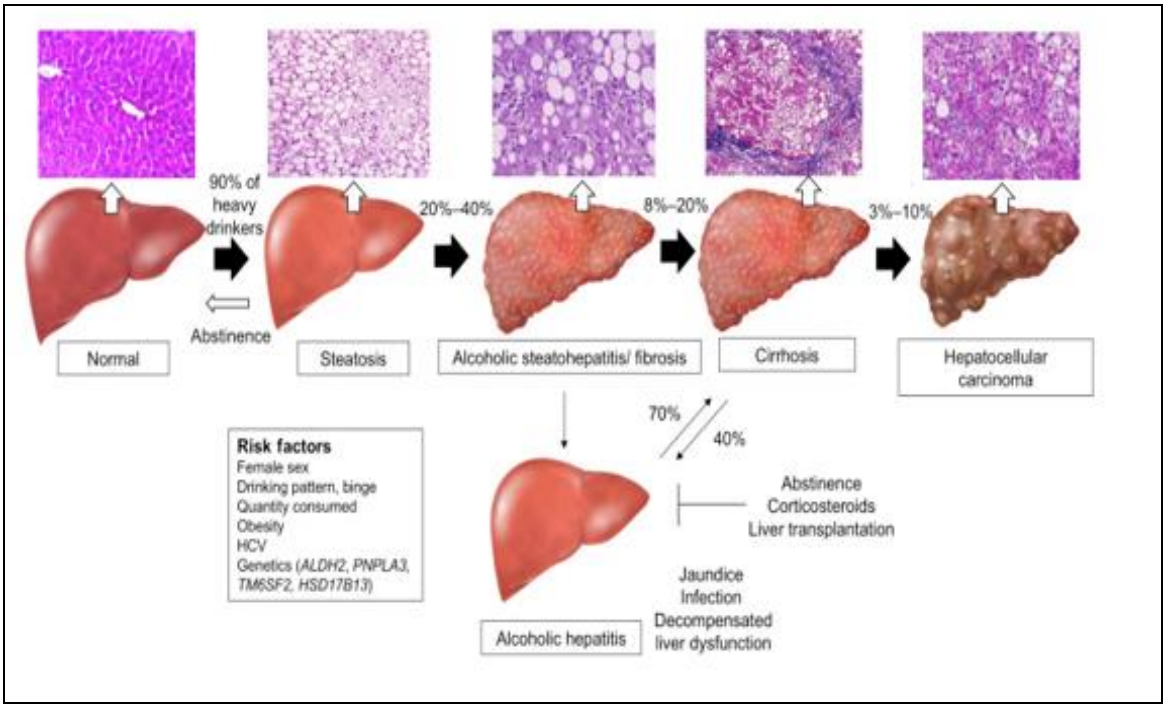


Figure 2. Stages of liver cirrhosis

Pathophysiology: [15, 16]: Cirrhosis represents an advanced stage of liver fibrosis characterized by the distortion of the hepatic vasculature. This condition results in the redirection of portal and arterial blood supply directly into the central veins of the liver, hindering the exchange between hepatic sinusoids and the adjacent liver tissue, specifically the hepatocytes. Normally, the hepatic sinusoids are lined with fenestrated endothelial cells resting on a layer of permeable connective tissue known as the space of Disse, which contains hepatic stellate cells (HSC) and some mononuclear cells.

On the opposite side of the space of Disse, hepatocytes carry out the majority of liver functions. However, in cirrhosis, the space of Disse becomes filled with scar tissue and the endothelial fenestrations are lost, a process referred to as sinusoidal capillarization. Histologically, cirrhosis is characterized by the formation of fibrotic septa that connect portal tracts with each other and with central veins, resulting in hepatocyte islands surrounded by fibrotic septa and devoid of a central vein. The major clinical consequences of cirrhosis include impaired liver function, increased intrahepatic resistance leading to portal hypertension, and the potential development of hepatocellular carcinoma (HCC). Traditionally, cirrhosis and its associated vascular distortions have been considered irreversible; however, recent data suggest that cirrhosis regression or even reversal may be possible.

***Pentas lanceolata*:** The *Pentas lanceolata* belonging to the Rubiaceae family, encompasses approximately 40 species, with many utilized extensively by indigenous African populations for medicinal purposes. These flowering plants are predominantly found as herbs or shrubs, with some existing as herbaceous or subshrubby varieties. This genus is commonly used in the treatment of tropical and other diseases such as malaria (*P. micrantha* and *P. longiflora*), tapeworms (*P. longiflora*), itchy rashes and pimples (*P. longiflora* and *P. decora*), gonorrhoea, syphilis and dysentery (*P. brussei*), cough (P. micrantha), dysmenorrhea, headache and pyrexia (P. purpurea), hepatitis B, mental illness and epilepsy (*P. schimperiana*), lymphadenitis, abdominal cramps, ascariasis, snake poisoning, retained placenta and some veterinary diseases (P. lanceolata) [17, 18].

Therapeutic effects of *Pentas lanceolata*: [19-22]

A. **Potent Antioxidant Capacity**: Pentas lanceolata boasts a rich reservoir of phenolic compounds like flavonoids and phenolic acids renowned for their robust antioxidant activity. By neutralizing harmful free radicals, it shields cells from oxidative stress, potentially mitigating ailments like cardiovascular issues, neurodegenerative disorders, and certain cancers.
B. **Inflammation Alleviation**: Pentas lanceolata extracts exhibit notable prowess in quelling inflammatory responses by impeding cytokines and prostaglandins. This anti-inflammatory action holds promise in easing conditions such as arthritis, asthma, eczema, and psoriasis, where inflammation plays a pivotal role.
C. **Broad-spectrum Antimicrobial Attributes**: Pentas lanceolata showcases impressive antimicrobial efficacy against a gamut of pathogens encompassing bacteria and fungi. Such properties hint at its potential in combatting various infections ranging from skin to gastrointestinal, as well as respiratory infections.
D. **Accelerated Wound Healing**: Long-prized in traditional medicine for its wound-healing prowess, Pentas lanceolata aids in collagen synthesis, fosters tissue regeneration, and combats microbial intrusion, thereby expediting the wound healing process. Its anti-inflammatory and analgesic properties further soothe discomfort associated with wounds.
E. **Promising Antidiabetic Capacities**: Experimental investigations suggest that Pentas lanceolata extracts hold promise in modulating glucose metabolism by enhancing insulin sensitivity, facilitating cellular glucose uptake, and curbing hepatic glucose production. Additionally, it exhibits potential in safeguarding pancreatic beta cells and mitigating oxidative stress implicated in diabetic complications.
F. **Anticancer Potential**: The bioactive constituents within Pentas lanceolata exhibit promising anticancer attributes by impeding cancer cell proliferation, inducing apoptosis, and inhibiting angiogenesis. These properties not only impede tumour growth but also augment the efficacy of conventional cancer therapies while mitigating their adverse effects.

In essence, Pentas lanceolata presents a multifaceted therapeutic profile owing to its antioxidant, anti-inflammatory, antimicrobial, wound-healing, antidiabetic, and anticancer properties. While supported by both traditional wisdom and preclinical evidence, further scrutiny through clinical trials is warranted to validate its efficacy and elucidate underlying mechanisms. Given variations in preparation and individual health contexts, consultation with healthcare professionals remains paramount before integrating Pentas lanceolata into medicinal regimens.

MATERIALS AND EQUIPMENTS [23-25]

MATERIALS:

1. Plant species – *Pentas lanceolata.*
2. Induction Drug: -Paracetamol is used to induce hepatotoxicity
3. Silymarin – Silymarin is used as the standard drug for treatment.
4. Carboxy methyl cellulose
5. Ethanol
6. N-hexene
7. Phosphatidylcholine

- Animal Model: Male wistar rats (200-250g)
- No. of animals: 30

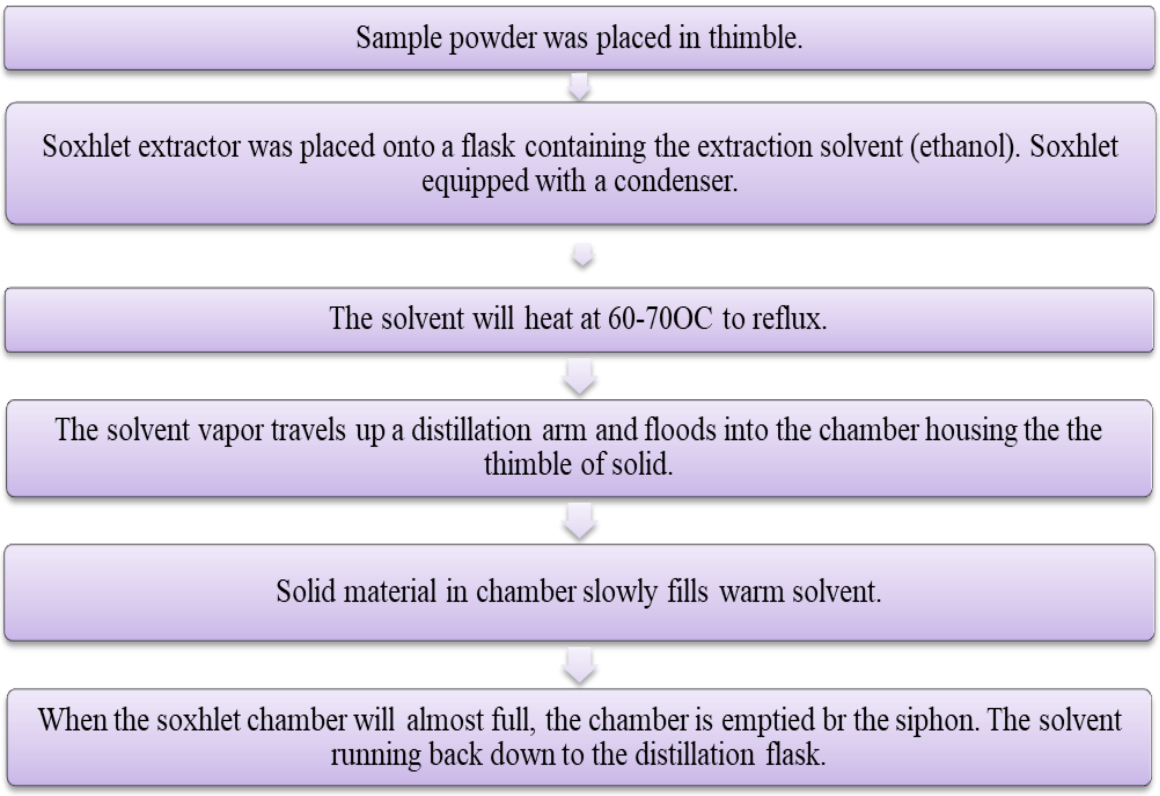
METHODOLOGY

Collection of Leaves: Fresh leaves of *Pentas lanceoleta* were collected from the Baramati region of Pune district, Maharashtra, India. After confirmation of the sample, the Leaves were washed with distilled water

Authentication of Plant: Authentication is an important part of research because it ensures that the plants being used are correctly identified. This process helps to ensure the validity of the research and produce reliable results. It is a quality assurance process that ensures the correct herbal species and plant parts are used as raw materials for herbal products Authentication of a plant is done by R. B. Deshmukh (Head Dept. of Botany), a Shardabai Pawar Mahila Arts, Commerce & Science College, Shardanagar, Malegaon, Tal- Baramati, Dist- Pune. 41311

Primary processes:

- **Washing and drying**: After collection of plant leaves of PL, the leaves were washed by distilled water. Shade drying process was used for drying of leaves which helps to retain its chemical components for longer period of time
- **Preparation of powder**: Powder of shade dried leaves of PL was prepared by using grinder. The prepared powder was passed through sieve to get uniform sized powder
- **Extraction**: The Soxhlet extraction process was carried out and the following steps were followed for the process



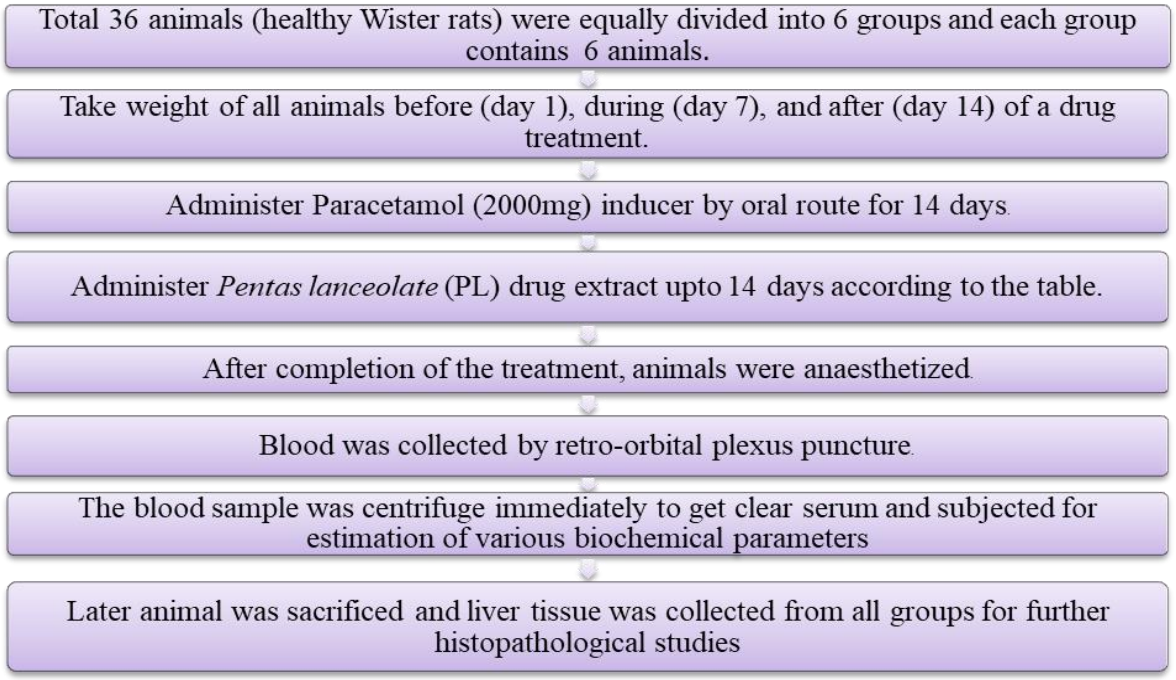
Phytochemical Screening:

Sr. No.	Phytochemicals	Presence (+)/ Absence (-)
1	Alkaloids	+
2	Carbohydrates	+
3	Coumarin	+
4	Flavonoids	+
5	Glycosides	+

6	Phenol	+
7	Quinones	+
8	Resins	+
9	Saponins	+
10	Sterols	+
11	terpenoids	+

Experimental work:

- **Method of Model- Paracetamol induced rat model:**



- Experimental Protocols:

No. of groups	**Name of group**	**Treatment given (mg/kg body weight of rat)**	**No. of Animals**
1	Normal control	5% Gum acacia suspension	6
2	Disease control	Paracetamol 2000mg /kg	6
3	Standard control	Silymarin (54mg/kg) + Paracetamol(2000mg/kg)	6
4	50mg (PL) drug extract	Paracetamol (2000mg) + 50mg PL drug extract	6
5	100mg (PL)drug extract	Paracetamol (2000mg) + 100mg PL drug extract	6
6	200mg (PL)drug extract	Paracetamol (2000mg) + 200mg PL drug extract	6

- **Test item Preparation:**

Test control group 1: (low dose) were treated with Pentas lanceolata at a dose of 50 mg/kg once daily for 14 days along with Paracetamol (2000mg/kg) in 0.5%(CMC) Carboxyl Methyl Cellulose suspension(1ml/kg)
Test control group 2: (Middle Test dose) were treated with Pentas lanceolata at a dose of 100 mg/kg once daily for 14 days along with Paracetamol (2000mg/kg) in 0.5%(CMC) Carboxyl Methyl Cellulose suspension(1ml/kg)
Test control group 2: (High Test dose) were treated with Pentas lanceolata at a dose of 200 mg/kg once daily for 14 days along with Paracetamol (2000mg/kg) in 0.5%(CMC) Carboxyl Methyl Cellulose suspension(1ml/kg)
Animal Welfare: All procedure such as housing, dosing, sacrifice, rehabilitation was done in accordance with the standard operating procedures and the guidelines provided by the committee for the purpose of control and supervision of experiments on animals (CPCSEA) as published in the Gazette of India, December15, 1998 and biological evaluation of medical devices part 2: Animal Welfare requirements. Study has been approved institutional Animal ethics committee meeting of crystal biological solutions.

OBSERVATION AND RESULTS

Effect of Pentas lanceolata on change in Body Weight: Animal body weight changes were observed on weekly basis from acclimatization day to day 14 in the experimental rats. The body weight of disease control group showed decreased body weight when compared to normal and treatment group. The body weight of Normal control and Test *Pentas lanceolata* groups were increased throughout the study period when compared with Disease control group as showed in Table 2.

Table 1. Mean Body weight

Groups	Day 0	Day 7	Day 14
Normal Control	230.83 ± 4.58	238.50±4.76	245.83±4.79
Disease Control	231.17±5.64	219.67±5.50	211.67±4.55
Standard	233.33±4.50	240.67±4.46	247.67±4.13
Test - 1	234.67±4.76	241.17±4.31	248.33±4.27
Test - 2	230.17±5.64	237.83±5.53	244.67±5.35
Test - 3	232.33±4.76	239.17±5.12	246.50±5.36

Values are mean ± SD, n = 6 in each group. P ≥ 0.05 non- significant, *P <0.05 when compared with Disease Control, **P <0.01 when compared with Disease Control, ***P <0.001 when compared with Disease Control, ****P <0.0001 when compared with Disease Control.

As compare to Disease Control group all the animals showed non-significant increase in body weight. P value is 0.999 that is higher than 0.05. Results were found to be non- significant. Two-way Anova were used for Body Weight comparison.

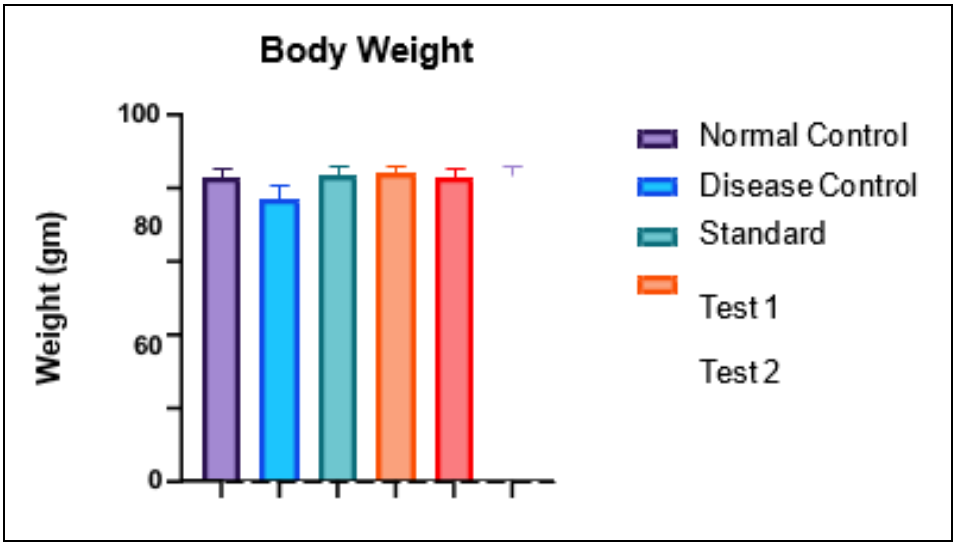


Figure 3. Body Weight

Effect of *Pentas lanceolata* on Clinical sign and Symptoms: Daily observations were recorded no any changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, and autonomic and central nervous systems, somatomotor activity, behaviour pattern work found abnormal as animals were weak and lethargic. No any signs of tremors, convulsions, salivation, and diarrhoea.

Table 2. Clinical sign and symptoms

Animal mark		Days														% Mortality
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
GROUP 1: NORMAL CONTROL																
1	H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0
	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	HB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	BT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	W	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
GROUP 2: DISEASE CONTROL																
2	H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0
	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	HB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	BT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	W	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
GROUP 3: Standard																
	H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0
	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	

	HB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	BT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	W	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
GROUP 4: TEST 1																
3	H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0
	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	HB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	BT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	W	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
GROUP 5: TEST 2																
4	H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0
	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	HB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	BT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	W	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
GROUP 6: TEST 3																
	H	N	N	N	N	N	N	N	N	N	N	N	N	N	N	0
	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	T	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	HB	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	BT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	W	N	N	N	N	N	N	N	N	N	N	N	N	N	N	

Effect of *Pentas lanceolata* on Liver Weight & Relative Liver Weight: In Disease Control group animals, weights of the Liver & Relative weight were increased when compared with control group.

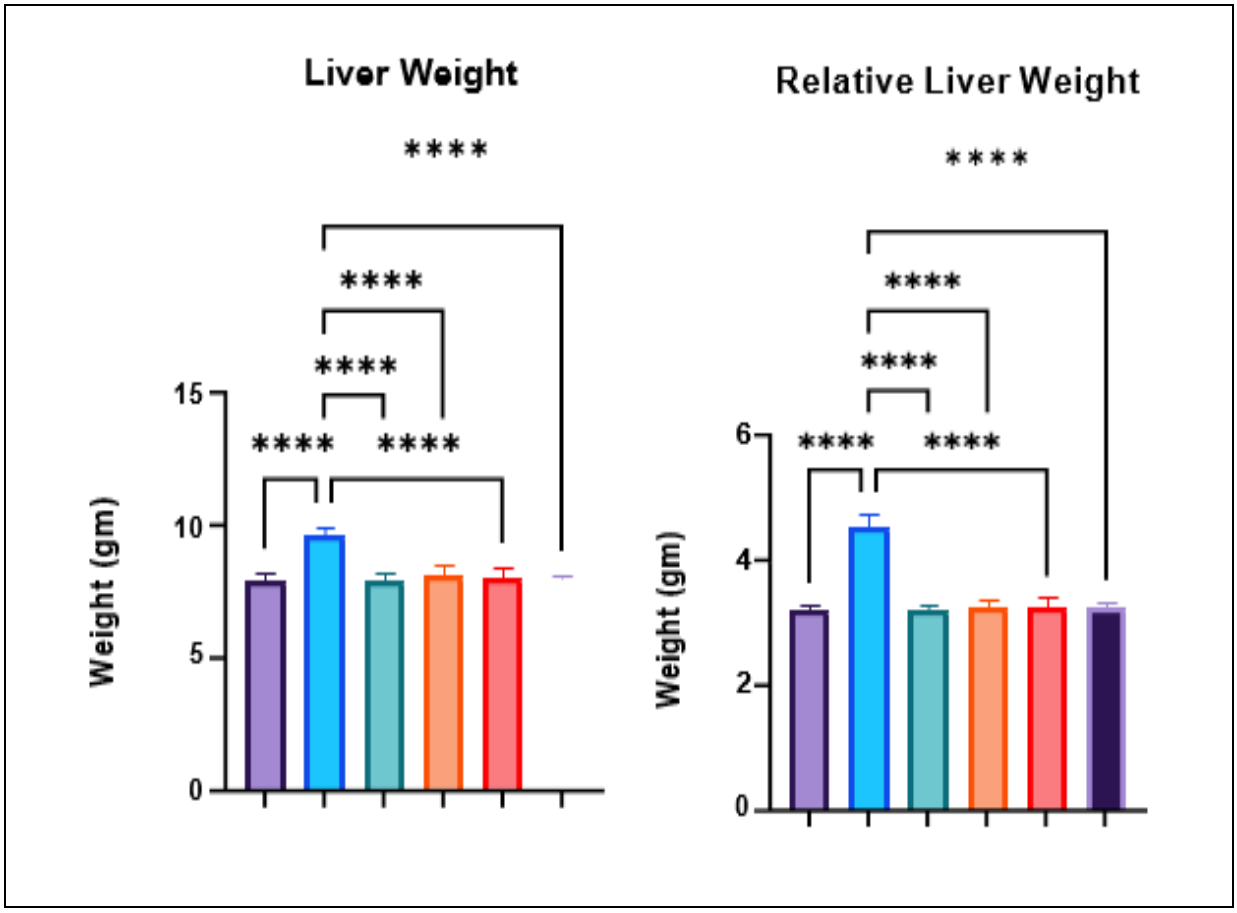


Figure 4. Organ Weight

Table 3. Mean Organ Weight

Groups	Liver Weight	Relative Liver Weight
Normal Control	8.15±0.49 ****	3.22 ± 0.10 ****
Disease Control	6.96±0.52	4.54 ± 0.23
Standard	7.94±0.22 ****	3.21±0.09 ****
Test - 1	8.03±0.63 ****	3.25 ± 0.13 ****
Test - 2	8.04±0.71 ****	3.26 ± 0.15 ****
Test - 3	8.11±0.60 ****	3.23 ± 0.12 ****

Values are mean ± SD, n = 6 in each group. P ≥ 0.05 non- significant *P <0.05 when compared with Disease Control, **P <0.01 when compared with Disease Control, ***P <0.001 when compared with Disease Control, ****P <0.0001 when compared with Disease Control.

Organ weight of Liver of Normal Control, Standard and test group animal significantly decreased when compared to disease control group. Disease control group increased organ weight after Paracetamol drug treatment. P value of DC liver was lower than 0.0001.

Relative organ weight of Liver in Normal Control, Standard and test group animal showed significantly decreased when compared to Disease control group. Disease control group increased Relative liver weight after Paracetamol drug treatment. P value of DC liver was lower than 0.0001 (p<0.0001).

Organ weight & Relative weight of Liver of Normal Control, Standard and test group animals was showed highly significant when compared to the Disease control group. One-way Anova was used to find out difference between DC and Test group.

Effect of Pentas lanceolata on Biochemical Parameter: The hepatotoxic agent caused significant liver damage as indicated by an increase in the level of liver chemistry biomarkers

such as; Bilirubin, AST, ALT, GGT and ALP. Blood sample was collected in 0 and 15th days and serum biochemical will be done.

A. **Biochemical Parameter Day-0:** Before induction of Paracetamol the biochemical parameters did not show any significant ($p>0.05$) change in their value when compared to the value of disease control.

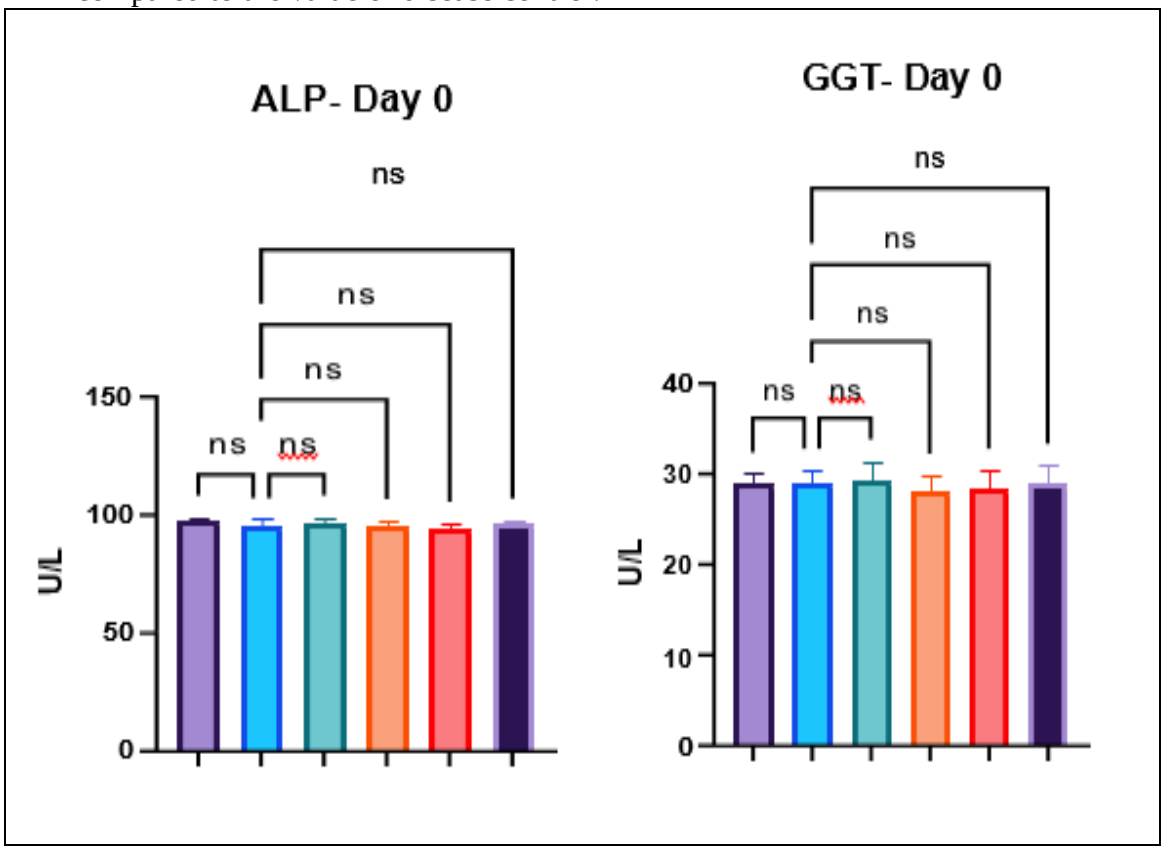


Figure 5. Biochemical Parameter (Day 0)

Table 4. Mean biochemical parameter- Day 0

Groups	ALP	GGT
Normal Control	97.96±1.75	29.05±1.14
Disease Control	96.09±2.45	29.20±1.39
Standard	96.92±2.79	29.50±2.13
Test - 1	96.05±2.06	28.36±1.69
Test - 2	94.85±2.17	28.37±2.20
Test - 3	96.82±1.65	29.01±2.16

Values are mean ± SD, n = 6 in each group. P ≥ 0.05 non- significant *P <0.05 when compared with Disease Control **P <0.01 when compared with Disease Control, ***P <0.001 when compared with Disease Control, ****P <0.0001 when compared with Disease Control.

As compare to Disease Control group all the animals showed non-significant increase in all parameter. P value is 0.999 that is higher than 0.05. Results were found to be non-significant. One way ANOVA was used to find out difference between DC and Test group.

B. **Biochemical Parameter Day -14:** After induction of Paracetamol the biochemical parameters Total Bilirubin, SGPT, SGOT, ALP and GGT show significant change in their value when compared to the value of disease control. Normal control, Standard n Test group values of bilirubin, SGPT, SGOT, GGT and ALP was decreased when compared to the value of disease control.

However, the values of Bilirubin, SGPT, SGOT, GGT and ALP were high in disease control after Paracetamol drugs induction. That means normal control, Standard and

Test group animals showed significantly reduced Bilirubin, SGPT, SGOT and ALP when compared to the disease control group.

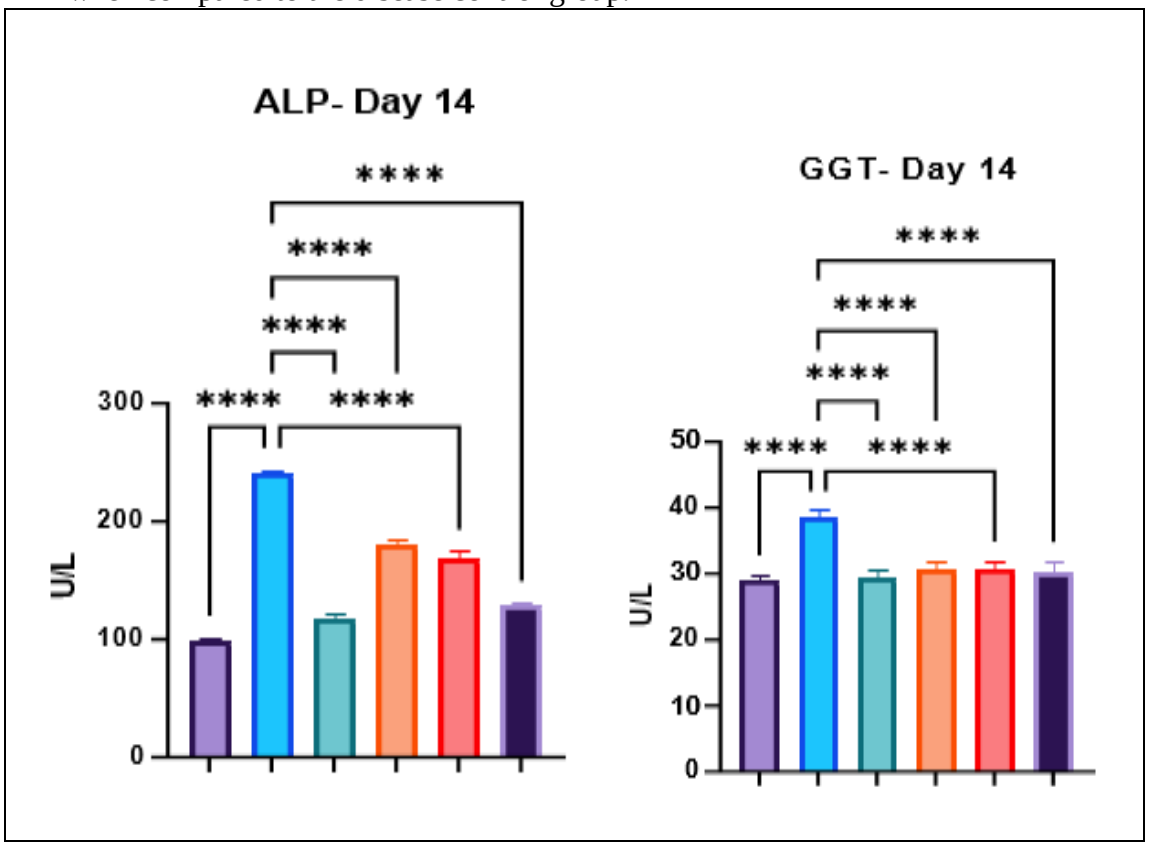


Figure 6. Biochemical Parameter (Day 14)

Table 5. Mean biochemical parameter- Day 14

Groups	ALP	GGT
Normal Control	99.19±2.20 ****	29.14±0.84 ****
Disease Control	240.45±3.49	38.63±1.03
Standard	117.49±3.40 ****	29.59±1.19 ****
Test - 1	180.75±2.74 ****	30.65±1.45 ****
Test - 2	168.89±5.37 ****	30.89±1.16 ****
Test - 3	128.62±2.12 ****	30.20±1.77 ****

Values are mean ± SD, n = 6 in each group. P ≥ 0.05 non- significant *P <0.05 when compared with Disease Control, **P <0.01 when compared with Disease Control, ***P <0.001 when compared with Disease Control, ****P <0.0001 when compared with Disease Control.

Normal Control, Standard and Test group animals were showed significant lower values of SGPT, SGOT, ALP, GGT and bilirubin. Highly significant changes were obtained in Normal control, Standard and Test group animals (p<0.0001) when compared to the disease control group. Disease control animals showed significantly increased SGPT, SGOT, ALP, GGT and Bilirubin after treatment of Paracetamol drug.

Effect of Pentas lanceolata on Biomarkers: Plasma samples from each Sacrificed rat were used. Levels of IL-6 & IL-8 in disease control & normal control group were determined by a rat kit provided by KRISHGEN.

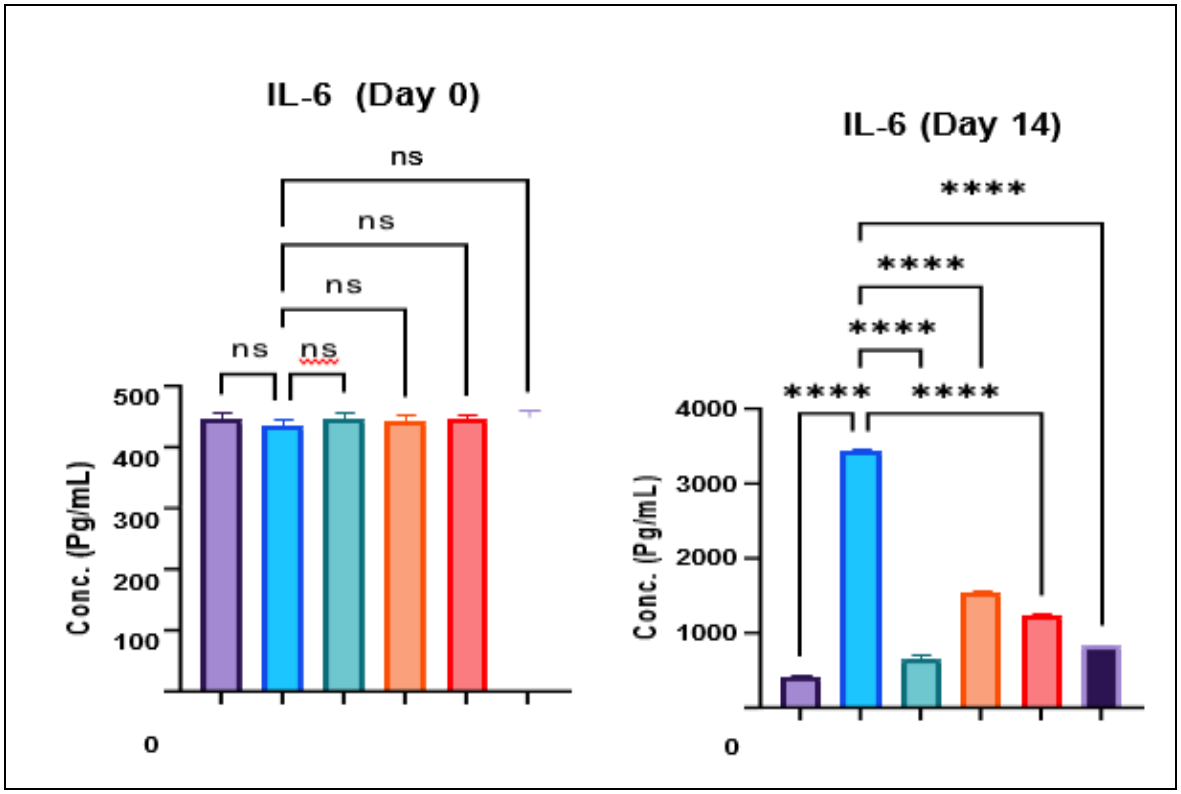


Figure 7. - IL-6

Table 6. Mean of IL-6 parameter

Groups	IL-6 Conc. (Pg/mL) Day 0	IL-6 Conc. (Pg/mL) Day 14
Normal Control	447.33±9.60	435.67±12.11 ****
Disease Control	437.89±9.11	3430.67±26.98
Standard	445.67±13.98	681.78± 29.47 ****
Test - 1	441.78±11.04	1554.56±13.61****
Test - 2	445.67±8.69	1239.56±21.54****
Test - 3	450.67±9.60	840.11±20.94****

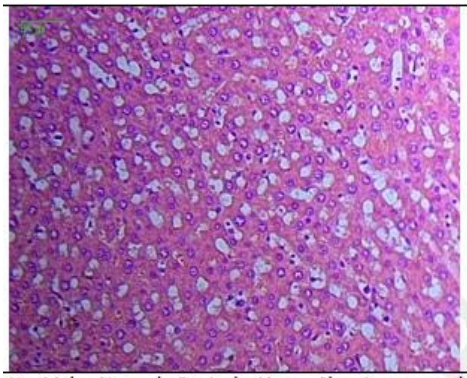
Values are mean ± SD, n = 6 in each group. P ≥ 0.05 non- significant *P <0.05 when compared with Disease Control, **P <0.01 when compared with Disease Control, ***P <0.001 when compared with Disease Control, ****P <0.0001 when compared with Disease Control.

Day 0 - Concentration of IL-6 in Normal Control and test group animal showed non-significant increase when compare to Disease Control group. P value is 0.999 that is higher than 0.05. Results were found to be non-significant.

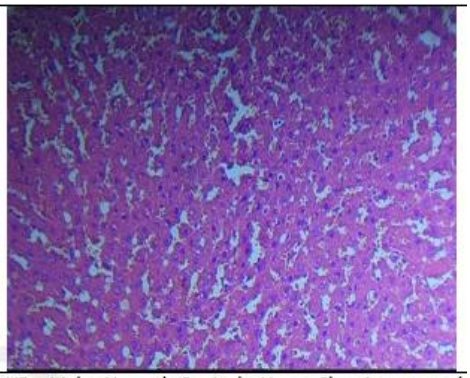
Day 14 - Concentration of IL-6 in Normal Control and test group animal showed significantly decreased when compared to Disease control group. Disease control group increased Concentration of IL-6 after Paracetamol drug induction. P value of DC liver was lower than 0.0001 (p<0.0001).

IL-6 Conc. of Normal Control and test group animals was showed highly significant when compared to the Disease control group. One-way ANOVA was used to find out difference between DC and Test group.

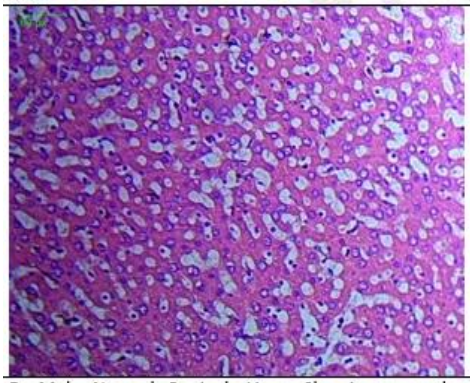
Effect of Pentas lanceolata on Histological Parameter: Microscopic examination of organs from 4 groups was carried out. It is mentioned in the chart given below.



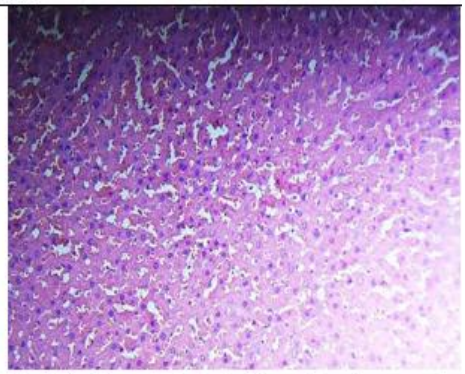
H- Male Normal Control: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



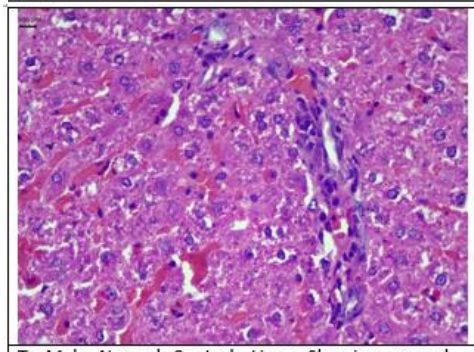
HB- Male Normal Control: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



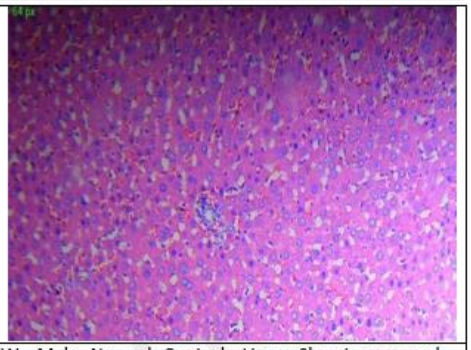
B- Male Normal Control: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



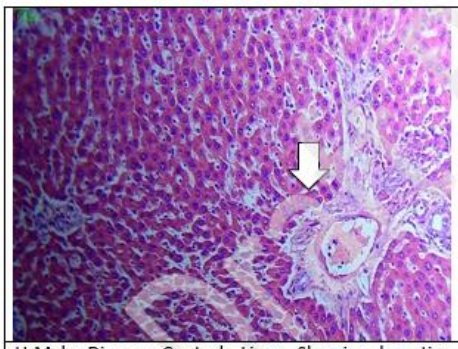
BT- Male Normal Control: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



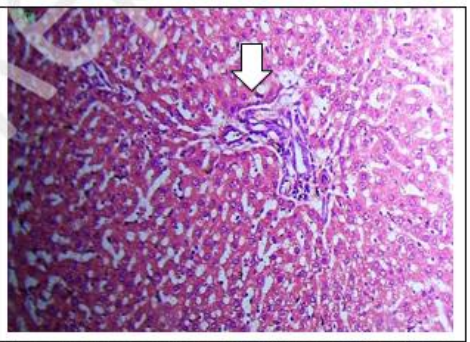
T- Male Normal Control: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



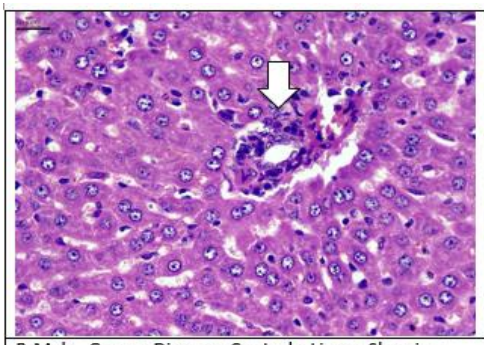
W- Male Normal Control: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



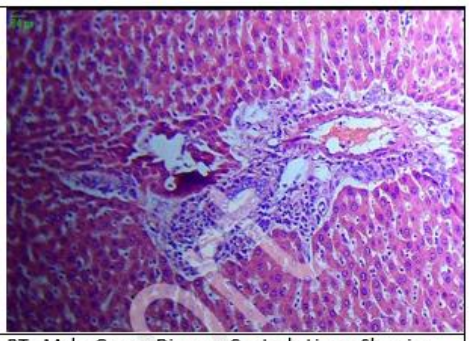
H-Male Disease Control: Liver: Showing hepatic degeneration with infiltration of inflammatory cells (arrow). 40X, H & E Stain



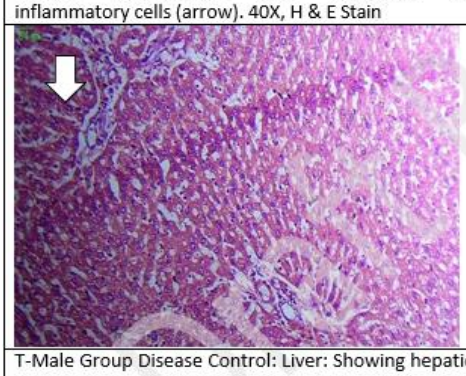
HB- Male Group Disease Control: Liver: Showing hepatic degeneration with infiltration of inflammatory cells (arrow). 40X, H & E Stain



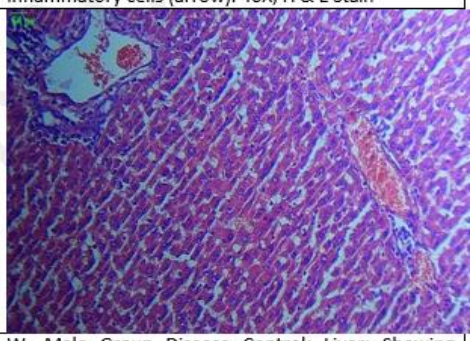
B-Male Group Disease Control: Liver: Showing hepatic degeneration with infiltration of inflammatory cells (arrow). 40X, H & E Stain



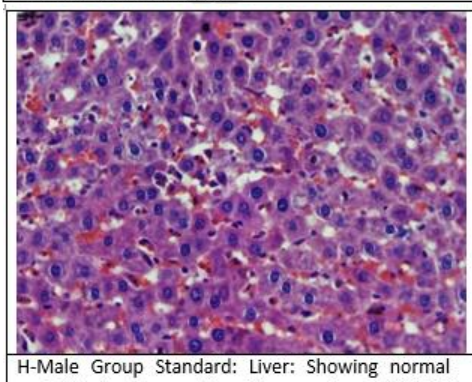
BT- Male Group Disease Control: Liver: Showing hepatic degeneration with infiltration of inflammatory cells (arrow). 40X, H & E Stain



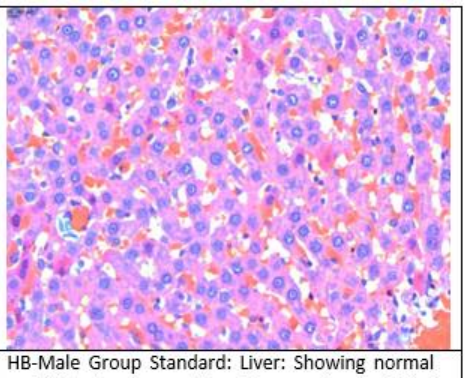
T-Male Group Disease Control: Liver: Showing hepatic degeneration with infiltration of inflammatory cells (arrow). 40X, H & E Stain



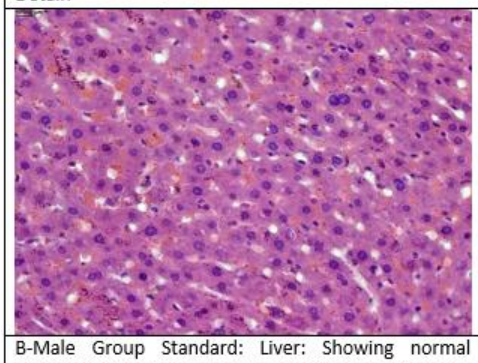
W- Male Group Disease Control: Liver: Showing hepatic degeneration with infiltration of inflammatory cells (arrow). 40X, H & E Stain



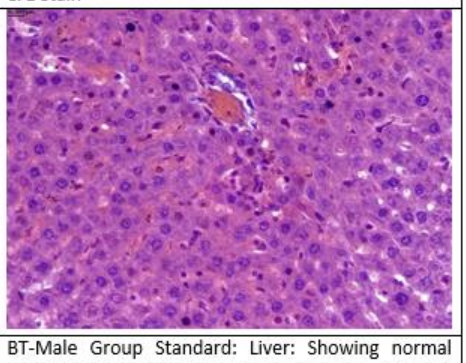
H-Male Group Standard: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



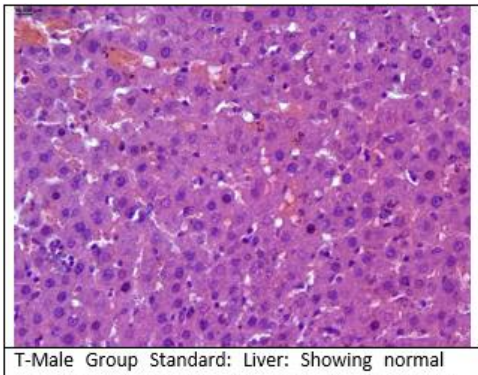
HB-Male Group Standard: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



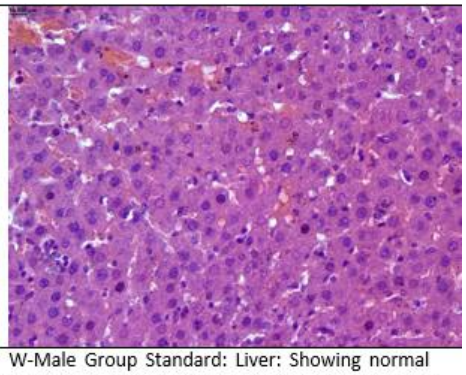
B-Male Group Standard: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



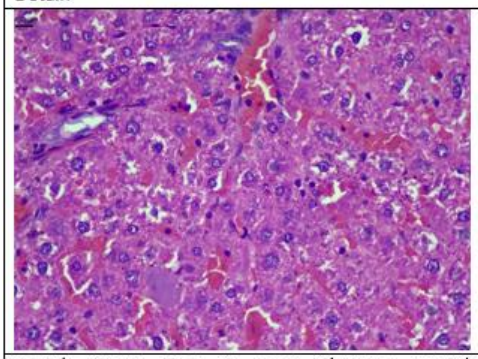
BT-Male Group Standard: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



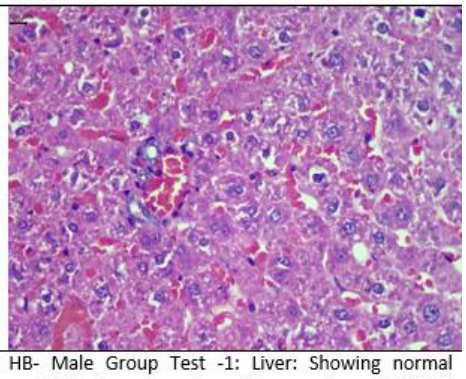
T-Male Group Standard: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



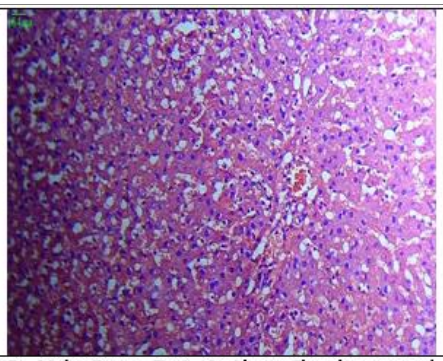
W-Male Group Standard: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



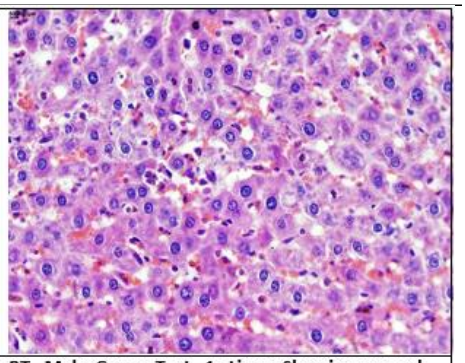
H-Male Group Test -1: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



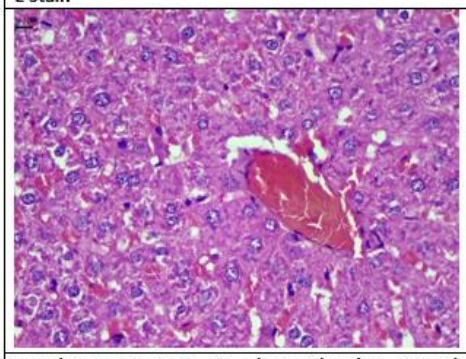
HB- Male Group Test -1: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



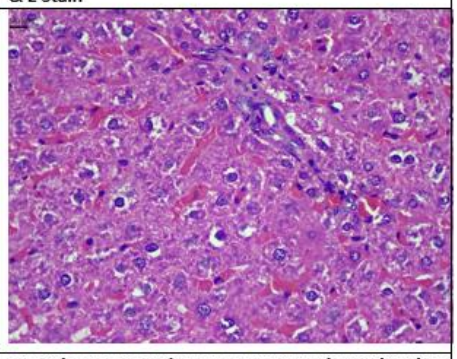
B- Male Group Test -1: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



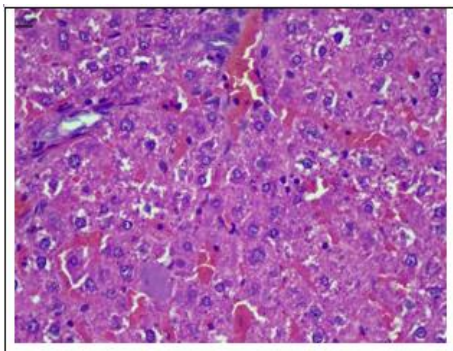
BT- Male Group Test -1: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



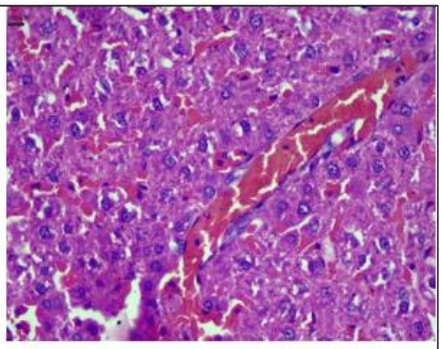
T-Male Group Test -1: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



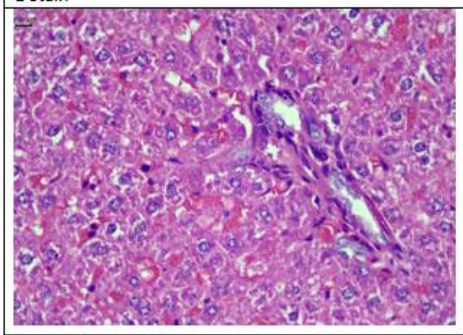
W-Male Group Male Group Test -1: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



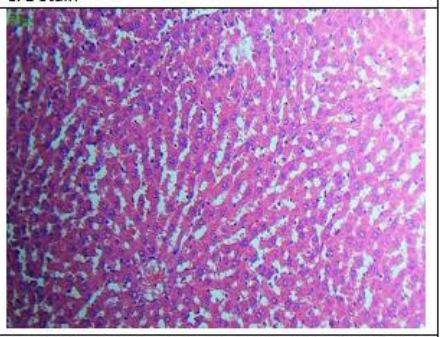
H-Male Group Test -2: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



HB- Male Group Test -2: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



B- Male Group Test -2: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



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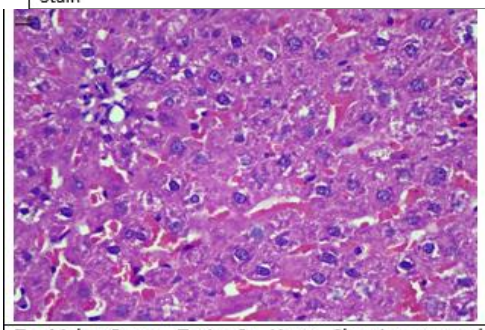
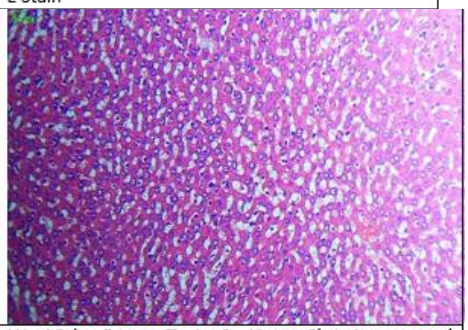
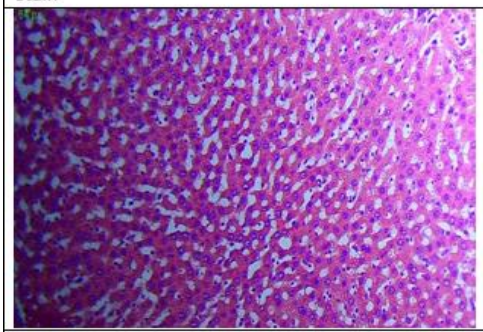


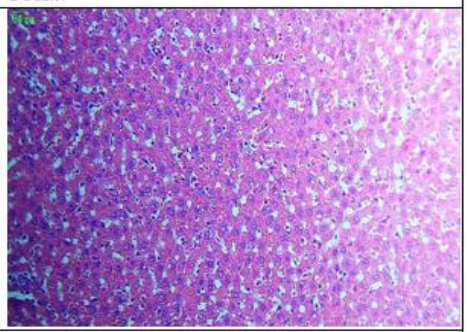
T- Male Group Test -2: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



W- Male Group Test -2: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



H-Male Group Test -3: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain



HB-Male Group Test -3: Liver: Showing normal centrilobular area and hepatic parenchyma. 40X, H & E Stain

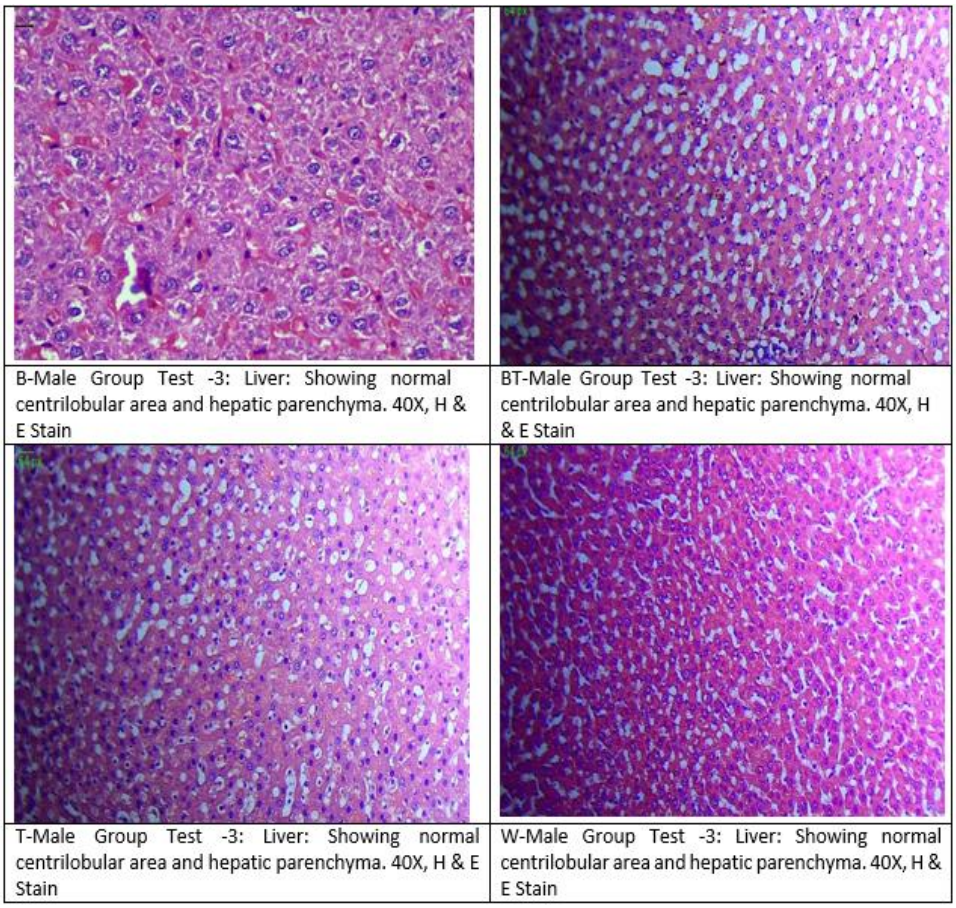


Figure 8. Histopathological Observation

Table 7. Histopathological Observation

Sr. No.	**Group**	**Animal**						**Total**	**Average**
		H	B	T	HB	BT	W		
1. Liver	Normal Control	0	0	1	0	0	0	1	0.17
2. Liver	Disease Control	3	3	3	2	3	2	16	2.67
3. Liver	Standard	1	0	0	1	0	0	2	0.33
4. Liver	Test 1	2	1	1	0	0	1	6	1.00
5. Liver	Test 2	1	1	1	0	1	1	5	0.83
6. Liver	Test 2	0	1	0	0	1	0	2	0.33

DISCUSSION: There were no notable differences in the body weight of animals between the normal, standard, and treatment groups when compared to the disease control group. However, the body weight of the disease control group was lower compared to the normal control and treatment groups. Throughout the study period, no deaths occurred in any of the animal groups. Additionally, none of the animals exhibited clinical signs such as lacrimation, salivation, irregular respiratory patterns, convulsions, tremors, or any unusual behaviours in any of the groups [3, 5].

Throughout the 21-day observation period, rats in the Normal Control, Disease Control, Standard, and Treated groups exhibited normal behaviour. All animals consumed comparable amounts of feed and water throughout the duration of the experiment.

Organ weight serves as a valuable indicator for assessing organ injury. When comparing the liver weight collected from Wistar rats on day 14 to the Disease Control group, a significant decrease in liver weight was observed in the test group animals. Additionally, both liver weight and relative liver weight were significantly increased in the Disease Control group compared to the Normal Control group. Moreover, significant changes were noted in the biochemical markers of liver function tests, as summarized in the results [8, 9].

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Bilirubin, GGT and Alanine phosphatase (ALP) levels were raised in disease control group after Paracetamol induction. Statistical difference was observed in day 0 and day 15 biochemical parameter. Before induction (Day 0) of Paracetamol drug the biochemical parameters did not show any significant ($p>0.05$) change in their value when compared to the value of disease control [11]. Following the induction of Paracetamol drugs on Day 14, the disease control group exhibited elevated levels of ALP and GGT. However, in both the standard and test groups, the levels of GGT and ALP decreased after treatment with Pentas lanceolata. Notably, Test Group 3 receiving 200mg/kg Pentas lanceolata treatment displayed a notable reduction in SGPT, SGOT, bilirubin, GGT, and ALP levels compared to Test Groups 1 (50mg/kg) and 2 (100mg/kg). Furthermore, in comparison to the disease control group, the normal control, standard, and test group animals demonstrated significantly decreased levels of IL-6 biomarkers. Thus, it is concluded that while no statistically significant changes were observed before the induction, there was a slight increase in IL-6 after the induction of Paracetamol drugs. However, treatment with Pentas lanceolata led to a decrease in IL-6 levels [15-17].

The test drug Pentas lanceolata group was found to be more effective in hepatotoxicity study similar to the Silymarin treated group.

CONCLUSION:

- Paracetamol is known for its strong hepatotoxic effects in rats, causing significant liver damage in exposed animals.
- The group of animals receiving Paracetamol for 14 days (Disease Control group) exhibited highly significant changes, including a notable increase in liver weight.
- Histopathological and biochemical examinations revealed various changes in animals exposed to Paracetamol, indicating its harmful effects.
- Treatment with Pentas lanceolata effectively reversed the hepatotoxic effects induced by Paracetamol. Biochemical markers such as SGPT, SGOT, bilirubin, GGT, and ALP levels were regulated by the treatment.
- During the 14-day observation period, Test Group 3 receiving Pentas lanceolata (200mg/kg) demonstrated efficacy in mitigating liver damage.
- The study concludes that Pentas lanceolata effectively protects against liver damage induced by Paracetamol...

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