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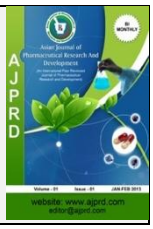

Open Access Research Article

Evaluation of Hepatoprotective Activity of Leaves Extract of *Pithecellobium Dulce* In Experimental Animals

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A B S T R A C T

Objective: To evaluate the hepatoprotective activity of leaves extract of *Pithecellobiumdulce* in experimental rats.
Methods: For the generation of Hepatotoxicity ,paracetamol (2g/kg) was administered orally for 7days, accompanied by a 7-day dose of Ethanolic extract of *Pithecellobiumdulce*leaves(100mg/kg, 200mg/kg, and400mg/kg, p.o.)using 1 % w/v CMC as a vehicle.
Results: From the present investigation, it was observed that ethanolic extract of *Pithecellobium dulce* have shown significant reduction in serum glutamate oxaloacetate transminase (SGOT),serum glutamate pyruvate transminase (SGPT),alkaline phosphatase (ALP) triglyceride, bilirubin level in paracetamol induced hepatotoxicity.
Conclusion: The findings in this study revealed the effectiveness of ethanolic extract of *Pithecellobiun dulce* against hepatotoxicity activity.

Keywords: *Pithecellobium dulce* ,Hepatotoxicity, Silymarin, Paracetamol, SGOT,SGPT,ALP

A R T I C L E I N F O: Received 25 June 2021; Review Complete; 16 July 2021 Accepted; 20 July 2021 Available online 15 August 2021

Cite this article as:

Sul k, Vitthal C, Redasani Vivek R, Evaluation of Hepatoprotective Activity of Leaves Extract of *Pithecellobium Dulce* In Experimental Animals., Asian Journal of Pharmaceutical Research and Development. 2021; 9(4):39-46.
DOI: http://dx.doi.org/10.22270/ajprd.v9i4985

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1. INTRODUCTION:

Hepatotoxicity is also termed as aquired high blood triglycerides; high bilirubin,highserum glutamate pyruvate transminase (SGPT); high serum glutamate oxaloacetate transminase (SGOT). It is an elevation of one or more of the plasma lipids, including triglycerides, cholesterol,always corresponding with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels[1]. In addition serum levels ofmany biochemical markers like bilirubin,triglyceride, alkaline phosphatase(ALP),Serum glutamate pyruvate transminase(SGPT),Serum glutamate oxaloacetate transminase(SGOT), triglycerides, cholesterol are exalted.The liver is the principal glandular organ in the body and has more functions than any other human organ.Liver is not only the second-largest organ of the body but also the largest gland. Liver is weighing about 1.4 kg in grown person and is underline to the diaphragm occupying most of the right hypochondriac and a part of the epigastric region of abdominopelvic cavity[2].Liver deals with many pathways which include energy provision ,nutrient supply,relevant to growth, fight against disease, and reproduction are corresponded with liver[3,4].The liver has an inventory task of maintaining the body's metabolic homeostasis. The liver is expected to perform physiological functions as well as protects against the hazards of chemicals and harmful drugs[5].Though tremendous scientific advancement in the field of hepatology , liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders and having higher mortality rate[6].In the makett , for the treatment of liver

disorders there is only few hepatoprotective drugs are available .

Pithocellobium dulce Benth belongs to the family of Leguminasae.It is a small to medium sized ,evergrren and found in the India and Southern Asia.Locally *Pithocellobium dulce* is known by various names at different region such as Vilayti Babul in hindi and Vilayti chinch in Marathi and in English it is known as Manila tamarind[7]. The fruit of plant *Pithecellobium dulce* is well known as edible fruits and they have been consumed for various ailments in a traditional manner.The Fruits are greenish-brown to red-pinkish, indehiscent pods and linear, curved legumes (pods) that range in length from 10 to 13 cm. usually,pods are 10-15 cm; the colour becomes reddish brown.pods are irregular in shape and flattened,Each pods contain 5-10 shiny seeds, set in spirals of 1 to 3 whorls and strangled between the seeds (lomentaceous). The seeds are black in colour and shiny nature and 1cm long surrounded by an edible white pulp. The pod is peeled on both sides[8] (Orwa et al., 2009).*Pithecellobium dulce leaves* has been reported to contain Quercetin, Kaempferol, cyclitol, dulcitol afezilin[9]. *Pithecellobium dulce* also contains Quercetin 3-O-rhamnoside ,Seven saponins named Pithedulosides A-G,Pinitol,triterpenoids,glycoside etc. Literature survey indicates that *Pithecellobium dulce* possesses Antidiarrhoeal activity[10],Anticonvulsant activity[11], Adulticidal activity[12], Hypolipidemic activity[13], Anti-oxidant activity[14], Anti-diabetic[15], Antifungal Activity[16], Analgesic[17] ,Anti-inflammatory[17] ,Larvicidal and ovicidal activities[18] and antimicrobial activity[19]etc .Therefore, the present investigation was under taken to evaluate hepatoprotective effect of ethanolic extract of *Pithecellobium dulce* in paracetamol induced hepatotoxicty in rats which has not been earlier reported . It is our belief that this examination will take us another step forward in our quest to understand the mechanism of action of *Pithecellobium dulce* in prevention and medicaments of liver related diseases.

2. MATERIALS AND METHODS:

2.1.Drugs& chemical

Paracetamol was acquired from S.D.fine.chemialsMumbai.Silymarin(silybon140,Cipla Ltd.) was purchased in a tablet form at strength 140 mg. All other chemicals and reagents used were of analytical grade and aquired from approved chemical suppliers. The serum glutamate oxaloacetate (SGOT), Serum glutamate pyruvate transminase (SGPT) and Serum alkaline phosphate (ALP)and Total bilirubin were measured with the help of commercial kits.

2.2. Collection and extraction of plant material

The fresh leaves of *Pithecellobium dulce* was collected form local area of, Solapur District, Maharastra, India and were identified by a botanist. They were washed with tap water and dried leaves were powdered and passed through sieve for coarse powder. This shaded dried powdered was extracted successively with (1:6)Petrolium ether, Chloroform, Ethanol in a soxhlet apparatus allowed to stand for a period of 18 hr, and filtered by using Whatman filter paper (No. 1). The filtrate was concentrated at 45°C in a water bath for complete dryness. Crude extract obtained was stored at 4°C for further use.

2.3. Experimental animals

The complete experiment was carried out using 36 wistar albino rats of both sex weighing 150 - 200g.The study protocol was approved by Institutional Animal Ethics Committee (IAEC) ,YTC, YSPM, Satara. The animals were aquired from registered breeder and familiarized in the quarantine area for one week. Animals were housed in clean polypropylene cages in a controlled room temperature 22°C ± 2°C, relative humidity of 50 ± 15% and 12 hr dark/ 12 hr light cycle at our Institution's animal house and allowed to aculturate for two weeks. The animals were fed with water and standard pellet diet ad libitum. Animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines.

2.4. Preparation of standard drug

Silymarin tablets were crushed into powder, dissolved in distilled water at dose 200mg/kg b. w. and administered orally.

2.5. Induction of hepatotoxicity

All the experimental animals excepts control group ,were administered Oraly with Paracetamol at a dose of 2 gm/kg dissolved in 1% Carboxy Methyl Cellulose(CMC).

2.6. Experimental design

A total 36 wistar albino rats of either sex were randomly divided into 6 groups containing 6 animals in each group. Group I (Normal control) did not receive any treatment apart from vehicle 10ml/kg b. w. /day for 7 days. Group II (Negative control) were induced with 2.0g/kg b. w. dose of Paracetamol without treatment . Group III(Standard control) were induced with 2.0g/kg dose of Paracetamol and treated with silymarin at a dose of 200mg/kg b. w. /day for 7 days. Group IV, V and VIwere induced with 2.0g/kg dose of Paracetamol and treated with test drug at dose 100 (low), 200 (medium) and 400 (high) mg/kg b. w. /day for 7days respectively.

2.7.Blood Sample Collection

At end of the experimental period animals were kept fasted over night and anaesthetized with diethyl ether. Blood samples were collected serially by retro orbital puncture.The blood was allowed to clot for 30 min at room temperature then serum was separated by centrifugation and used for biochemical parameters like serum glutamate

oxaloacetate (SGOT),Serum glutamate pyruvate transminase (SGPT) and Serum alkaline phosphate (ALP),Bilirubin, Triglycerides.

2.8.Evaluation parameters

2.8.1. Physical Parameter

Determination of wet liver weight:

Animals were sacrificed and livers were isolated and washed with saline and weights determined by using an electronic balance.The liver weight were expressed with respecte to its body weight i.e.gm/100gm.

Determination of Wet liver Volume:

After recording the weight all the livers were dropped individual in a measuring cylinder containing a fixed volume of distilled water or saline and the volume displaced was recorded.

2.8.2. Biochemical parameters

The principle, details of kits and methodology used in the estimation of various bio-chemical parameters by Autoanalyser in the present investigation are as follows

- Estimation of AST
- Estimation of ALT
- Estimation of ALP
- Estimation of bilirubin
- Estimation of triglycerides

2.8.3. Liver histopathology

The fixed specimens of liver were evolved by washing through running tap water, dehydration through ascending grades of alcohol, clearing through xylene and embedding completely with in paraffin into blocks. The serial sections of not exceeding 3 mm thickness were cut using microtome and were mounted on polylysine coated slides, removal of paraffin from tissues by using xylene, rehydrated and stained with hematoxylin and eosin, dehydrated, cleared and mounted on DPX under glass cover slips. The slides were then observed under light microscope which was connected to a camera to capture images.

2.9.Statistical analysis

The statistical analysis was carried out with Graphpad prism 5.0 software. The data was statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison tests and $p< 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1.Yield of the extract

The yield of the extract was found to be 24.35%. Further preliminary phytochemical screening revealed the presence of flavonoids, saponins, phenol, carbohydrates, tannins, alkaloids, sterols and glycoside.

3.2.Liver weight and Liver volume

Paracetamol treatment in rats showed the hepatic damage which was evident by increase in the liver weight and volume.when treated with standard (Silymarin) showed good reduction in weight and volume of liver.

Table1: Effect of ethanolic extract of Pithecellobiumdulce on Liver weight and volume of Paracetamol induced hepatotoxicity in experimental animals.

Sr. No	Experimental group	Liver weight gm/100gm	Liver volume ml/100gm
1	Normal control	2.82 ±0.11	5.62 ±0.24
2	Negative control	4.81 ± 0.12	9.62 ±0.17
3	Standard control	2.87 ±0.09	6.43 ±0.20
4	Test I	3.73 ±0.19	8.78 ±0.16
5	Test II	3.30 ±0.13	7.51 ±0.17
6	Test III	3.12 ±0.10	6.93 ±0.13

Normal control: distilled water ; Negative control : Paracetamol; Standard control: Silymarin; Test I :EEPD(100mg/kg) ; Test II EEPD (200mg/kg)Test III EEPD(400mg/kg)

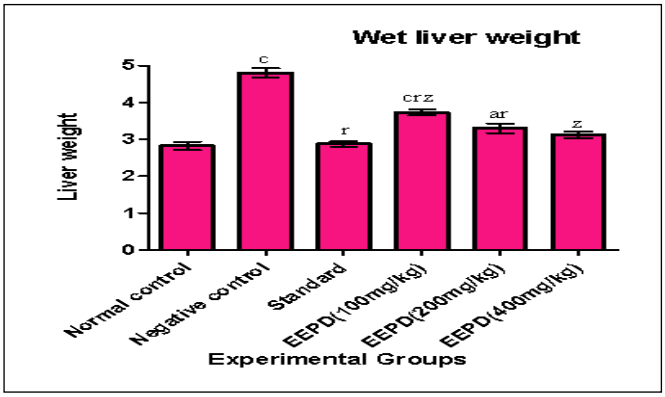

Figure: 1.Effect of ethanolic extract of Pithecellobiumdulce leaves on Wet liver weight

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

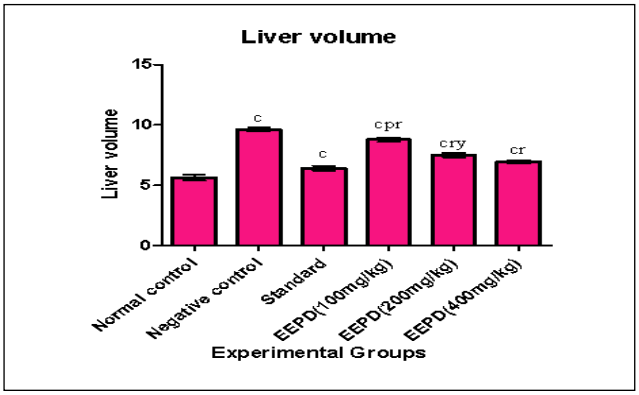

Figure 2: Effect of ethanolic extract of Pithecellobiumdulce leaves on Wet liver volume

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

3.2. Liver functional test

Paracetamol administration developed acute hepatotoxicty in rats by significantly increasing the level of triglycerides, SGOT,SGPT,ALP,Bilirubin level in rats treated with paracetamol group as compared to normal control group.

Effect of EEPD on AST,ALT,ALP levels in paracetamol induced hepatotoxicity in wistar rats:

Rats treated with paracetamol developed a significant liver damage observed as elevated serum levels of hepatospecific enzymes like aspartate amino transferase ,Alaninetransferase,when compared with normal control.Medicaments with silymarin showed protection against paracetamol induced hepatotoxicity.Test groups treated with ethanolic extract of leaves of pithecellobiumdulce showed significant effect which can be comparable with toxic control. In One way ANOVA Dunnet's test indicates a significant reduction on elevated serum enzymes levels with extract treated animals compared to toxic control animals.

Effect on total bilirubin:

The total bilirubin concentration was found to increase in animals with liver damage by paracetamol .In standard treated group (Silymarin) administration reduced total bilirubin and animal medicaments with EEPD have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group.

Effect on Triglyceride:

Induction of hepatic damage by administration of paracetamol was increase the concentration of triglycerides.treatment with silymarin has shown significantly reduction in the triglycerides levels while EEPD have shown dose dependent decrease in triglyceride level compared to toxic control group**.**

Table 2 Effect of ethanolic extract of pithecellobiumdulce on serum liver profile of Paracetamol induced hepatotoxicity in experimental animals.

Sr. No.	Experimental	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubn	Triglyceride (mg/dL)
1	Normal Control	54.17 ±1.88	79.67 ± 3.28	103.1± 1.67	0.53 ± 0.029	108.3 ± 2.02
2	Negative Control	149.5 ±1.43	307.4 ±5.07	248.1 ± 1.78 c	2.19 ± 0.028	265.3± 2.56
3	Standard Control	103.5±1.49	119.0 ± 4.69	126.2 ± 0.42	0.92± 0.036	121.2± 1.07
4	Test I	149.0± 1.58	190.5±4.70	191.5± 1.66	1.98± 0.016	197.1± 1.42
5	Test II	143.8± 1.78	175.5± 5.54	169.7± 1.80	1.56± 0.035	165.0± 1.50
6	Test III	110.8± 0.48	149.2± 3.82	147.3± 1.09	0.95± 0.03	134.8± 1.46

Normal control: distilled water; Negative control: Paracetmol; Standard control: Silymarin; Test I: EEPD (100mg/kg); Test II: EEPD (200mg/kg); Test III: EEPD (400mg/kg).

Values represented mean ± SEM; n=6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. a<0.05, b<0.01, c<0.001; Data compared with normal control; p<0.05, q<0.01, r<0.001 Data compared with negative control; x<0.05, y<0.01, z<0.001 Data compared with standard control (by one way ANOVA followed by Tukey's multiple comparison test).

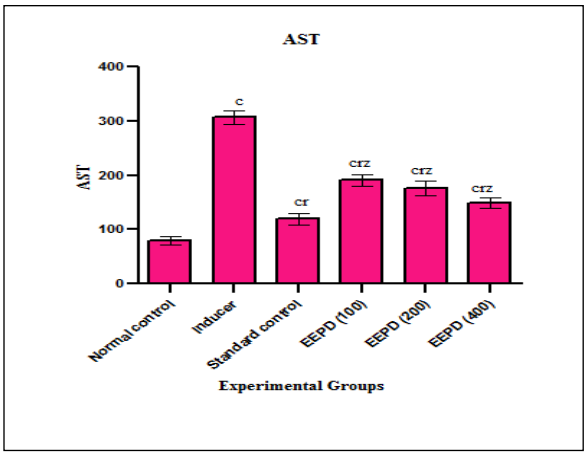

Figure 3: Effect of ethanolic extract of Pithecelobiumdulce on AST (IU/L)

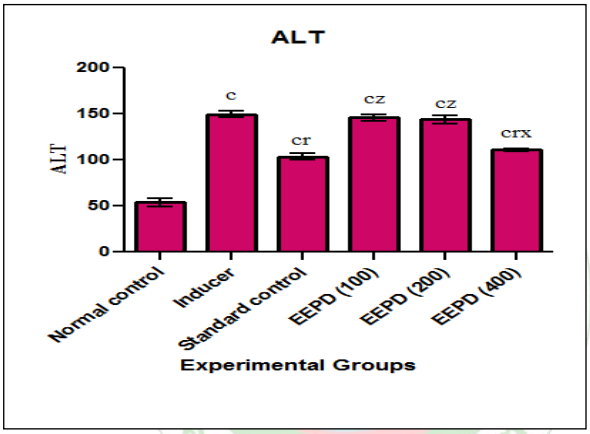

Figure 4: Effect of ethanolic extract of Pithecellobiumdulce on ALT (IU/L)

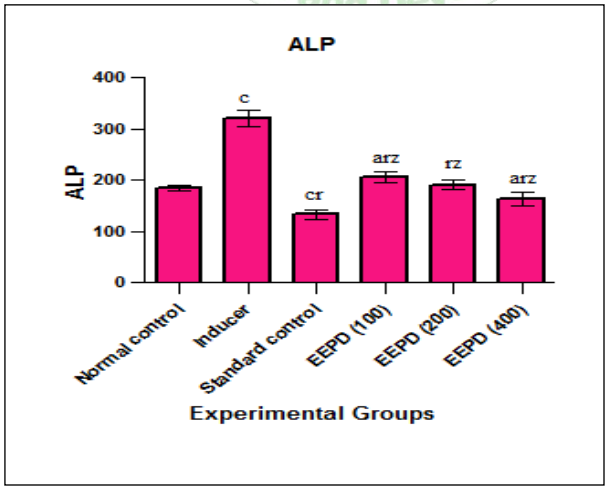

Figure 5: Effect of ethanolic extract of Pithecellobiumdulce on ALP (IU/L)

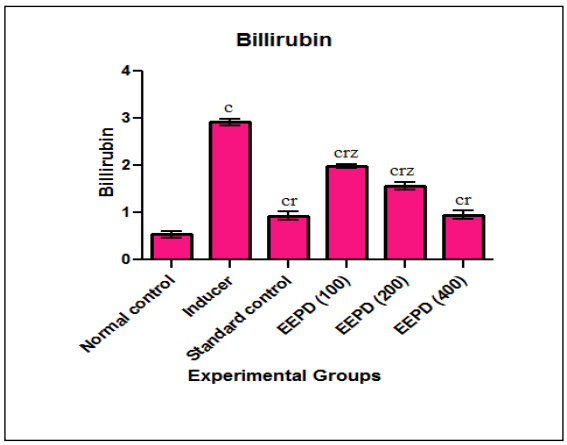

Figure 6: Effect of ethanolic extract of Pithecellobiumdulce on Bilirubin (IU/L)

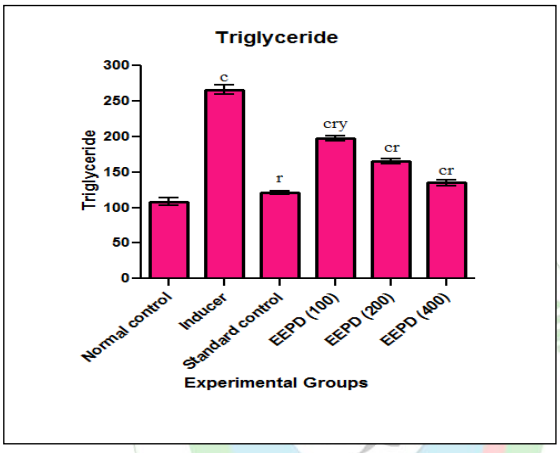

Figure 7: Effect of ethanolic extract of pithecellobiumdulce on triglyceride (mg/dL)

3.4.1. Histopathological observation of liver in normal control group

1.The normal control group animals showed normal hepatocyte architecture such as healthy nucleus and parenchymal structure [Fig.1].

2.Section of liver in negative control group shows partially effaced architecture compared to normal control group the negative control group animals showed apoptotic changes, perivenular mononuclear inflammatory infiltration, scattered inflammatory infiltration within the parenchyma which is due to toxicity . [Fig.2].

3.When compared with negative control group the test III group animals has reduced fatty changes and restored the hepatocytes near to the normal group [Fig.3].

4.Section of liver in the test drug treated group(200 and 400mg/kg)shows intact architecture few, regenerative hepatocytes, sinusoidal congestion with diffuse congestion of sinusoids.[Fig4,5]

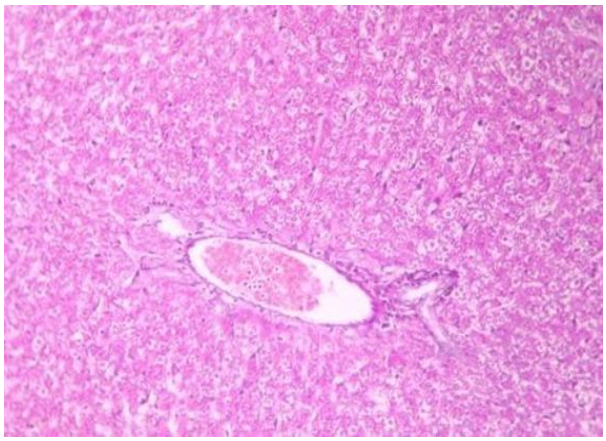

Figure:1 Normal Contol group, showing normal hepatocytes.

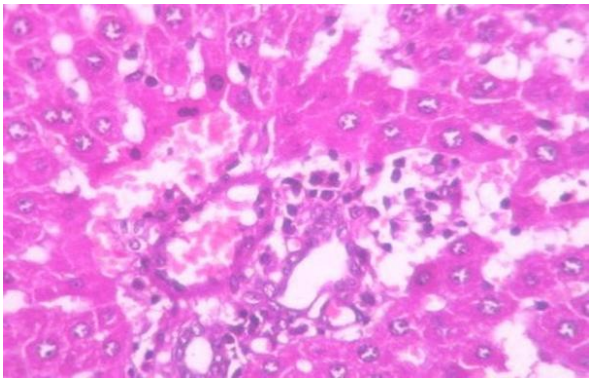

Figure: 2 Paracetamol treated animal group shows that hepatic cells damage And congestion of the liver.

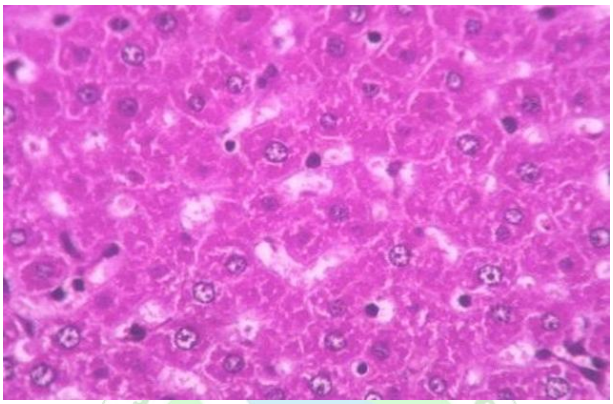

Figure 3: Hepatocytesin grouptreatedwith Standard(Silymarin200mg/kg)

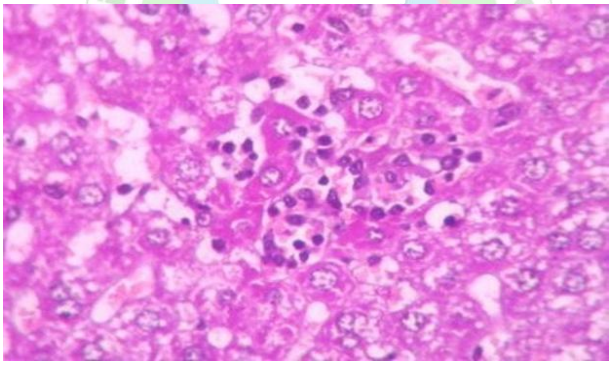

Figure 4: EEPD of 200 mg/kg shows that few regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells

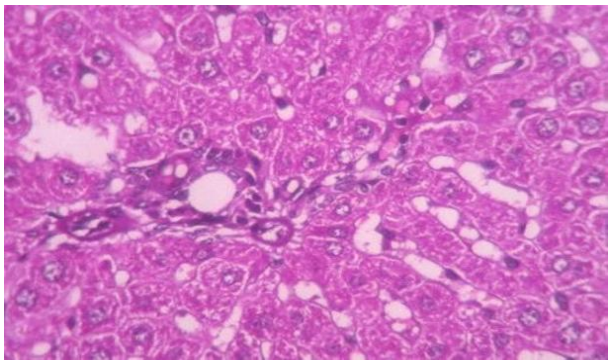

Figure 5: EEPD of 400mg/kg shows that few regenerative hepatocytes ,sinusoidal congestion and scattered mono nuclear inflammatory cells

5. CONCLUSION

In conclusion, the present study has demonstrated that ethanolic extract of *Pithecellobium dulce* has hepatoprotective effect in paracetamol induced hepatotoxicity. *Pithecellobium dulce* leaves ethanolic extract has showed dose dependent activities on liver weight and volume, various liver functional test Furthermore the better activities has has revealed by the EEPD at dose of 400mg/kg. Utilizing this model,*Pithecellobium dulce* ethanolic extract was showen to be effective in significantly lowering total triglycerides,AST,ALP and ALT levels; thus it can be used in the treatment and/or prevention of liver diseases.

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