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## Anti-Oxidant and hepatoprotective activities of ethanolic root extract of *Bauhinia variegata* Linn

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**Abstract**

To study the hepatoprotective activity of BVEE. *In-vitro* Antioxidant Studies was carried out by using Nitric oxide scavenging activity method and DPPH radical scavenging activity method. Albino wistar rats of either sex weighing 150-200g were divided into five groups. Liver injury was produced by CCL<sub>4</sub> 1 ml/kg/d dissolved in olive oil (1:1) orally. Silymarin (100mg/kg) orally was used as standard drug. Test groups received BVEE in the doses of 200,400 mg/kg/day orally along with CCL<sub>4</sub>. Treatment was given to all the groups daily for 5 days. The hepatoprotective effect of BVEE was evaluated by assessment of biochemical parameters AST, ALT, ALP and Level of total protein Histopathological examination of the liver was also done. BVEE (200mg/kg and 400 mg/kg) exhibited highly significant reduction (p<0.01) in AST, ALT, ALP, GGT and total protein. Histopathological examination of the liver suggested hepatoprotective effect of the extract by decreasing the extent of centrilobular necrosis, fatty changes and congestion of sinusoids when compared to carbon tetrachloride group. BVEE showed significant dose dependent protection against CCL<sub>4</sub> induced liver injury in rats.

**Keywords:** Carbon tetrachloride (CCL<sub>4</sub>), *Bauhinia variegata* ethanolic extract (BVEE), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase gamma-glutamyltransferase (ALP)

**1. Introduction**

Liver which is the key organ of metabolism and excretion is exposed to a variety of xenobiotics and therapeutic agents continuously. Thus the disorders associated with this organ are numerous and varied [1]. Liver diseases, such as jaundice, cirrhosis and fatty liver are very common worldwide [2]. Several environmental toxins and carcinogens are also converted into reactive intermediates during metabolism, resulting in tissue damage. Since the metabolic function of the liver is primarily responsible for detoxification of diverse therapeutic agents, toxins and carcinogens, drug-induced liver injury may manifest as acute hepatitis, cholestasis which may also lead to the development of cirrhosis [3]. There is increasing evidence that free radicals and reactive oxygen species play a crucial role in various steps that initiate and regulate the progression of liver diseases independently of the original agent [4]. Treatment options for common liver diseases such as drug induced hepatitis, fatty liver and chronic hepatitis are very few and only supportive. The effectiveness of agents available for the treatment of liver disease are inconsistent and have greater incidence of side-effects [5]. *Bauhinia variegata* commonly known as Kachnar is a medium-sized, deciduous tree, found throughout India. Its stem bark, flowers, flower buds, leaves and root are used in folklore medicine for the treatment of various problems of the gastrointestinal tract as carminative, antihelminthic and liver tonic. There are few reports regarding hepatoprotective activity of the plant. *Bauhinia variegata* has been reported to have Tannis, Total phenols, Flavonoids and other polyphenolic compounds in Root. Therefore the present study was framed to assess the hepatoprotective activity of the root of *Bauhinia variegata*.

**2. Material and Methods****2.1 Plant material and Preparation of Extracts**

The Plant specimen for the proposed study was collected from Nellore, Andhra Pradesh. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai (PARC/ 2010/ 628). The freshly collected Root of *Bauhinia variegata* Linn of this plant was chopped, shade dried and coarsely powdered. The powder was

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defatted with petroleum ether (60-80 °C) then successively extracted with ethanol (90% v/v) using soxhlet extractor. The ethanolic extracts were dried under reduced pressure using a rotary vacuum evaporator. The percentage yield was 3.912 % w/w for ethanol extract.

## 2.2 Chemicals

Carbon tetrachloride (CCl<sub>4</sub>) and Silymarin (Silybon) was obtained from nice chemicals and Microlabs respectively.

**IAEC approval** The Institutional Animal Ethics Committee (IAEC) approved the protocol of the study on Registration no. 1243/bc/08/ CPCSEA dated 9.02.2011. All animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) under the "Guidelines for Care and Use of Animals in Scientific Research".

## 2.3 Experimental Animals

Wistar albino rats of either sex (175-200g) were obtained from saastra Bioscience Research Laboratories in Saastra College of pharmaceutical education and research centre, Varigonda village, Totapalligudur Mandal, SPSR Nellore dist. Animals were housed in plastic cages at an ambient temperature (25±2 °C) and relative humidity of 45-55%. A 12:12 hr light- dark cycle was maintained during the experiments. They were fed with balanced rodent pellet diet from saastra Bioscience Research Laboratories, Nellore, and water *ad libitum* throughout the experimental period. Animals were acclimatized to their environment for at least one week before experimentation. The animals were randomly divided into different groups. Each animal was housed separately after recording its body weight and had kept separate marks for identifying the dose level, group and individual number.

## 2.4. In-vitro Antioxidant Studies

### 2.4.1. Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5mM) in standard phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations of BVEE (25-1000 µg/ml) and ascorbic acid as reference standard (5-1000 µg/ml) and the tubes were incubated at 25 °C for 5 hrs. [6, 7, 8, 9] Control was used without the test compounds, but with an equivalent amount of buffer was added in an identical manner. After 5 hrs, 0.5 ml of incubated solution was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm. All the determinations were performed in 6 replicates. Percentage inhibition of nitric oxide radical was calculated by using the formula,

Percentage inhibition (%) =

$$\frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}}$$

### 2.4.2. DPPH radical scavenging activity

The free radical scavenging activity was measured in terms

of hydrogen donating or radical scavenging ability using the stable radical DPPH. About 0.1mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of the different concentration (25-1000 µg/ml) of BVEE (25-1000 µg/ml), (5-1000 µg/ml) of standard Ascorbic acid and control (without the test compound, but with an equivalent amount of ethanol) in different test tubes. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC<sub>50</sub> value (50% of inhibitory concentration in µg/ml) of the crude extract and its fractions were compared with that of Ascorbic acid, which was used as the standard. Decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage inhibition of DPPH radical was calculated using the formula,

Percentage inhibition (%) =

$$\frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}}$$

## 2.5. Acute Toxicity Study

The toxic effect of the drug is evaluated and the LD<sub>50</sub>, ED<sub>50</sub> and the therapeutic index was determined for the drug under investigation. Acute toxicity study – up and down procedure – was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD) 423.<sup>[10]</sup> In this experiment three groups of albino Swiss mice n=6 were used. Animals were fasted overnight with water *ad libitum* and food was withheld for 3-4 hrs after oral administration of the BVEE of *Bauhinia variegata*. First group of animals was treated with starting dose of 1000 mg/kg body weight of BVEE orally. Second group of animals was treated with a maximum dose of 2000 mg/kg body weight of BVEE. The Control group was treated with normal saline. Animals were observed individually after dosing. Observation included mortality and gross behaviors e.g. body positions, locomotion, rearing, tremors were observed. The effect of BVEE on passivity, grip strength, pain response, stereotypy, righting reflex, and mortality were assessed. No abnormality in the gross behavioral studies also no mortality were noted. Based on these observations three different doses (200 and 400 mg/kg) were selected for the pharmacological studies.

## 2.6. Experimental design

Albino wistar rats of either sex weighing 175-200 g were divided into five groups, containing six animals each. Group I (control): Distilled water (1 ml/kg, p. o.) daily for 5 days and olive oil (1 ml/kg, s. c.) on 2<sup>nd</sup> and 3<sup>rd</sup> day of the treatment. Group II (CCl<sub>4</sub> control): Distilled water (1 ml/kg, p. o.) daily for 5 days and CCl<sub>4</sub>: olive oil (1:1, 2 ml/kg, s. c.) on 2<sup>nd</sup> day and 3<sup>rd</sup> day of the treatment. Group III (Standard): Silymarin (50 mg/kg, p. o.) daily for 5 days and CCl<sub>4</sub>: olive oil (1:1, 2 ml/kg, s. c.) on 2<sup>nd</sup> day and 3<sup>rd</sup> day, 30 min after administration of standard drug. Groups IV, V (Test): The ethanolic extracts of *Bauhinia variegata* root at doses of 200, 400 mg/ (kg p. o.) for 5 days and CCl<sub>4</sub>: olive oil (1:1, 2 ml/kg, s. c.) on 2<sup>nd</sup> day and 3<sup>rd</sup> day, 30 min after administration of ethanol extracts of *Bauhinia variegata* root. All the groups were given the above treatment daily for 5 days<sup>[11]</sup>.

## 2.7. Assessment of hepatoprotective activity

On 6th day the animals were sacrificed under ether anaesthesia and blood was collected by direct cardiac puncture [12]. Liver was dissected out and 500 mg of liver tissue was taken for determination of the levels of antioxidant Enzymes. Rest of the liver was kept in 10% formalin for histopathological examination.

## 2.8. Determination of serum biochemical parameters

The collected blood was centrifuged at 5000 rpm for 10 minutes and serum was separated. Serum was analyzed for biochemical parameters like Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) gamma-glutamyltransferase (GGT) and Level of Total protein [12].

## 2.9. Histopathological examination

Liver tissue was fixed in 10% formalin, dehydrated in graded ethanol and embedded in paraffin wax. Sections were prepared and stained with hematoxylin and eosin [13]. The slides thus prepared were observed for histopathological features under the microscope.

## 2.10. Statistical Analysis

The results were expressed as Mean  $\pm$  Standard Error of Mean (SEM). [14, 15] The groups were compared by one way analysis of variance (ANOVA) followed by post hoc "Dunnett's Multiple comparison test" to analyze statistical significance.  $P < 0.05$  was considered to be significant.

## 3. Results and Discussion

### 3.1. Antioxidant

Several concentrations ranging from 25-1000  $\mu\text{g/ml}$  of the ethanolic extract of *Bauhinia variegata* Linn root were tested for their antioxidant activity in different in-vitro models. It was observed that free radicals scavenging property of the test samples were found to increase in a concentration dependent manner in all the models.

### 3.2. Nitric oxide scavenging activity

The scavenging of nitric oxide by BVEE and ascorbic acid (reference standard) were concentration dependent.

The  $\text{IC}_{50}$  value of BVEE and ascorbic acid were found to be 76.1  $\mu\text{g/ml}$ , 75.2  $\mu\text{g/ml}$  respectively. The values were shown in (Table-1, Figure-1).

**Table 1:** Nitric oxide radical scavenging property of, BVEE and Ascorbic acid

S. No	Concentration ( $\mu\text{g/ml}$ )	% inhibition	
		BVEE	STANDARD
1	100	47.79 $\pm$ 0.8346**	52.15 $\pm$ 0.3124**
2	200	52.11 $\pm$ 0.9670**	62.52 $\pm$ 0.1832**
3	400	62.76 $\pm$ 0.3264**	74.35 $\pm$ 0.0647*
4	600	74.33 $\pm$ 0.4494**	82.00 $\pm$ 0.0520**
5	800	82.43 $\pm$ 0.4452**	86.34 $\pm$ 0.2050**
6	1000	88.76 $\pm$ 0.3715*	91.36 $\pm$ 0.0728**
7	$\text{IC}_{50}$	76.1 $\mu\text{g/ml}$	75.2 $\mu\text{g/ml}$

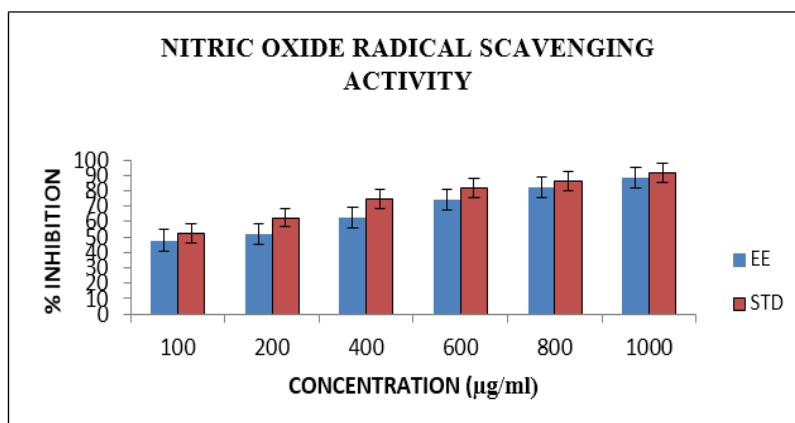
BVEE - *Bauhinia variegata* Ethanol extract

Values are mean  $\pm$  SEM of 6 parallel measurement.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6)

All the values are significant \*\* $P < 0.01$  when compared against control.

All the values are significant \* $P < 0.01$  when compared against standard



**Fig 1:** Nitric oxide radical scavenging property of, EE and Ascorbic acid

BVEE - *Bauhinia variegata* Ethanol Extract

STD - Standard

Values are mean  $\pm$  SEM of 6 parallel measurement.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6)

All the values are significant \*\* $P < 0.01$  when compared against control.

All the values are significant \* $P < 0.01$  when compared against standard

**3.3. Inhibition of DPPH radical**

The potential decrease in the concentration of DPPH radical due to the scavenging ability of EE was found to be about  $45.72 \pm 0.5340$ ,  $55.21 \pm 0.5541$ ,  $68.44 \pm 0.3955$ ,  $74.29 \pm 0.5411$

$79.50 \pm 0.5068$  and  $86.47 \pm 0.3366$  respectively at higher doses with the  $IC_{50}$  value being  $77 \mu\text{g/ml}$  respectively. The activity was increasing dose dependently. The results were listed in (Table –2, Figure-2).

**Table 2:** DPPH free radical scavenging property of, BVEE and Ascorbic acid

S.No	Concentration	% inhibition	
	( $\mu\text{g/ml}$ )	BVEE	STANDARD
1	100	$45.72 \pm 0.5340^{**}$	$55.33 \pm 0.0637^{**}$
2	200	$55.21 \pm 0.5541^{**}$	$67.68 \pm 0.0716^{**}$
3	400	$68.44 \pm 0.3955^{**}$	$74.15 \pm 0.0388^*$
4	600	$74.29 \pm 0.5411^{**}$	$78.50 \pm 0.0857^{**}$
5	800	$79.50 \pm 0.5068^{**}$	$84.37 \pm 0.2223^{**}$
6	1000	$86.47 \pm 0.3366^*$	$88.98 \pm 0.0309^{**}$
7	$IC_{50}$	$77.0 \mu\text{g/ml}$	$75.0 \mu\text{g/ml}$

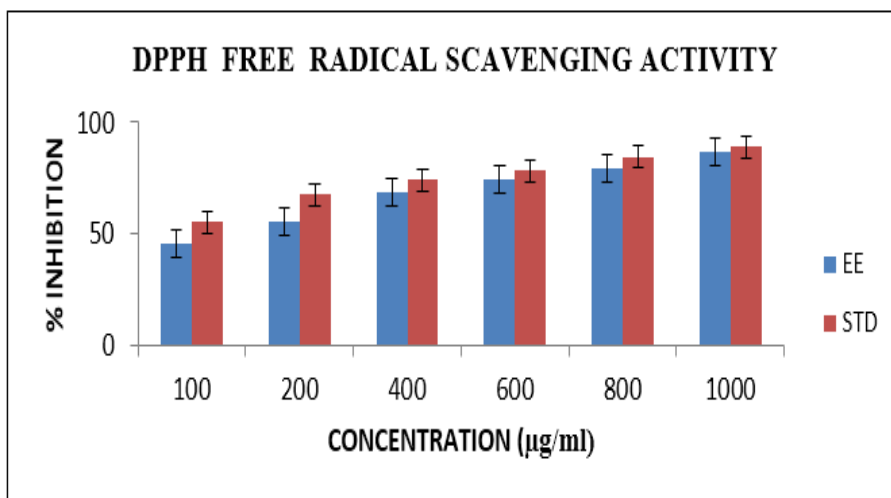
BVEE - Bauhinia variegata Ethanol extract

Values are mean  $\pm$  SEM of 6 parallel measurement.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet’s ‘t’ test (n=6)

All the values are significant  $^{**}P < 0.01$  when compared against control.

All the values are significant  $^*P < 0.01$  when compared against standard



**Fig 2:** DPPH free radical scavenging property of, BVEE and Ascorbic acid

BVEE - Bauhinia variegata Ethanol Extract

STD - Standard

Values are mean  $\pm$  SEM of 6 parallel measurement.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet’s ‘t’ test (n=6)

All the values are significant  $^{**}P < 0.01$  when compared against control.

All the values are significant  $^*P < 0.01$  when compared against standard

**3.4. Acute toxicity study**

Table-3 predicted that BVEE does not show any marked sign of toxicity and mortality upto 1000 mg/kg body weight orally

in mice for 24 hrs and was considered as safe for pharmacological activity. According to OECD – 423 guidelines, the  $LD_{50}$  dose was fixed as 200 and 400 mg/kg.

**Table 3:** Acute Toxicity Study

Parameters	Group I	Group II	Group III
Alertness	Normal	Normal	Normal
Passive/acute	Positive	Positive	Positive
Restlessness	Absent	Absent	Absent
Aggressiveness	Absent	Absent	Absent
Touch response	Normal	Normal	Normal
Tremors	Absent	Absent	Absent
Pain response	Normal	Normal	Normal
Convulsion	Absent	Absent	Absent
Righting reflex	Positive	Positive	Positive
Gripping strength	Present	Present	Present
Pinna reflex	Present	Present	Present
Corneal reflex	Normal	Normal	Normal
Writhing	Absent	Absent	Absent
Pupils	Normal	Normal	Normal
Urination	Normal	Normal	Normal
Salivation	Absent	Absent	Absent
Skin colour	Normal	Normal	Normal
Lacrimation	Absent	Absent	Absent
Respiration	Normal	Normal	Normal

### 3.5. Hepatoprotective activity

The hepatoprotective activity of ethno fraction shown in Figure 4, 5, 6, 7, 8, all the marker enzyme AST, ALT, ALP, GGT registered enhanced activity in group II rats as compared to group I. In group IV and group V the levels of

these enzymes were found retrieving towards normalcy. The total protein content of the serum and liver was lesser in group II animals and it attained an almost normal value group IV and V. Shown in Table 4.

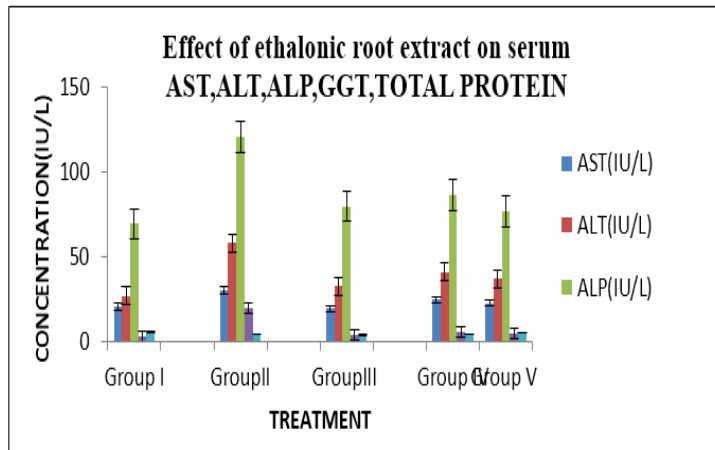
**Table 4:** Effect of Ethanolic extract of *Bauhinia variegata* Linn root on Biochemical parameters in serum

S. No	Treatment	Bio chemical parameters				
		AST(IU/L)	ALT(IU/L)	ALP(IU/L)	GGT(IU/L)	Total protein(mg/dl)
1	Group I(water: Olive oil)	20.43 ± 0.051*	26.80±0.07*	69.21±0.05*	3.11 ± 0.04**	5.49 ±0.08**
2	Group II(Water:CCL <sub>4</sub> : Olive oil)	30.21 ±0.048*	58.13±0.23*	120.41±0.15*	19.25±0.13*	4.42 ±0.18*
3	Group III (Silymarin:CCL <sub>4</sub> : Olive oil)	19.36 ± 0.10**	32.52±0.08*	79.59±0.69**	3.46 ± 0.07*	4.01 ±0.05**
4	Group IV(EEBV- 200 mg:CCL <sub>4</sub> :Olive oil)	24.45 ± 0.10*	40.98±0.09*	86.2±0.5*	5.21± 0.9**	4.33 ± 0.12*
5	Group V (EEBV- 400 mg:CCL <sub>4</sub> : Olive oil)	22.57± 0.03*	37.01±0.08**	76.51±0.02*	4.55 ± 0.06*	5.05 ± 0.11*

Values are mean ± SEM of 6 parallel measurement.

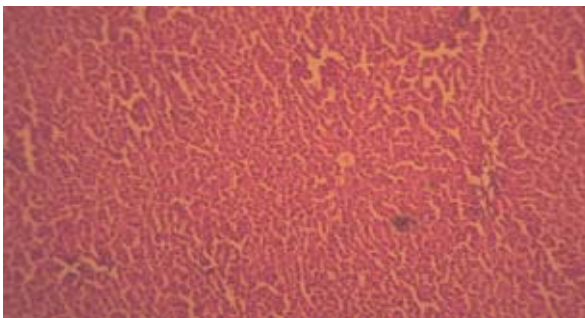
Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6)

All the values are significant  $**P < 0.01$  when compared against control.  
 All the values are significant  $^aP < 0.01$  when compared against standard

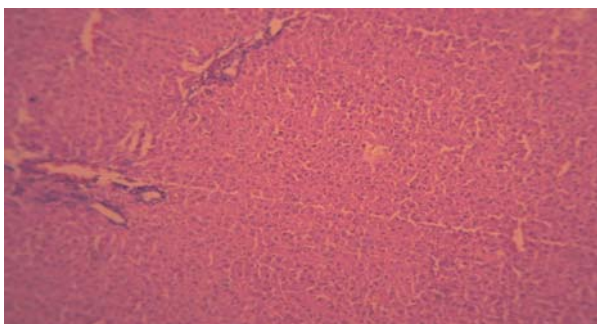


**Fig 3:** Effect of Ethanolic extract of *Bauhinia variegata* Linn root on Biochemical parameters in serum  
 Values are mean  $\pm$  SEM of 6 parallel measurement.  
 Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6)  
 All the values are significant  $**P < 0.01$  when compared against control.  
 All the values are significant  $^aP < 0.01$  when compared against standard

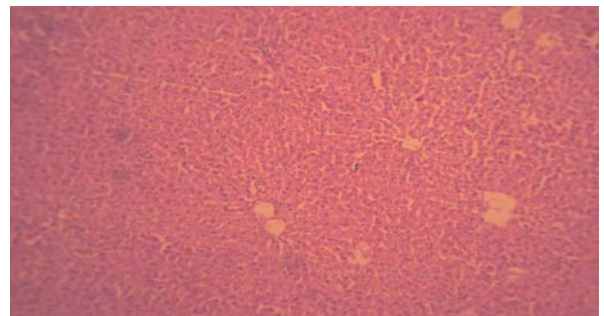
### 3.6. Histopathology



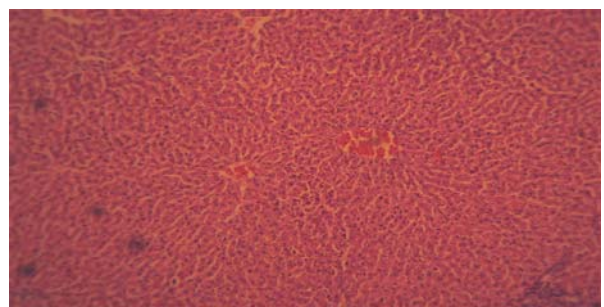
**Fig 4:** Normal Liver normal liver with normal hepatocytes



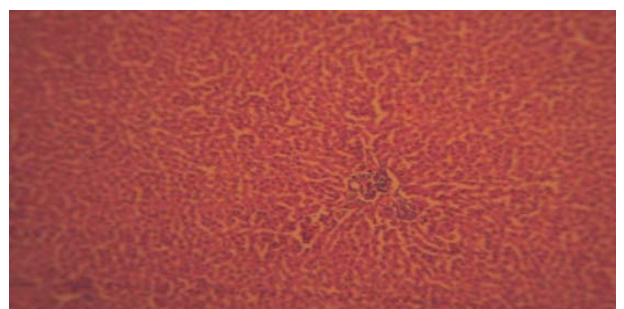
**Fig 5:** Inflamed Liver liver with hepatic inflammation



**Fig 6:** Liver treated with Standard (SILYMARIN 50 mg/kg) rapid recovery of inflamed cells



**Fig 7:** Liver treated with ethanolic (200 mg/kg) normal liver with normal hepatocyte



**Fig 8:** Liver treated with ethanolic extract (400 mg/kg) Section shows rapid recovery of inflamed cells

#### 4. Summary and Conclusion

*In vitro* antioxidant study was screened for BVEE using nitric oxide and DPPH method and the IC<sub>50</sub> values were found to be 76.1 µg/ml, 77 µg/ml, all these results revealed that the BVEE exhibits good antioxidant activity. Acute toxicity study was carried out for the extract upto 2000 mg/kg in mice. No mortality was observed in all the groups. Ethanolic extracts were subjected to hepatoprotective activity by CCL<sub>4</sub> induced method. In conclusion, BVEE showed dose dependent protection against CCL<sub>4</sub> induced acute liver injury, maximal effect was observed in the dose of 200 and 400 mg/kg/d. Further studies for longer duration are required to find out the protective potential of *Bauhinia variegata* against chronic liver injury.

#### 5. Acknowledgement

I would like to thank Dr. Srinivasa Rao, Chairman- saastra Bioscience Research Laboratories in Saastra College of pharmaceutical education and research centre, Varigonda village, Totapalligudur Mandal, SPSR Nellore dist and Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai for their support in this research work.

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