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## Phytochemical and Pharmacological Evaluation of *Vigna radiata* Stem Bark Extracts for Its Hepatoprotective Activity



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**Shrinivas Sarje<sup>1\*</sup>, Dinesh Shinde<sup>1</sup>, Sagar Dukare<sup>1</sup>,  
Tukai Kulkarni<sup>1</sup>, Danish Farooqui<sup>1</sup>**

<sup>1</sup>Department of Pharmacology, Nanded Pharmacy  
College, Nanded, Maharashtra, India.

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**Keywords:** *Vigna radiata* (v.r.), Ethyl acetate (EA.) and Ethanollic (Eth.) extract, Phytochemical screening, Antioxidant activity, Hepatoprotective activity

### ABSTRACT

Medicinal plants have always been the principle sources of medicine worldwide. Human beings have used those as medicine from the very beginning of time. India sustains a very rich traditional medicinal plant wealth and inherits unique plant and animal communities. The objective of the study was undertaken to investigate the hepatoprotective activity of Ethyl acetate & Ethanollic extract of *Vigna radiata* stem bark which is an important medicinal plant in Indian folk. In the present study, the stem bark of *Vigna radiata* which belongs to family Fabaceae were chosen because various parts of the plant possess several bioactivities & is used in traditional medicinal system. The antioxidant activity of the extracts was done by using DPPH method. The results showed that ethyl acetate extract and ethanollic extract at 125µg/ml concentration showed the significant antioxidant effect as compared with ascorbic acid as standard. The *In-vivo* hepatoprotective activity of VREA and VR Eth extract were estimated by using carbon tetrachloride induced hepatotoxicity model. The degree of protection was estimated by measuring levels of biochemical markers like SGOT, SGPT, Total Bilirubin and Total Protein. The histopathological study was also carried out and compared with carbon tetrachloride treated group. **Results:** The interpretations of results were done by subjecting the data to statistical analysis using mean  $\pm$  S.E.M. The VREth extract at dose of 200mg/kg shows promising Hepatoprotective effect than VREA extract. The VREth extract of plant shows significant antioxidant activity at the concentration of 125µg/ml than VREA extract at the concentration of 125µg/ml. **Conclusion:** The results suggest that the stems of *Vigna radiata* stem bark possess potential antioxidant and hepatoprotective activity.

## INTRODUCTION

Liver is vital organ which plays important role in metabolism, storage, detoxification, synthesis and regulation of various body processes. Liver is largest and heaviest gland of the body weighing about 1.4 kg. In the average adult it is second largest organ of the body located in the diaphragm and occupies most of right hypochondrium and part of epigastrium of the abdomen.

The causes of liver disease are viruses, excessive drug therapy, environmental pollution, alcoholic intoxication etc. Liver receive blood supply from hepatic artery (20%) and portal circulation (80%) up to 20-25% of total cardiac output. Toxin, infectious agent medication and serum inflammatory mediator enter into the liver through the blood, may result in diverse range of disease processes, causing the loss of normal histological architecture reduced cell mass and loss of blood flow this may lead to decline liver function.

No effective hepatoprotective therapy is available. Conventional medicines used in liver treatments are often insufficient. Many chronic irreversible and acute hepatic disorders culminate in ultimately death due to lack of adequate remedies in modern medicines. It is therefore necessary to search for alternative drugs for treatment of liver diseases to replace currently used drugs of controversial efficacy and safety. In this condition there is greater demand of herbal formulation to treat liver diseases in developed as well as in developing countries for primary health care. Herbal medicines have minimum side effects, good biological activity, medicinal property, large safety margin and minimal cost. Modern drug are little to offer for curing of hepatic disorder, whereas most important representative of phytoconstituents used to treat liver disorder depend on the region are *Silyrium* (*Silybum marianum*) and catechin (*Anacardium occidentale*) in Europe, glycyrrhizin (*Glycyrrhiza glabra*) in Japan and chizandrins (*Schizandra chinesis*) in China.

Mung bean (*Vigna radiata* L.) is a food source of vitamins, minerals and essential amino acids and has a high nutrient value comparable to that of soyabean (*Glycine max* L.) and kidney bean (*Phaseolus vulgaris* L.). Mung bean is traditionally known as a functional food, and its functional components have been identified over decades with the development of analytical techniques. In recent years, the physiological functionality of mung bean has received attention, particularly with respect to the content of anti-angiotensin-I converting enzyme and to antitumor, antioxidant, anti-diabetic, and anti-melanocyte effects.

## MATERIAL AND METHODS:

### 1. Collection, identification and authentication of plant material

The fresh Leaf of *Solanum nigrum* L. was collected from local region of Nanded i.e. from local market and authenticated by **Dr. Shirang S. Bodke**, Head, Department of Botany & Horticulture, Yeshwant Mahavidyalaya, Nanded.

### 2. Processing of crude drug:

Shade drying of the leaves up to complete removal of moisture was done. (Took around 15 days) Dried leaves were powdered by hand crushing and sieved through sieve number 30 #.

### 3. Preparation of Extracts:

Three extracts of whole plant of *vigna radiata* was prepared.

- Petroleum ether extract by continuous hot extraction method.
- Ethyl acetate extract by continuous hot extraction method.
- Ethanol extract by continuous hot extraction method.

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$\text{(\% ) yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

#### 1. Preparation of Petroleum ether extract:

Dried powdered plant was successfully extracted with petroleum ether by Soxhlet extractor apparatus according to the standard method till colorless solution was observed in siphon tube. 300 gm of the powdered plant and 1000 ml petroleum ether was used for extraction. After completion of extraction, extract was cooled and dried. The extract was stored in airtight container and kept in desiccator till use. Percentage yield of extract was calculated.

#### 2. Preparation of Ethyl acetate extract:

Dried powdered plant was successfully extracted with Ethyl acetate by Soxhlet extractor apparatus according to the standard method till colorless solution was observed in siphon

tube. 250 gm of the powdered plant and 1000 ml chloroform was used for extraction. After completion of extraction, extract was cooled and dried. The extract was stored in airtight container and kept in desiccator till use. Percentage yield of extract was calculated.

### 3. Preparation of Ethanol extract:

Dried powdered plant was successfully extracted with ethanol by Soxhlet extractor apparatus according to the standard method till colorless solution was observed in siphon tube. 250 gm of the powdered plant and 1000 ml ethanolic was used for extraction. After completion of extraction, extract was cooled and dried. The extract was stored in airtight container and kept in desiccator till use.

### Phytochemical Evaluation:

#### 1. Total Phenolic Content

Total Phenolic Content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1ml) of extract or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10 ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% NaHCO<sub>3</sub> solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 746 nm with an UV-Visible spectrophotometer.

#### 2. Total Flavonoids Content

Total Flavonoid Content was measured by the aluminum trichloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% NaNO<sub>2</sub>, after five minutes 0.3 ml 10 % AlCl<sub>3</sub> was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 258 nm.

### 3. *IN VITRO* ANTI-OXIDANT ACTIVITY

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the

cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions.

## 2, 2 Diphenyl- 1 picryl-hydrazyl radical scavenging (DPPH) Activity-

### Principle:

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 1, 1-diphenyl-2-picryl-hydrazyl. *Vani T, Rajani, M et al., (1997)*

### Reagents Required:

- 1) DPPH
- 2) Pure Methanol

### Preparation of samples and standard solutions:

Accurately weighed 10 mg of Ethyl acetate and Methanolic extracts and the standard ascorbic acid and dissolved separately in 10 ml of phosphate buffered saline. These solutions were serially diluted with methanol to obtain the lower dilutions.

### Procedure:

The reaction mixture (3.0 ml) consists of 1 ml of 0.1mM DPPH solution in methanol was mixed with 1 ml of drug solution and 1.0 ml of methanol. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample was served as control.

The percentage of inhibition can be calculated using the formula:

$$(\%) \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where,

A<sub>control</sub>: Absorbance of control.

A<sub>test</sub>: Absorbance of test.

**Animal used:**

For the study, Wistar rats of either sex, of weight 150-200gm were selected.

**Test group:**

For the study, six groups of animals were made. Each group having six rats.

**Route of administration:** Oral route administration.

**Housing Condition:**

Animals were housed six groups in separate cages under controlled conditions of temperature ( $22 \pm 2^{\circ}\text{C}$ ). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were divided randomly into six treatment groups; each group consisting of three mice.

**Hepatoprotective Activity:**

Hepatoprotective study of whole plant of *Vigna radiata* was carried out using  $\text{CCl}_4$  induced hepatotoxicity in rats.

**IAEC Approval**

Wistar rats of either sex weighing 150 to 200 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature ( $24 \pm 2^{\circ}\text{C}$ ) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

## 1. Carbon tetrachloride induced hepatotoxicity

### Experimental design:

Male and female Wistar rats with weighing 200–250 g will be used. Animal will be randomly divided into 11 groups containing six animals in each group.

1. **Group I** (Negative control) Animals will receive Dimethyl Sulfoxide/Saline water orally.
2. **Group II** (Positive control) Animal will receive Carbon Tetrachloride.
3. **Group III** Animals will receive standard drug (Silymarin).
4. **Group IV** Test group 1 (Ethyl acetate extract, dose 100 mg/kg) for seven days.
5. **Group V** Test group 2 (Ethyl acetate extract, dose 200 mg/kg) for seven days.
6. **Group VI** Test group 3 (Ethanol extract, dose 100 mg/kg) for seven days.
7. **Group VII** Test group 4 (Ethanol extract, dose 200 mg/kg) for seven days.

### Procedure:

- Albino Wistar rats of either sex [200–250 g] were used in the study.
- Animals were randomly divided into seven groups containing six in each six each namely.
- Food was withdrawn 16 hrs before administration to enhance acute liver toxicity.
- **Group II, III, IV, V, VI and VII** were treated with **CCl<sub>4</sub> was administered (1 ml/kg) diluted in olive oil (1:1) was administered on 7<sup>th</sup> day after 1hr** of test sample treatment and scarified 24 hours after administration of CCl<sub>4</sub>.
- On 7<sup>th</sup> day the blood was collected by carotid artery or retro orbital under mild ether anesthesia, serum was collected by allowing the blood samples to coagulate for 30 min at 37°C followed by centrifugation (3000 rpm for 15 min) and biochemical parameters like Serum Glutamate Pyruvate Transminase (SGPT/ALT), Serum Glutamate Oxaloacetate Transminase (SGOT/AST), Total bilirubin and Total protein were estimated in the blood serum using Autoanalyser.

- Animals were sacrificed by overdose of diethyl ether (inhalation anesthesia) and livers from all animals were removed, washed, collected and preserved in 10% formalin for histopathological studies.

#### **Evaluation procedure for biochemical parameter**

The blood was collected by carotid artery or retro orbital puncture from the ether anesthetized rats. The blood was allowed to clot and then centrifuged at 3000 rpm for 15 min. The haemolysed free serum sample was used for determination of biochemical parameters. The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the kit (Autoanalyser).

Evaluation was carried out by estimating parameters such as Total bilirubin, Total protein, SGPT, SGOT by using enzymatic kit.

#### **Statistical Analysis**

The data were expressed as mean + standard of mean (SEM). Statistical analysis were performed by one way analysis of variance (ANOVA).

#### **RESULTS:**

##### **Phytochemical Test of *Vigna radiata* stem bark extracts**

**Table No. 1: Observations for Phytochemical qualitative analysis**

Test for	Petroleum Ether	Ethyl acetate	Ethanol
<b>Carbohydrate</b>			
a) Molisch's test	+	+	+
b) Barfoed's test	+	+	+
c) Fehling's test	+	+	+
d) Benedict's test	+	+	+
<b>Proteins</b>			
a) Biuret test	+	+	+
b) Millons test	-	+	+
<b>Alkaloids</b>			
a) Dragendorff's test	-	+	+
b) Mayer's test	+	-	-
c) Wagner's test	-	-	-
<b>Glycosides</b>			
a) Borntrager's test	-	-	+
b) Modified Borntrager's test	+	+	+
<b>Flavonoids</b>			
a) Shinoda test	-	+	+
b) Zinc hydrochloride test	-	-	+
c) Alkaline reagent test	-	+	+
<b>Tannins</b>			
a) Lead Acetate Test	-	-	-
b) FeCl <sub>3</sub> test	-	-	-
<b>Amino acids</b>			
a) Million's test	-	+	+
b) Ninhydrine test	+	+	+

(+); Present, (-); Absent

Above observation table shows the presence of phytoconstituents in the extracts. It reveals all two (i.e. Ethyl acetate and Ethanolic) extracts contains carbohydrates, glycosides, steroids, proteins, tannins and phenols, alkaloids, flavonoids.

#### TLC Fingerprinting:

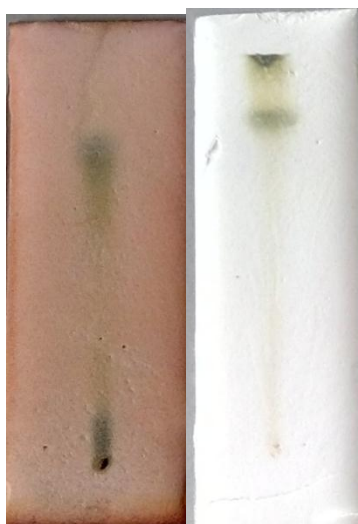


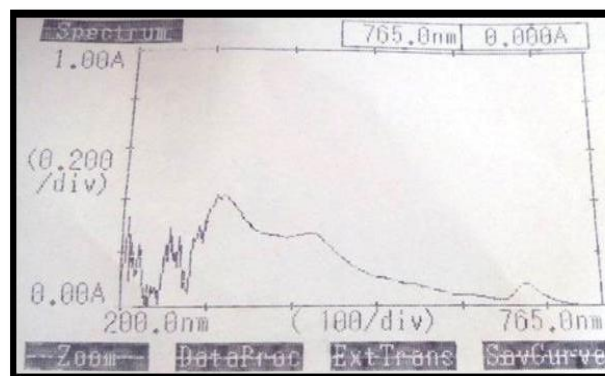
Figure No. 1: TLC of Ethyl acetate and ethanolic extracts

Table No. 2: Results of TLC profile of extracts

Pigment/solvent band	R <sub>f</sub> Value
Pheophytin - a (green)	0.5
Chlorophyll <i>a</i> (blue green)	0.37
Chlorophyll <i>b</i> (Green)	0.3
Xanthophylls (yellow)	1.1

Pigment/solvent band	R <sub>f</sub> Value
Pheophytin - a (green)	0.57
Chlorophyll <i>a</i> (blue green)	0.61
Xanthophylls (yellow)	0.5

**Total Phenolic Content:**



**Figure No. 2: Amax Determination of Gallic acid**

**Table No. 3: Total phenolic content of standard Gallic acid**

Sr. No.	Conc. µg/ml	Absorbance
1	20	0.067
2	40	0.144
3	60	0.201
4	80	0.256
5	100	0.331



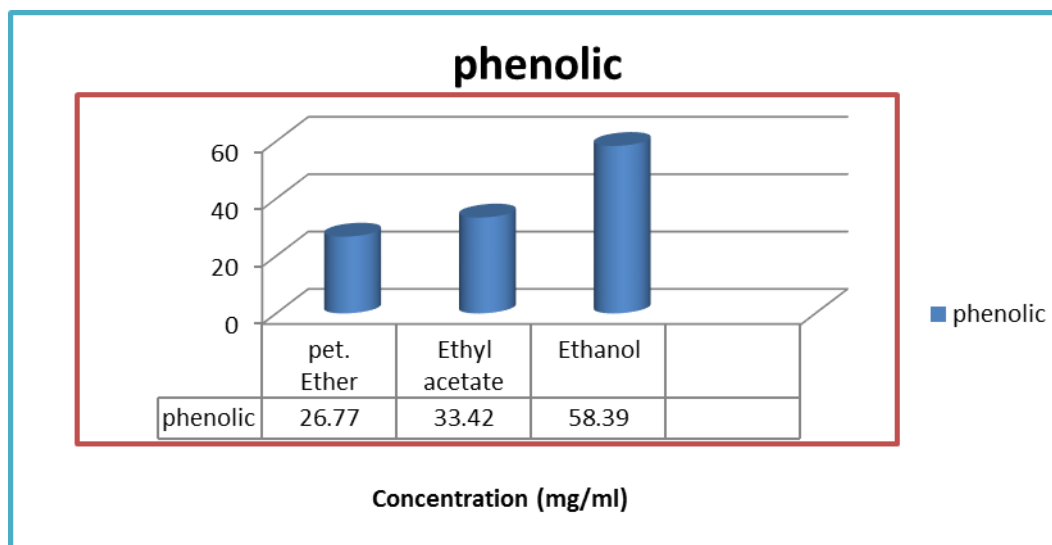
**Figure No. 3: Calibration Curve of Gallic acid**

**Table No. 4: Total phenolic content of *Vigna radiata* stem bark extracts**

Sr. No.	Conc. µg/ml	Extracts	Absorbance	Phenolic content (mg GAE/g DW)
1	100	Petroleum ether	0.090	26.77 ± 0.18
2	100	Ethyl acetate	0.107	33.42 ± 0.37
3	100	Ethanol	0.196	58.39 ± 0.21

(N=3) Note: GAE/g DW denotes Gallic Acid Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, Ethyl acetate and Ethanol have Phenolic content as 26.77 (mg GAE/g DW), 33.42 (mg GAE/g DW), 58.39 (mg GAE/g DW) respectively. Ethanol extract shows more phenolic content than Petroleum ether and Aqueous as per comparative evaluation of phenolic content of extracts.



**Chart No. 1: Effect of Phenolic content of extracts**

The concentration absorbance calibration curve for sequentially and separately prepared stock solution of standards of Gallic acid solution was taken. The absorbance measured at 765 nm for 20, 40, 60, 80, 100 µg/ml concentration Gallic acid solution are in a range of 0.067 to 0.331 within the range of concentrations, the calibration curve of Gallic acid has clearly exhibited linearity. Above table indicate that the Ethanolic extract contain more phenolic content (58.39 mg GAE/g DW) than Ethyl acetate and Petroleum ether extract (33.42 mg GAE/g DW, 26.77 mg GAE/g DW) respectively equivalent to Gallic acid.

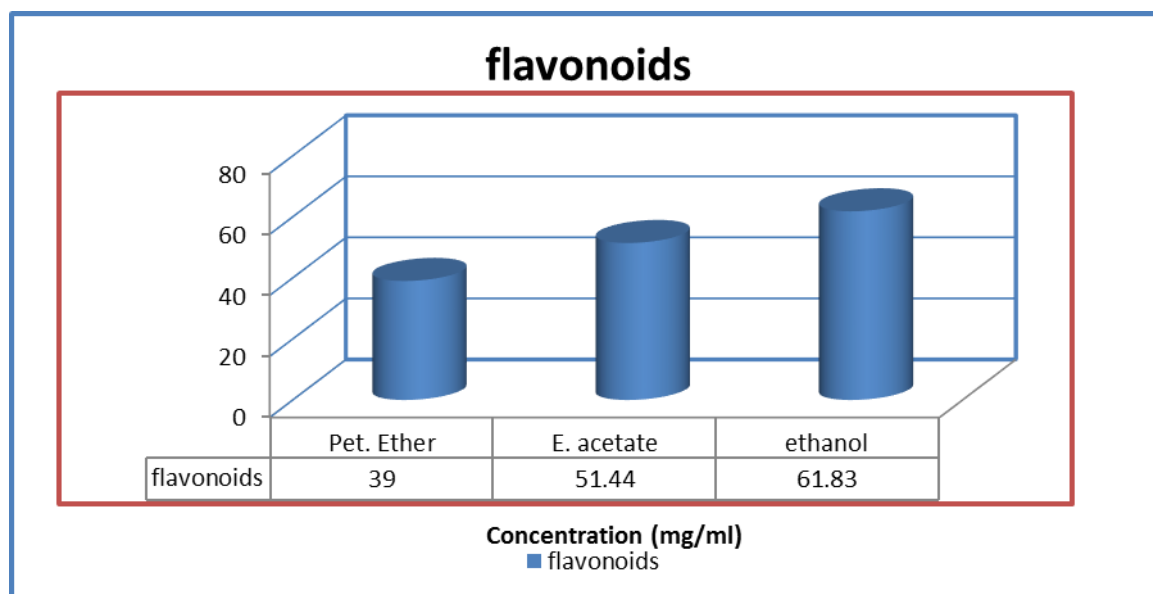
### Total Flavonoid Content

**Table No. 5: Total flavonoid content of *Vigna radiata* stems extracts**

Sr. No.	Conc. µg/ml	Extracts	Flavonoid content(mg Ru/g DW)
1	100	Petroleum ether	39.00 ± 0.19
2	100	Ethyl acetate	51.44 ± 0.16
3	100	Ethanol	61.83 ± 0.17

(N=3) Note: Ru/g DW denotes Rutin Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, Aqueous and Ethanol have Flavonoid content as 39.00 (mg Ru/g DW), 51.44 (mg Ru/g DW), 61.83 (mg Ru/g DW) respectively. Ethanol extract shows more Flavonoid content than Petroleum ether and Aqueous as per comparative evaluation of Flavonoid content of extracts.



**Chart No. 2: Effect of Flavonoid content of extracts**

The calibration curve for sequentially and independently prepared stock solution of rutin that depicts the concentration of rutin against the absorbance. The absorbance values increased proportionally upon increasing the concentration of rutin from 20 µg/ml to 100 µg/ml. Above table indicates that the Ethanol extract contain more flavonoids (61.83 mg Ru/g DW) of extract than Aqueous and Petroleum ether extract (51.44 mg Ru/g DW and 39.00 mg Ru/g DW) respectively equivalent to rutin.

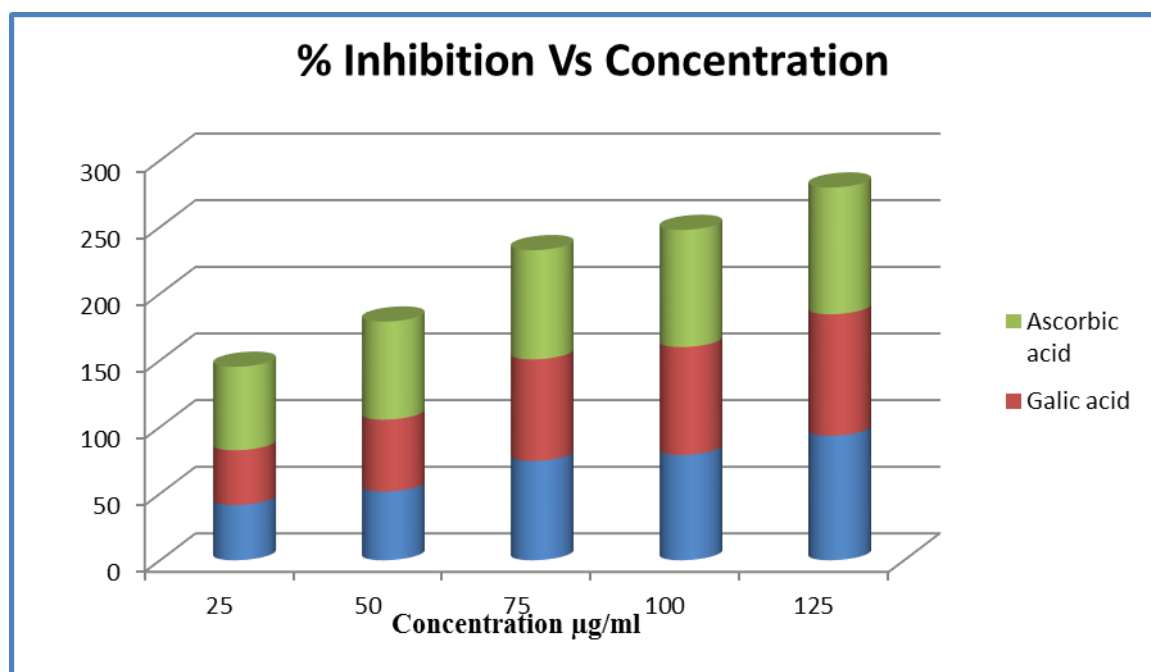
### Total Antioxidant Activity

**Table No. 6: Total Antioxidant Content of Standard**

Sr. No.	Conc. µg/ml	Absorbance of Ascorbic acid	Absorbance of Gallic acid	Absorbance of Rutin
1	25	0.182 ± 0.0008	0.287 ± 0.0013	0.285 ± 0.0013
2	50	0.129 ± 0.0012	0.224 ± 0.0004	0.236 ± 0.0011
3	75	0.088 ± 0.0019	0.116 ± 0.0009	0.124 ± 0.0013
4	100	0.059 ± 0.0015	0.093 ± 0.0006	0.102 ± 0.0003
5	125	0.024 ± 0.0009	0.043 ± 0.0010	0.032 ± 0.0003

**Table No. 7: % inhibition of Standards:**

Sr. No.	Conc. µg/ml	Ascorbic acid % inhibition	Gallic acid % inhibition	Rutin % inhibition
1	25	62.62 ± 0.23	41.06 ± 0.33	41.47 ± 0.19
2	50	73.51 ± 0.22	54.00 ± 0.26	51.54 ± 0.17
3	75	81.93 ± 0.21	76.18 ± 0.27	74.53 ± 0.25
4	100	87.88 ± 0.04	80.90 ± 0.27	79.05 ± 0.31
5	125	95.07 ± 0.25	91.17 ± 0.28	93.42 ± 0.31



**Chart No. 3: Effect of % Inhibitions of Standard**

**Table No. 8: Total Antioxidant Content of *Solanum nigrum* leaves Extracts**

Sr. No.	Conc. µg/ml	Absorbance of Pet. Ether Extract	Absorbance of Ethyl acetate Extract	Absorbance of Ethanolic Extract
1	25	0.219 ± 0.0049	0.208 ± 0.0011	0.197 ± 0.0025
2	50	0.197 ± 0.0035	0.187 ± 0.0008	0.144 ± 0.0034
3	75	0.144 ± 0.0025	0.112 ± 0.0026	0.095 ± 0.0008
4	100	0.121 ± 0.0009	0.088 ± 0.0012	0.062 ± 0.0017
5	125	0.111 ± 0.0007	0.055 ± 0.0015	0.041 ± 0.0019

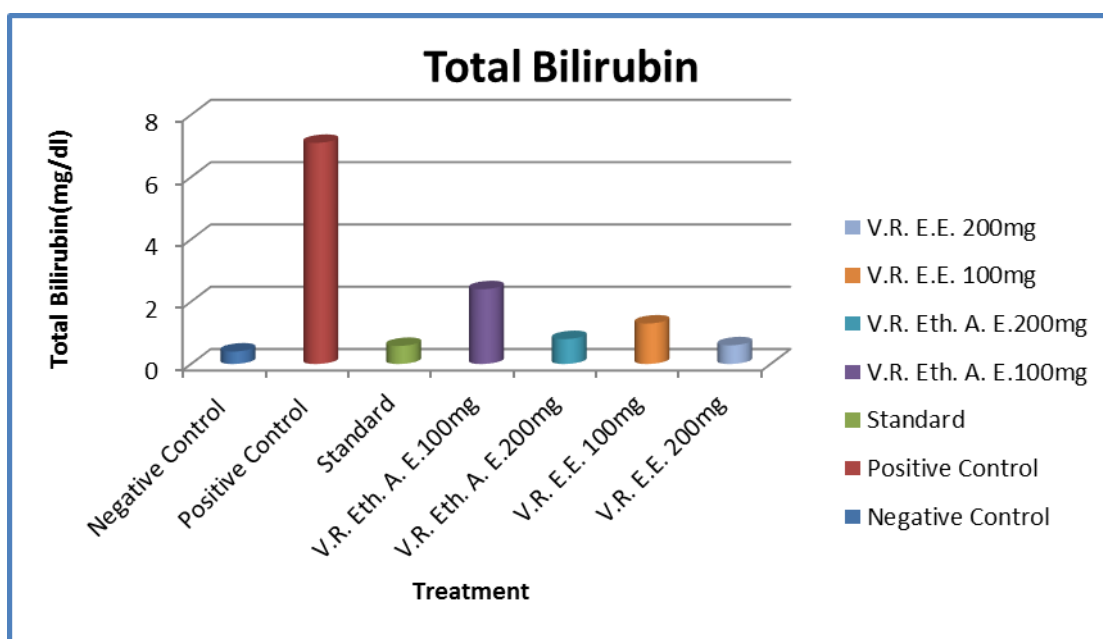
**Table No. 9: Comparative DPPH Scavenging assay method of *Solanum nigrum* leaves powder (Pet. Ether, Ethyl acetate and Methanolic) leaves extracts**

Sr. No.	Conc. µg/ml	Petroleum ether % inhibition	Ethyl acetate % inhibition	Ethanol % inhibition	Ascorbic acid % inhibition
1	25	53.62 ± 0.19	56.11 ± 0.35	58.34 ± 0.35	62.62 ± 0.23
2	50	60.93 ± 0.36	59.89 ± 0.32	69.84 ± 0.06	73.51 ± 0.22
3	75	68.84 ± 0.27	76.31 ± 0.36	79.08 ± 0.25	81.93 ± 0.21
4	100	74.00 ± 0.35	82.88 ± 0.34	86.26 ± 0.31	87.88 ± 0.04
5	125	78.01 ± 0.28	88.90 ± 0.33	90.89 ± 0.31	95.07 ± 0.25

**Pharmacological Screening of *Vigna radiata* stem bark Extracts for “Hepatoprotective” activity**

**Table No. 10: Total Bilirubin levels in all groups of animal**

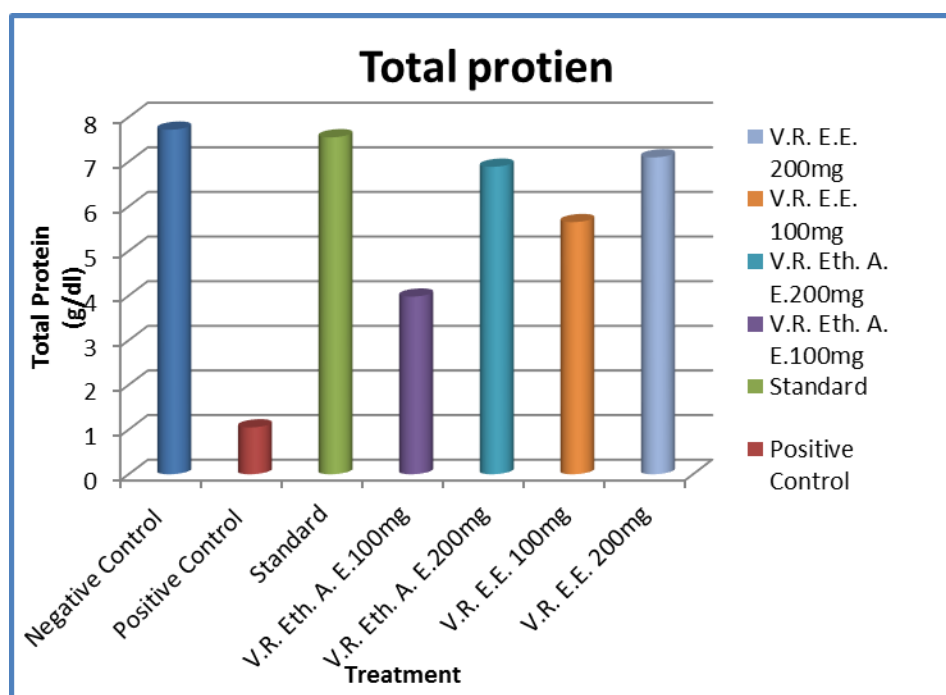
Sr. No.	Groups	Treatment	Total Bilirubin (mg/dl)
1	Group I	Negative Control (vehicle)	0.40 $\pm$ 0.03
2	Group II	Positive Control (CCl <sub>4</sub> )	7.10 $\pm$ 0.19
3	Group III	Standard( Silymarin)	0.58 $\pm$ 0.01
4	Group IV	V.R. Eth. A. Extracts 100mg/ kg	2.40 $\pm$ 0.20
5	Group V	V.R. Eth. A. Extracts 200mg/ kg	0.80 $\pm$ 0.01
6	Group VI	V.R. ethanol extracts 100mg/ kg	1.30 $\pm$ 0.13
7	Group VII	V.R. ethanol extracts 200mg/ kg	0.60 $\pm$ 0.04



**Chart No. 4: Total bilirubin level in all group**

**Table No. 11: Total Protein levels in all groups of animal**

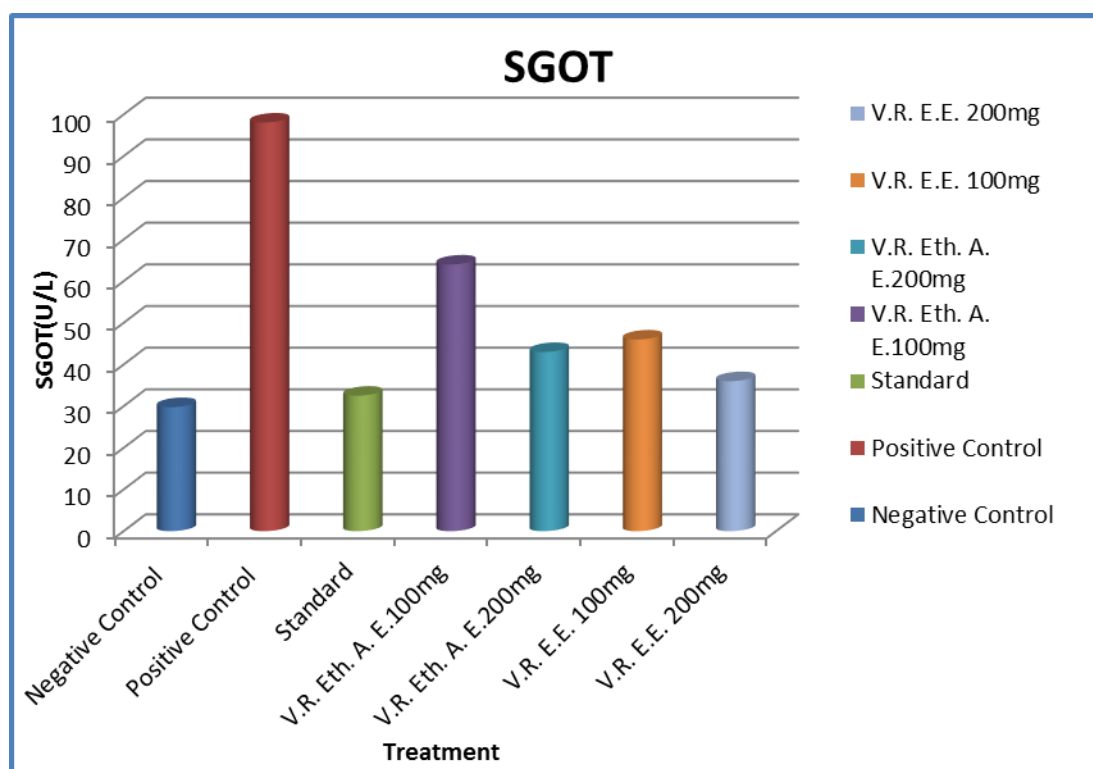
Sr. No.	Groups	Treatment	Total Protein (g/dl)
1	Group I	Negative Control (vehicle)	7.72 $\pm$ 0.19
2	Group II	Positive Control (CCl <sub>4</sub> )	1.05 $\pm$ 0.10
3	Group III	Standard( Silymarin)	7.55 $\pm$ 0.15
4	Group IV	V.R. Eth. A. Extracts 100mg/ kg	3.98 $\pm$ 0.21
5	Group V	V.R. Eth. A. Extracts 200mg/ kg	6.89 $\pm$ 0.12
6	Group VI	V.R. ethanol extracts 100mg/ kg	5.65 $\pm$ 0.14
7	Group VII	V.R. ethanol extracts 200mg/ kg	7.10 $\pm$ 0.12



**Chart No. 5: Total protein level in all group**

**Table No. 12: SGOT levels in all groups**

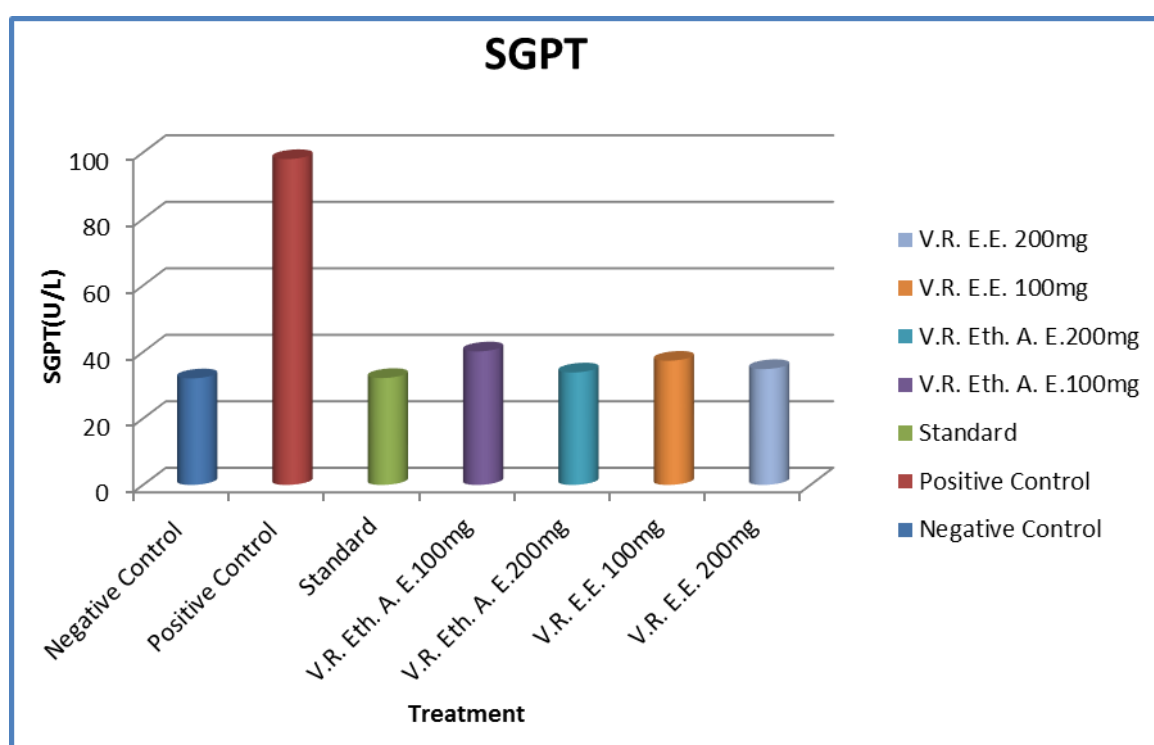
Sr. No.	Groups	Treatment	SGOT(U/L)
1	Group I	Negative Control (vehicle)	29.78 $\pm$ 1.80
2	Group II	Positive Control (CCl <sub>4</sub> )	98.09 $\pm$ 5.44
3	Group III	Standard( Silymarin)	32.52 $\pm$ 0.61
4	Group IV	V.R. Eth. A. Extracts 100mg/ kg	64.01 $\pm$ 0.12
5	Group V	V.R. Eth. A. Extracts 200mg/ kg	43.01 $\pm$ 0.28
6	Group VI	V.R. ethanol extracts 100mg/ kg	46.01 $\pm$ 0.23`
7	Group VII	V.R. ethanol extracts 200mg/ kg	36.01 $\pm$ 0.12



**Chart No. 6: SGOT level in all group**

**Table No. 13: SGPT levels in all groups of animal**

Sr. No.	Groups	Treatment	SGPT(U/L)
1	Group I	Negative Control (vehicle)	32.04 $\pm$ 0.45
2	Group II	Positive Control (CCl <sub>4</sub> )	98.01 $\pm$ 0.89
3	Group III	Standard( Silymarin)	32.16 $\pm$ 0.19
4	Group IV	V.R. Eth. A. Extracts 100mg/ kg	40.16 $\pm$ 1.48
5	Group V	V.R. Eth. A. Extracts 200mg/ kg	33.83 $\pm$ 0.23
6	Group VI	V.R. ethanol extracts 100mg/ kg	37.36 $\pm$ 0.49
7	Group VII	V.R. ethanol extracts 200mg/ kg	34.84 $\pm$ 0.19



**Chart No. 7: SGPT level in all groups**

**Table No. 14: All Biochemical parameter**

Sr. No.	Groups	Total Bilirubin (mg/dl)	Total Protein (g/dl)	SGOT (U/L)	SGPT (U/L)
1	Group I (Negative control)	0.43 $\pm$ 0.03	7.72 $\pm$ 0.19	29.78 $\pm$ 1.80	32.04 $\pm$ 0.45
2	Group II ( Positive control)	7.19 $\pm$ 0.18	1.05 $\pm$ 0.10	98.09 $\pm$ 5.4	98.01 $\pm$ 0.89
3	Group III (Std. Group)	0.58 $\pm$ 0.01**	7.55 $\pm$ 0.15**	32.52 $\pm$ 0.61**	32.16 $\pm$ 0.19**
4	Group IV V.R. Eth. A. Ext. 100mg	2.40 $\pm$ 0.20**	3.98 $\pm$ 0.21**	64.01 $\pm$ 0.12**	40.16 $\pm$ 1.48**
5	Group V V.R. Eth. A. Ext. 200mg	0.80 $\pm$ 0.01**#	6.89 $\pm$ 0.12**	43.01 $\pm$ 0.28**	33.83 $\pm$ 0.23**#
6	Group VI V.R. ethanol ext. 100mg	1.30 $\pm$ 0.13**	5.65 $\pm$ 0.14**	46.01 $\pm$ 0.23**	37.36 $\pm$ 0.49**
7	Group VII V.R. ethanol ext. 200mg)	0.60 $\pm$ 0.04**#	7.10 $\pm$ 0.12**#	36.01 $\pm$ 0.12**#	34.84 $\pm$ 0.19**#

Each value represents the mean  $\pm$  S. E. M. (n=6), P < 0.05: when compared to control (One way ANOVA followed by Dennett's test);\*-Significant difference (P<0.05), when test and standard compared with positive control; \*\*-Highly significant difference (P<0.001), when test and standard compared with positive control;#- No significant difference, when test is compared with standard,\$- Significant difference when test is compared with standard but more activity than standard.

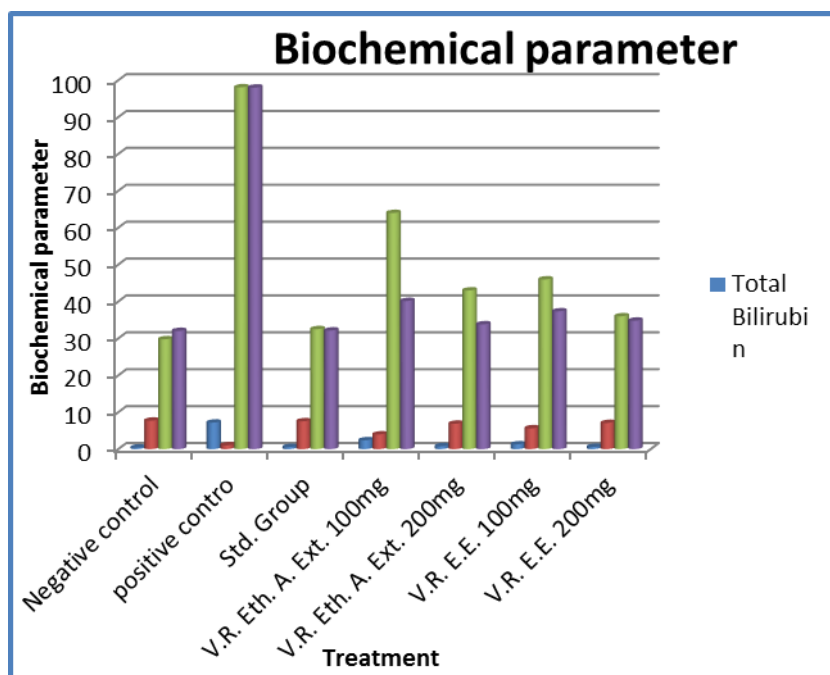


Chart No. 8: Biochemical Parameters of all groups

Table No. 15: Effect of drug on the liver weight

Sr. No.	Dose (mg/kg)	Liver Weight (g/100g)
1	Negative control (Vehicle)	4.2
2	Positive Control (CCl <sub>4</sub> )	5.3
3	Standard (Silymarin)	4.1
4	V.R. Eth. A. Extracts 100mg/ kg	4.8
5	V.R. Eth. A. Extracts 200mg/ kg	4.6
6	V.R. ethanol extracts 100mg/ kg	4.2
7	V.R. ethanol extracts 200mg/ kg	4.1

Above observation table shows that liver weight increases due to CCl<sub>4</sub> administration and it falls down with pretreatment of test or standard drug.

**Gross anatomy of liver:**

**Negative control (Vehicle)**

**Positive control (CCl<sub>4</sub> 1ml/kg)**



**Figure No. 4: Standard (Silymarin 100 mg/kg) + CCl<sub>4</sub>**

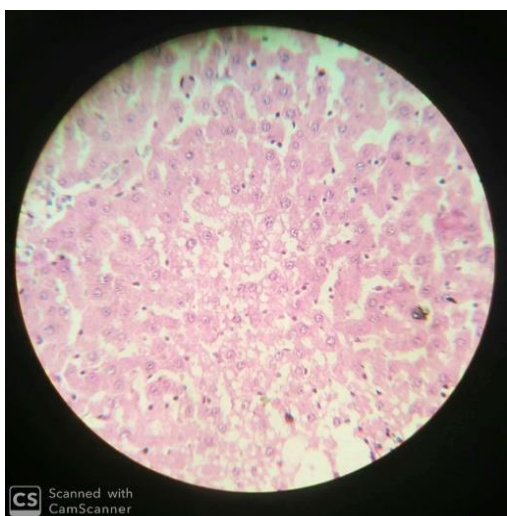


**Figure No. 5: V.R. Eth. A. E.100mg/ kg +CCl<sub>4</sub> V.R. Eth. A. E.n200mg/ kg +CCl<sub>4</sub>**

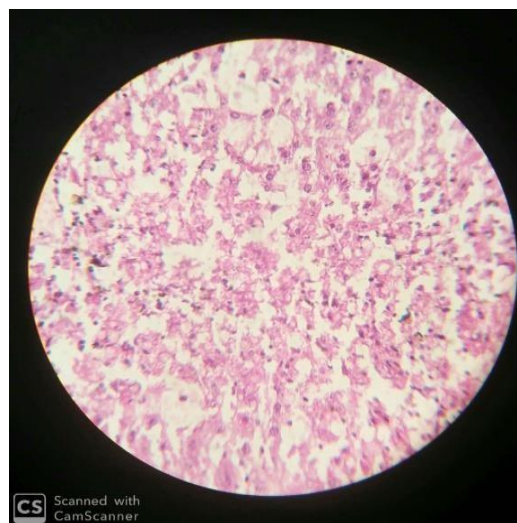


**Figure No. 6: V.R. Ethanol E. 100mg/ kg +CCl<sub>4</sub> V.R. Ethanol E. 100mg/kg+ CCl<sub>4</sub>**

**Histopathological evaluation of V.R. Ethyl acetate and V.R. Ethanol extract for Hepatoprotective activity**



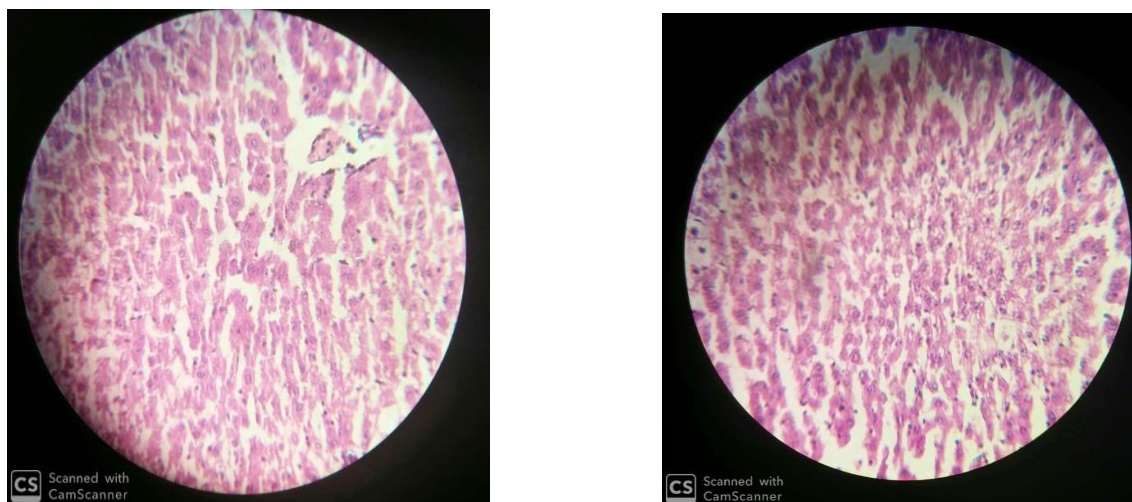
**Figure No. 7: Negative control (Vehicle)**



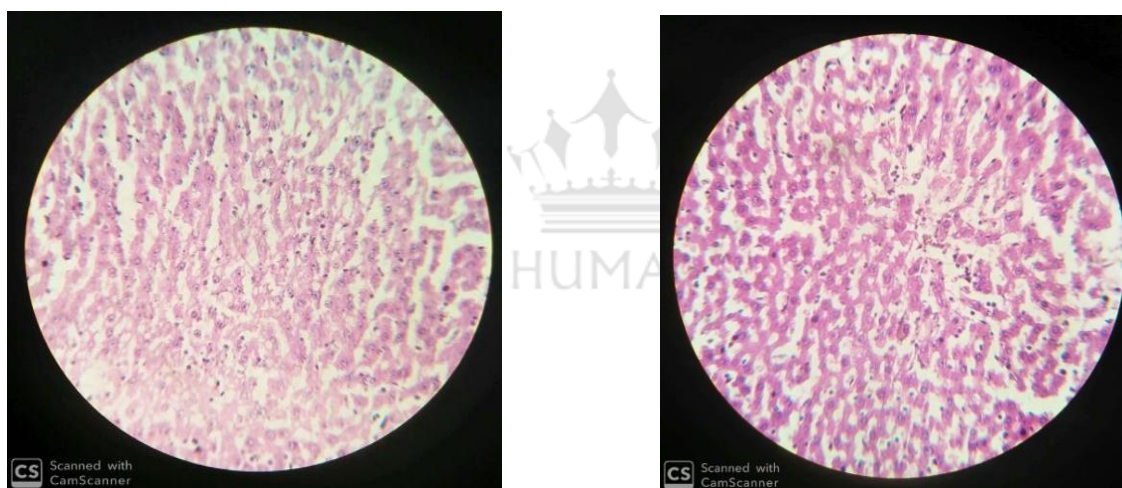
**Positive control (CCl<sub>4</sub> Treated)**



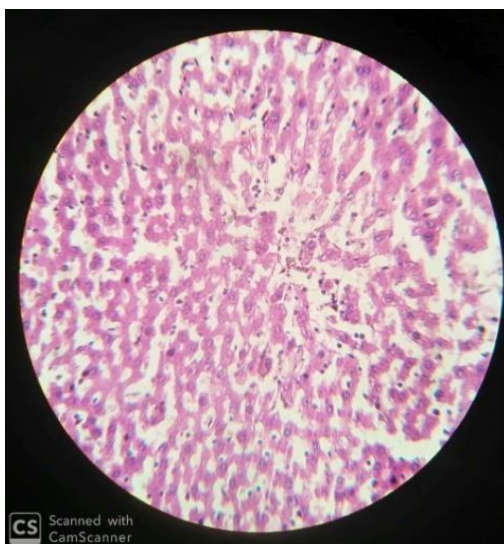
**Figure No. 8: Standard (Silymarin 100 mg/kg + CCl<sub>4</sub>)**



**Figure No. 9: V.R. Ethyl acetate 100 mg/kg + CCl<sub>4</sub>**



**Figure No. 10: V.R. ethanol extracts 100mg/ kg + CCl<sub>4</sub> V.R. Ethyl acetate 200 mg/kg + CCl<sub>4</sub>**



**Figure No. 11: V.R. ethanol extracts 200mg/ kg + CCl<sub>4</sub>**

## DISCUSSION

A lot of medicinal plants, traditionally used for thousands of years, are present in group of herbal preparation of the Indian traditional health care system. Silymarin; a phytoconstituents from (*Silybum marianum*) has been widely used from ancient times because of its excellent hepatoprotective action.

Liver protective herbal drugs contains a variety of chemical constituents like phenols, coumarins, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, lipids, alkaloids and xanthenes.

Extraction of *Vigna radiata* stem bark was done by using successive extraction process; two extracts were prepared with ethyl acetate and ethanol solvent. Phytoconstituent were found to be carbohydrate, alkaloids, glycosides, protein, phenol and flavonoid.

The V.R. Eth. A. and V.R. Ethanol extracts shows antioxidant activity which was demonstrated by DPPH radical scavenging method and compared with standard ascorbic acid. The V.R. Eth. A. and V.R. Ethanol extracts shows significant antioxidant activity. When there is comparison between two extracts, minimum dose of V.R. Eth. A. extract shows more significant activity than V.R. Ethanol extract.

Liver injury due to carbon tetrachloride in the rats was first reported in 1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of CCl<sub>3</sub>O<sup>-</sup>,

reactive oxidative free radical, which initiates lipid peroxidation. Administration of single dose of  $\text{CCl}_4$  to a rat within 24 hours produces a centrilobular necrosis and fatty changes. The toxicant reaches its maximum concentration in the liver within 3 hours of administration.

AST/SGOT predominantly found in mitochondria of hepatocytes. ALT/SGPT is more specific to the liver, is one of the most sensitive tests employed in the diagnosis of hepatic diseases and thus it is a better parameter for detecting liver injury. The AST/SGOT, ALT/SGPT, Total Bilirubin and Total protein levels are largely used as most common biochemical markers to evaluate liver injury.

Administration of Carbon tetrachloride caused a significant elevation in enzyme levels such as AST, ALT, Total bilirubin and Total protein, this indicated the damaged structural integrity of liver because they are cytoplasmic in the location and released into circulation after cellular damages indicating development of hepatotoxicity. SGPT has comparatively more activity in liver tissue. The increased activities in liver damage are due to necrotic or damaged hepatocytes and the enzyme is sensitive to hepatic dysfunction. Due to Carbon tetrachloride inducing agent the levels of SGOT, SGPT, Total bilirubin were elevated and the total protein levels was decline than normal. The pre-treatment of V.R. Eth. A. and V.R. Ethanol stem bark extracts at dose levels of 100 and 200 mg/kg were shown a restored the ALT, AST, Total bilirubin and Total protein levels towards normalization and the effects were comparable with standard drug (Silymarin 100mg/kg).

The serum bilirubin increased due to large number of chemicals, drugs and diseases. Carbon tetrachloride, as inducing agent causes increase in the bilirubin that is not hyperbilirubinemia, as the raise is much less than double. But V.R. Eth. A. and V.R. Ethanol stem bark at 100 mg/kg and 200 mg/kg were restored the approximate elevated level. Histopathological evaluation of livers revealed that the V.R. Eth. A. and V.R. Ethanol stem bark extracts reduced inflammation of hepatocytes, swelling, necrosis and no. liver lesions induced by  $\text{CCl}_4$ .

The above results suggest that the V.R. Eth. A. and V.R. Ethanol stem bark extracts inhibits  $\text{CCl}_4$  induced oxidative hepatic damage. It protects tissue from the effects of  $\text{CCl}_4$  and reduces insidious progressive inflammation leading hepatic cell necrosis.

Phytochemical study of V.R. Eth. A. and V.R. Ethanol stem bark extracts shows the presence of carbohydrate, alkaloids, glycosides, protein, phenol and flavonoid thus both the extracts

proved to be an antioxidant and herbal remedies.

## SUMMARY AND CONCLUSION

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The liver is a major target organ for toxicity of xenobiotics and drugs because most of the orally ingested chemicals and drugs firstly passes through the liver where they metabolized into inactive and toxic intermediates. At present, drug or chemical- induced liver injury has become a major clinical problem. Much attention should be paid to the mechanisms involving drug or chemical-induced liver injury. In addition, the search for effective therapeutical methods for the treatment of drug or chemical induced liver injury is also very important. Liver injury induced hepatotoxicity and is commonly used model for screening hepatoprotective drugs.

Liver injury due to carbon tetrachloride in the rats was first reported in 1936 and has been widely and successfully used by many investigators. Carbon tetrachloride is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of  $\text{CCl}_3\text{O}^\cdot$ , reactive oxidative free radical, which initiates lipid peroxidation. Administration of single dose of  $\text{CCl}_4$  to a rat within 24 hours produces a centrilobular necrosis and fatty changes. The

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