IJPSR (2020), Volume 11, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 May 2019; received in revised form, 19 August 2019; accepted, 01 September 2019; published 01 February 2020

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *COLEUS VETTIVEROIDES* ROOTS ON EXPERIMENTALLY INDUCED HEPATO TOXIC MODELS

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Keywords:

Coleus Vettiveroides, Hepatotoxicity, Antioxidant, Silibinin

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ABSTRACT: The present study was aimed to assess the hepatoprotective activity of ethanolic extract of *Coleus vettiveroides* on experimentally induced hepatotoxic models. Phytochemical screening of EECV was carried out and the results showed the presence of carbohydrates, alkaloids, tannin, flavonoids, phenolics, proteins and aminoacid, steroids and triterpenoids. HPTLC screening of EECV was carried out along with different marker compounds to ensure the presence of phytoconstituents such as Quercetin, Rutin, and Gallic acid. Antioxidant activities of the herbal extract were determined using both DPPH and ABTS scavenging method. MTT assay is most widely used to determine the cytotoxic potential of medicinal agents and other toxic materials. It was observed that for different concentrations of EECV, the cell viability was greater than 90%. *In-vitro* hepatoprotective activity of EECV was evaluated on Hep G2 cells, the human hepatoma cell line. In Hep G2 treatment with EECV (12.5, 25, 50, 100 and 200 µg/ml) the cell viability was above 90% indicating that EECV showed no cytotoxicity up to 200 µg/ml. Evaluation of biochemical parameters showed a significant increase in the activities of AST, ALT, and ALP that indicates increased permeability, severe damage to hepatocytes after exposing to CCl_4. In the present study, hepatotoxicity model in Wistar rats was successfully produced by administering INH and RIF (15 and 10 mg/kg respectively) orally. The protective effects of EECV were further confirmed by histopathological examination of the livers, showing marked protection against liver tissue damage, normal lobular architecture, dilated sinusoidal spaces and vacuolization as compared to controls.

INTRODUCTION: The liver is one of the largest organs in the body weighing about 1.5 kg. It is by far the largest gland. It is included amongst the accessory organs of the alimentary system because it produces a secretion, called bile, which is poured into the duodenum (through the bile duct) and assists in the digestive process.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(2).765-73

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(2).765-73

All the blood circulating through the capillary bed of the abdominal part of the alimentary canal (excepting the lower part of the anal canal) reaches the liver through the portal vein and its tributaries. In this way, all substances absorbed into the blood from the stomach and intestines are filtered through the liver, where some of them are stored; and some toxic substances may be destroyed.

Numerous other functions essential to the wellbeing of the individual are performed in the liver, which is, therefore, regarded as one of the vital organs. The liver lies in the upper, right part of the abdominal cavity. It lies mainly in the right hypochondrium and the epigastrium, but part of it

extends into the left hypochondrium and part of it into the right lateral region. In general, hepatotoxic chemicals engender some reactive species, which form a covalent bond with the lipid which is responsible for damage liver cells [1]. Another most used hepatotoxic agent relating to liver disorders is ethanol (alcohol) [2]. In liver during the process of elimination, there is a chance of accumulation of different kinds of toxic materials inside the hepatocytes, and there is a chance of liver infection, and hepatic disorders such as hepatitis [3]. Even though different kind of allopathic molecules are available in the market all of them are suffering from some are the other toxic effect, so the need of developing a herbal medicine which has got both liver-protecting and nutritional value is required hence an attempt has been made to screen the hepatoprotective activity of roots of *Coleus vettiveroides*.

MATERIALS AND METHODS:

Plant Collection and Authentication: The roots of the plant of *Coleus vettiveroides* Jacob was collected from Tirunelveli district, Tamil Nadu, India. Taxonomic identification was done by a Botanist of Central Council for Research in Ayurveda and Siddha. (Reference - Flora of Tamil Nadu Vol. 2 Page 183, 1987). The plant was washed to remove all debris and was shade-dried. The dried plant material was made to coarse powder.

Extraction of the Plant Material: [4] The extraction of the roots of *Coleus vettiveroides* was done by using a Soxhlet apparatus. The coarse powders of the roots were extracted with ethanol. After extraction, the ethanolic extract of *Coleus vettiveroides* (EECV) was evaporated or concentrated by using rotary evaporator and dried at room temperature to give a viscous mass. The obtained crude extracts were weighed and stored at 40 ºC for further analysis.

***In-vitro*-Antioxidant Studies:**

DPPH Radical Scavenging Assay: [5, 6] The antioxidant activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical (Blois method). 0.3 mm solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 1 ml of various concentrations of sample (10, 20, 40, 60, 80 and 100 µg/ml) and the reference compound (5, 10, 15, 20, 25 and 30 µg/ml), shaken vigorously, left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm against a blank. Reference compound used here was quercetin. A control reaction was carried out without the test sample. All the tests were performed in triplicate in order to get the mean values.

The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. The antiradical activity was expressed as percentage inhibition (I%) and calculated using the following equation.

Percentage inhibition (I %) = (Abs control - Abs sample /Abs control) × 100

Different sample concentrations were used in order to obtain calibration curves and to calculate the IC_{50} values. (IC_{50} - concentration required to obtain a 50% radical scavenging activity).

Abts Radical Scavenging Assay: [7, 8] ABTS radical scavenging activity of the extract was measured by Rice-Evans method. ABTS was dissolved in water to a 7 mm concentration. ABTS radical cation (ABTS+) was produced by reacting ABTS stock solution with 2.45 mm potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 - 16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study, the ABTS solution was diluted with phosphate buffer saline pH 7.4 (PBS) to an absorbance of 0.70 (± 0.02) at 734 nm and equilibrated at 30 ºC. Reference compound used here was quercetin.

After addition of 1 ml of diluted ABTS+ solution to various concentrations of sample (5, 10, 15, 20, 25 and 30 µg/ml) and reference compound (0.25, 0.5, 0.75, 1, 1.25 and 1.5 µg/ml), the reaction mixture was incubated for 6min and then absorbance was measured at 734 nm against a blank. A control reaction was carried out without the sample. All the tests were performed in triplicate in order to get the mean values. The percentage inhibition of ABTS+ by the sample was calculated according to the formula:

Percentage inhibition (I %) = (Abs control - Abs sample / Abs control) × 100

Determination of *In-vitro* Cell Viability by MTT Assay: [8, 9]

Cell Culture: Human liver hepatoma cells (Hep G2) were grown in Eagles Minimum Essentials Medium (EMEM) containing 10% fetal bovine serum (FBS). Cells were maintained at 37 ºC, 5% CO_2, 95% air and 100% relative humidity. Cells were subcultured every third day by trypsinization with Trypsin-EDTA solution and medium changed daily.

Procedure: The monolayer cell culture was trypsinized to make single-cell suspensions and viable cells were counted using a hemocytometer and the cell count was adjusted to 2×10^4 cells per ml using EMEM containing 10% fetal bovine serum. To each well of 96 well microtiter plates, 0.1 ml of diluted cell suspension approximately 20,000 cells per well was added. After 24 h, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds at serial concentration was added to the cells in microtitre plates and kept for incubation at 37 ºC in 5% CO_2 incubator for 48 h.

After 48 h, the sample solution in wells was flicked off and 15 µl of MTT dye (5 mg/ml) in phosphate-buffered saline was added to each well. The plates were gently shaken and incubated for 4 h at 37 ºC in 5% CO_2 incubator. The supernatant was removed, 100 µl of DMSO was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured at 570 nm using microplate reader. The medium without samples was served as control and triplicates were maintained for all concentrations.

The percentage of cell viability was then calculated by using equation,

% Cell viability = Absorbance of treated cells / Absorbance of control × 100

***In-vitro* Hepatoprotective Activity:** [10, 11] Hep G2 cells were plated at a density of 2×10^4 cells per well in medium containing 10% FBS incubated for 24 h at 37 ºC under 5% CO_2 to attain confluency. On attaining confluency the medium was replaced with serum-free medium containing different concentrations of extract and standard silybinin along with toxicant carbon tetrachloride (CCl_4 -1%) for 4 h and viability of cells was determined by MTT assay.

Experimental Design:

Group I (Control): The cells were treated with 100 µl of serum-free culture medium for 4 h.

Group II (Toxin Treatment): The cells were treated with 100 µl of serum-free medium containing 1% CCl_4 for 4 h.

Group III (Silibinin Treatment): The cells were treated with 100 µl of serum-free medium containing 1% CCl_4 with silybinin at a concentration of 50, 100 and 200 µg/ml for 4 h.

Group IV (Extract Treatment): The cells were treated with 100 µl of serum-free culture medium containing 1% CCl_4 with EECV at concentration of 50, 100 and 200 µg/ml for 4 h.

***In-vivo* Hepatoprotective Activity:**

Animals and Management: Male Wistar rats of 6-8 weeks old and 160 - 180 g body weight were offered by KMCH College of Pharmacy, Coimbatore. All rats were kept at room temperature and allowed to accommodate in standard conditions at 12 h light and 12 h dark cycle in the animal house. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study.

The experimental procedure was approved by IAEC (Institutional animal ethical committee of KMCH, governed by CPCSEA, Government of India. Proposal number: KMCRET/ M.PHARM /11/2015-2016. Acute toxicity study based on a previously conducted study of *Coleus vettiveroides* route of administration and dosage was selected.

Isoniazid and Rifampicin Induced Hepatotoxicity in Rats: [12]

Experimental Procedure: Male Wistar rats of weight between 160 - 200 g were divided into five groups with six animals in each group. Distilled water was used as a vehicle as extract was soluble in distilled water.

Group 1 was a vehicle-treated group, administered with distilled water (1 ml/kg, p.o.). Group 2 was toxic control group, administered orally with isoniazid (15 mg/kg) and rifampicin (10 mg/kg)

and dissolved in water for 21 days. Group 3 was standard drug group administered with Silibinin (100 mg/kg, p.o.) suspended in 1% carboxymethyl cellulose. Group 4 and 5 were test groups, administered with EECV at 200 mg/kg, p.o, and 400 mg/kg, p.o. respectively with isoniazid and rifampicin. The vehicle, extract, and Silibinin was administered 1 h before isoniazid and rifampicin administration, each day for 21 days. 24 h after last dosing blood sample was collected through retro-orbital puncture and animals were sacrificed by cervical dislocation under general anesthesia. The liver was collected for estimation of enzymes involved in oxidative stress and histopathological examination.

Estimation of Biochemical Parameters: The cell supernatant was collected separately after *in-vitro* treatment and the supernatant was used to measure concentration of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) as an indication of cell necrosis. The results were expressed as units per liter (U/L). Also the biochemical parameters such as AST, ALT, ALP and total bilirubin were studied using serum after *in-vivo* drug treatment.

Aspartate Amino Transferase (AST): [13]
Method: Optimized UV test according to the IFCC (International Federation of Clinical Chemistry and laboratory medicine)

Assay Procedure: 800 µl of reagent 1 (R1) was mixed with 200 µl of reagent 2 (R2). 100 µl of serum was added to this mixture. Mixed well and sample was analyzed using Photometer 5010v5+. Normal range: < 37 u/l.

Alanine Amino Transferase (ALT): [14]
Method:
Kinetic UV Test: According to the international federation of clinical chemistry and laboratory medicine (IFCC).

Assay Procedure: 800 µl of reagent 1 (R1) was mixed with 200 µl of reagent 2 (R2). 100 µl of serum was added to this mixture. Mixed well and sample was analyzed using Photometer 5010v5+ Normal range: < 41 u/l.

Alkaline Phosphatase (ALP): [15]
Procedure: 800 µl of reagent 1 (R1) was mixed with 200 µl of reagent 2 (R2). 100 µl of serum was added to this mixture. The sample was mixed well and was analyzed using Photometer 5010v5+. Normal range: 53-128 u/l.

***In-vivo* Antioxidant Activity:** [16, 17]
Preparation of Tissue Homogenate: The animals were sacrificed after treatment and liver were isolated for *in-vivo* antioxidant studies. The separated liver, each weighing 0.5 g were homogenized with motor-driven Teflon coated homogenizer with 5ml of ice-cold 0.1 M Tris-HCl buffer of pH 7.4 to get 10% homogenate. The homogenate was centrifuged at 10000 rpm for 20 min at 50 ºC. The supernatant was collected and used for the estimation of *in-vivo* antioxidant activity.

Estimation of Total Protein: Total protein content of the granular tissue was determined by following Lowry *et al.,* (1951).

Procedure: 0.1 ml homogenate was made up to 1 ml with distilled water and 5 ml of alkaline solution was added, mixed well and allowed to stand for 10 min. Then, 0.5 ml Folin's reagent was added, mixed well and incubated at room temperature for another 10 min. The blue color developed was measured at 640 nm against blank. Bovine serum albumin (1 mg/ml) served as the standard and from the standard graph obtained the amount of protein in the sample was calculated and expressed as mg/ 100 mg tissue.

Enzymatic Anti-Oxidant Activity:
A) Estimation of Catalase (CAT): [18]
Procedure: To 1 ml of tissue homogenate 4 ml of hydrogen peroxide and 5 ml of phosphate buffer was added and mixed well. From this, 1 ml of solution was taken and mixed with dichromate acetic acid reagent and allowed to incubate for 30 min at room temperature. The absorbance was measured at 570 nm.

B) Estimation of Superoxide Dismutase (SOD):
Procedure: This method might be used for determination of the antioxidant activity of a sample, and it was described by McCord and Fridovich. The main purpose of this method that was estimated 5% of tissue homogenate after adding 75 mm, 30 mm and 2 mm from Tris-HCl (pH 8.2), EDTA, and pyrogallol respectively. Then, the absorbance was measured at 420 nm.

The percentage of inhibition was calculated depending on that the ability of the enzyme to inhibit oxidation. So, any changes might have happened on the absorbance; it will give a clear picture of the ability of enzyme activity to prevent oxidation.

C) Estimation of Glutathione Peroxidase (GPX):
Procedure: To 0.1 ml of the tissue homogenate, 0.2 ml of EDTA, sodium azide, hydrogen peroxide were added and mixed. Then 0.4 ml of phosphate buffer was added and allowed to incubate at room temperature. The reaction was arrested by the addition of 0.5 ml of TCA. The reaction mixture was centrifuged at 5000 rpm for 10 min, and the supernatant was collected. To 0.5 ml of the supernatant 4 ml of disodium hydrogen phosphate and 0.5 ml of DTNB were added and the color developed was read immediately at 420 nm. The activity of Glutathione peroxidase was expressed as units/mg protein.

Non-Enzymatic Antioxidant Activity:
Estimation of Reduced Glutathione (GSH):
Procedure: To 1 ml of the homogenate, 1 ml of the TCA solution was added and centrifuged. The supernatant was collected, and the precipitate formed was removed. To 0.5 ml of supernatant 2 ml of DTNB was added, the volume was made up to 3 ml with phosphate buffer. Then absorbance was read at 412 nm. The amount of glutathione was expressed as µg/mg protein.

Determination of Lipid Peroxidation:
Procedure: To 0.1 ml of the sample, 2 ml of TBA-TCA-HCl reagent (ratio of 1:1:1) was added, mixed and kept in a water bath for 15 min. Afterward, the solution was cooled and supernatant was removed. The absorbance was measured at 535 nm against reference blank. The level of lipid peroxidase was given as n moles of MDA formed/mg protein.

Histopathological Techniques: Histopathology is the microscopical study of tissues for pathological alterations. This involves the collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

Collection of Materials: Thin pieces of 3 to 5 mm, thickness were collected from liver tissues showing gross morbid changes along with normal tissue.

Fixation: Kept the tissue in fixative for 24 - 48 h at room temperature. The fixation was useful in the following ways:

a. Serves to harden the tissues by coagulating the cell protein.

b. Prevents autolysis.

c. Preserves the structure of the tissue.

d. Prevents shrinkage.

Common Fixatives: 10% Formalin.

Hematoxylin and Eosin Method of Staining: Deparaffine the section by xylol 5 to 10 min and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with hematoxylin for 3 - 4 min and again cleaned under tap water.

Allow the sections in tap water for a few minutes and counterstained with 0.5% eosin until section appears light pink for 15 to 30 sec and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol for 15 to 30 sec.

Mounted on Canada balsam or DPX Moutant and kept the slide dry and remove air bubbles.

Statistical Analysis: Statistical analysis data were expressed as mean ± SEM and statistical analysis was carried out by using Graph Pad 5.0 software by applying one-way ANOVA with Dunnet's test. $P < 0.05$ was considered to be significant.

Results:
***In-vitro* Antioxidant Activity:**
DPPH Radical Scavenging Activity:

TABLE 1: PERCENTAGE INHIBITION AND IC_{50} VALUES OF DPPH RADICAL BY QUERCETIN AND EECV

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
EECV	10	23.90	47.27
	20	30.94	
	40	40.87	
	60	51.29	
	80	59.61	
	100	76.63	
Standard (Quercetin)	5	45.79	6.560
	10	58.87	
	15	69.15	
	20	78.47	
	25	90.54	
	30	96.89	

BTS Radical Scavenging Activity:

TABLE 2: PERCENTAGE INHIBITION OF ABTS RADICAL BY ASCORBIC ACID AND EECV

Sample	Conc. (µg/ml)	% Inhibition	IC_{50} (µg/ml)
EECV	5	69.18	2.738
	10	76.6	
	15	82.61	
Standard	20	88.3	0.1902
(Quercetin)	25	98.67	
	30	99.38	
	0.25	62.57	
	0.5	69.17	
	0.75	78.04	
	1	85.75	
	1.25	99.38	
	1.5	99.48	

Determination of Cell Viability by MTT Assay:

TABLE 3: PERCENTAGE CELL VIABILITY AFTER TREATMENT WITH EECV AND STANDARD

Sample	Conc. (µg/ml)	% Cell viability
EECV	12.5	100.2169
	25	99.4938
	50	97.9031
	100	96.3123
	200	93.0585
Standard	6.25	100.5
(Silybinin)	12.5	99.60
	25	107.48
	50	104.92
	100	74.63

In-vitro Hepatoprotective Activity:

TABLE 4: *IN-VITRO* HEPATOPROTECTIVE ACTIVITY OF EECV AGAINST CCl_4 INDUCED HEPATO-TOXICITY IN HEP G2 CELL LINES

Group	%Cell Viability	AST (U/L)	ALT (U/L)	ALP (U/L)
Group 1: Normal (Only medium)	100	15.00 ± 1.732	11.33 ± 2.603	28.33 ± 2.906
Group 2: Toxicant (CCl_4)	33.33	64.33 ± 2.728	84.67 ± 6.936	200.3 ± 15.88
Group 3: CCl_4 + 25 µg/ml Standard	151.56	23.33 ± 1.453	27.00 ± 2.646	68.67 ± 4.055
CCl_4 + 50 µg/ml Standard	197.64	20.67 ± 2.848	16.67 ± 2.906	50.00 ± 3.786
CCl_4 + 100 µg/ml Standard	189.21	19.00 ± 1.528	15.67 ± 2.906	31.33 ± 3.180
Group 4: CCl_4 + 50 µg/ml EECV	83.45	29.67 ± 4.055	24.00 ± 1.000	149.7 ± 5.487
CCl_4 + 100 µg/ml EECV	110.56	20.33 ± 2.603	22.67 ± 1.202	64.00 ± 1.528
Cl_4 + 200 µg/ml EECV	134.42	18.00 ± 3.215	21.33 ± 3.180	60.67 ± 1.764

In-vivo Hepatoprotective Activity:

TABLE 5: EFFECT OF EECV ON BIOCHEMICAL PARAMETERS IN INH+RIF INDUCED HEPATOTOXIC RATS

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)
Normal	48.00 ± 2.769	45.17 ± 4.715	127.7 ± 3.694	0.2167 ± .0477
Control INH+RIF (15, 10 mg/kg)	171.5 ± 10.65***	160.3 ± 12.94***	352.0 ± 26.18***	0.5500 ± .0562***
Standard Silibinin (100 mg/kg)	65.6 7± 2.201***	61.33 ± 6.048***	143.3 ± 2.404***	0.2833 ± .0401**
EECV (200 mg/kg)	116.2 ± 4.301***	101.5 ± 6.433***	211.3 ± 5.548***	0.3500 ± .0619^{ns}
EECV (400 mg/kg)	95.50 ± 7.284***	88.50 ± 3.149***	179.5 ± 4.530***	0.3000 ± 0.0365*

Estimation of Total Protein:

TABLE 6: EFFECT OF EECV ON TOTAL PROTEIN IN LIVER TISSUE, VALUES EXPRESSED IN MG/G OF TISSUE

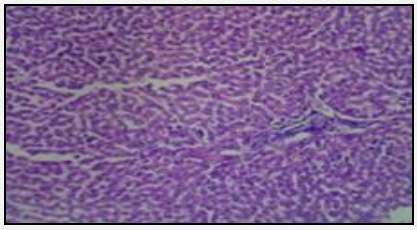
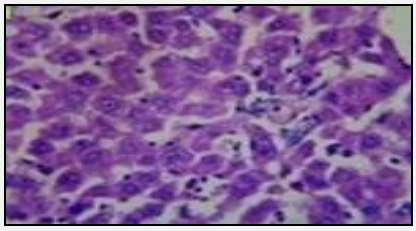
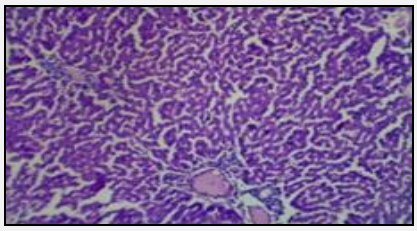
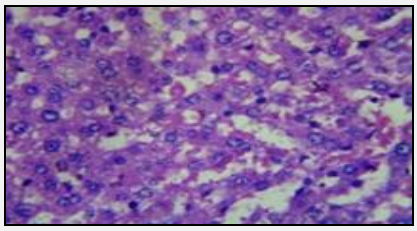
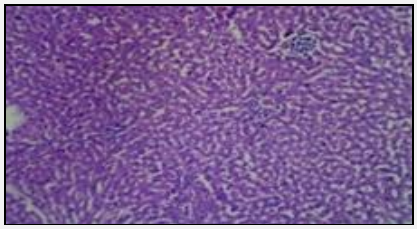
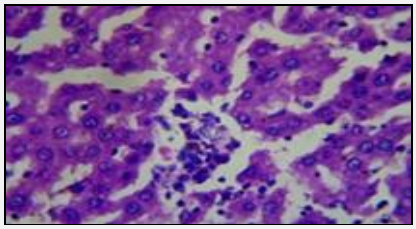
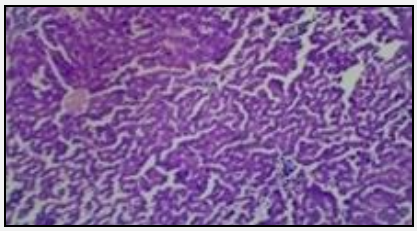
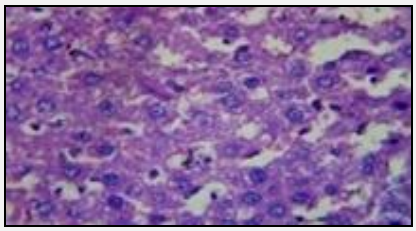
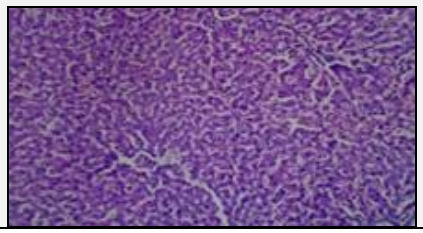
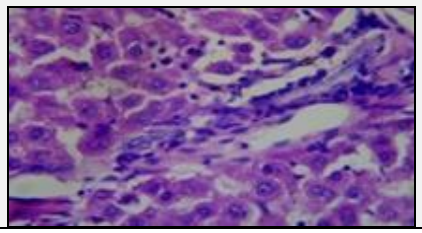
Treatment	Total proteins (mg/100 mg tissue)
Normal	7.14 5 ± 0.5921
Control (INH+RIF)	3.313 ± 0.6290***
Silibinin (100 mg/kg)	6.125 ± 0.5715**
EECV (200 mg/kg)	4.917 ± .0703ns
EECV (400 mg/kg)	5.533 ± 0.5602*

In-vivo Antioxidant Study:

TABLE 7: EFFECT OF EECV ON LIVER ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION

Treatment	Catalase (µM of H_2O_2/mg protein)	SOD (U/mg protein)	GP_X (U/mg protein)	GSH (µg of GSH/mg protein)	Lipid peroxidation (nM of MDA/mg protein)
Normal	34.67 ± 1.783	3.375 ± 0.1948	29.02 ± 0.5776	17.61 ± 0.8516	6.158 ± 0.5598
Control (INH+RIF)	15.50 ± 1.118***	0.9067 ± 0.025***	15.10 ± 1.32***	8.05 ± 1.41***	24.25 ± 1.182***
Standard (Silibinin)	29.50 ± 0.6191***	2.507 ± 0.1434***	25.15 ± 0.596***	14.17 ± 0.9426**	9.05 ± 0.5795***
EECV (200 mg/kg)	20.53 ± 0.8353*	1.527 ± 0.0729*	20.03 ± 0.578**	10.02 ± 1.644ns	17.07 ± 0.5737***
EECV (400 mg/kg)	27.05 ± 1.167***	2.027 ± 0.1884***	22.08 ± 0.583***	13.08 ± 0.7926*	12.02 ± 0.5776***

Histopathology:

GROUP	10X	40X
Group 1: Normal		
Group 2: Control (INH-RIF only)		
Group 3: Silibinin (100 mg/kg)		
Group 4: EECV (200 mg/kg)		
Group 5: EECV (400 mg/kg)		

DISCUSSION: The liver may be considered as one of the most important organs in drug toxicity for two reasons: on the one hand it is functionally interposed between the site of absorption and the systemic circulation and is the main site of metabolism and elimination of foreign substances, but on the other hand these features also render it a preferred target for drug toxicity. Phytochemical screening of EECV was carried out, and the results showed the presence of carbohydrates, alkaloids, tannin, flavonoids, phenolics, proteins and aminoacid, steroids and triterpenoids.

It is suggested that the phytoconstituents like flavonoids and phenolics mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions which aid in the hepatoprotective activity of EECV. HPTLC screening of EECV was carried out along with different marker compounds to ensure the presence of phytoconstituents such as quercetin, rutin, and gallic acid. From the result of HPTLC analysis it was found that EECV contains phytoconstituents like gallic acid and quercetin. Percentage of Gallic acid and Quercetin in 100 mg of extract by HPTLC quantification was found to be 1.37% and 1.08% respectively. Antioxidant activities of the herbal extract were determined using both DPPH and ABTS scavenging method. The IC_{50} value of EECV and quercetin was found to be 47.27 µg/ml and 6.560 µg/ml respectively.

From the results obtained in ABTS assay it was found that the extract scavenged ABTS radicals generated by the reaction between ABTS and potassium persulphate.

The activity was found to be increased in a dose-dependent manner. IC_{50} value of quercetin and EECV was found to be 0.1902 µg/ml and 2.738 µg/ml respectively. MTT assay is most widely used to determine the cytotoxic potential of medicinal agents and other toxic materials. It was observed that for different concentrations of EECV, the cell viability was greater than 90%. The cell viability of EECV at concentration 200 µg/ml is 93.0585% and for standard silybinin the percentage cell viability at concentration 100 µg/ml is 74.63%. In the present study, hepatotoxicity model in Wistar rats was successfully produced by administering INH and RIF (15 and 10 mg/kg respectively) orally. In the present study administration of INH and RIF in Wistar rats produced a significant increase in AST, ALT, ALP and total bilirubin levels confirming hepatocellular damage. Treatment with EECV 200, 400 mg/kg significantly attenuated these changes in a dose-dependant manner. There was significant increase in AST, ALT, ALP and total bilirubin in control group compared to normal group. Treatment with EECV and Silibinin showed significant decrease in AST, ALT, ALP, and total bilirubin levels. The results were tabulated in table and figures.

The hepatoprotective effect of EECV may be due to the ability to act as a free radical scavenger, thereby protecting membrane permeability. Enhanced oxidative stress in antitubercular drug-treated animals was confirmed based on altered profile of antioxidant enzymes and increased lipid peroxidation. SOD is a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.

Increasing one's glutathione levels help the productivity of the glutathione peroxidase and vice versa. Lipid peroxidase is the enzyme involved in oxidative degradation of lipids. In the process free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene groups that possess reactive hydrogen [13]. In the toxic control group, the level of SOD, GSH, GPX, and catalase was significantly lower, and LPO level was significantly higher. This confirmed the induction of oxidative stress due to administration of isoniazid and rifampicin in toxic control group. At both of the selected doses of extract, significant protection against oxidative stress was observed with increase in level of SOD, GSH, GPX, and catalase and decrease in LPO level.

Also the total protein level was significantly lower in the toxic group treated with INH and RIF only and the level was significantly higher in standard and extract-treated groups. The protective effects of EECV were further confirmed by histopathological examination of the livers, showing marked protection against liver tissue damage, normal lobular architecture, dilated sinusoidal spaces and vacuolization as compared to controls.

CONCLUSION: Liver is a versatile organ in the body concerned with the regulation of internal chemical environment. Therefore, damage to liver caused by hepatotoxic agents is of grave consequence. There is an ever-increasing need for an agent which could protect liver damage.

The present study reveals that the roots of *Coleus vettiveroides* possess hepatoprotective activity in CCl_4 and INH-RIF induced hepatotoxicity. It showed significant antioxidant activity by terminating the actions of free radicals. The extract reduced the elevated serum biochemical parameters such as AST, ALT, ALP and total bilirubin. The level of *in-vivo* antioxidant parameters such as catalase, SOD, GPX, and GSH was restored in liver tissues. The potential hepatoprotective activity of EECV may be due to the presence of phenols and flavonoids by scavenging free radicals and/or by chelating metal ions which aid in the hepatoprotective activity of EECV.

ACKNOWLEDGEMENT: Authors are thankful to the Management of KMCH College of Pharmacy, Coimbatore for their support in the form of providing facilities.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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How to cite this article:
Abarnadevika A and Nisha TR: Evaluation of hepatoprotective activity of *Coleus vettiveroides* roots on experimentally induced hepato toxic models. Int J Pharm Sci & Res 2020; 11(2): 765-73. doi: 10.13040/IJPSR.0975-8232.11(2).765-73.