RESEARCH ARTICLE

Hepatoprotective Activity of Whole Plant of *Hemigraphis colorata* against Paracetamol Induced Liver Toxicity in *Wistar albino* Rats

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ABSTRACT

Objective: The objective of the present study was to evaluate the hepatoprotective activity of whole plant of *Hemigraphis colorata* against paracetamol-induced liver toxicity in *Wistar albino* rats. Powdered mixture materials were extracted with Ethanol using the Soxhlet apparatus. The dried extracts were subjected to preliminary phytochemical analysis and the extracts were evaluated for acute oral toxicity by OECD guidelines 423. The ethanolic extract at a dose level of 200 mg/kg and 400 mg/kg body weight was selected and administered to Albino rats. Hepatotoxicity was induced by Paracetamol (2 mg/kg body weight with CMC). Silymarin (100 mg/kg body weight orally) was used as the Standard. The In-vitro antioxidant activity using DPPH scavenging assay and Nitric oxide free radical assay were estimated. Biochemical parameters like SGOT, SGPT, ALP, HDL, LDL, cholesterol, triglycerides, direct bilirubin, protein, albumin, globulin, and total bilirubin. Biochemical observations were also supplemented with a histopathological examination of the liver section. The preliminary phytochemical examination showed the presence of alkaloids, glycosides, saponins, Flavonoids, Tannins, Amino acids, Tryptophan, quinones, terpenoids, Starch, Vitamin C, Phenols and Carbohydrates. The acute toxicity study results showed that the extracts were found to be safe up to 2000 mg/kg b. wt. The In-vitro antioxidant activity using the DPPH scavenging assay and Nitric oxide free radical assay showed the presence of free radicals

and antioxidant activity. The extract dose-dependently shows hepatoprotective potential by restoring the elevated biochemical parameters. The same was further confirmed using histopathological studies. The results were comparable to that of the standard drug Silymarin. The results obtained from the study indicate that the whole plant of Hemigraphiscolorata showing hepatoprotective activity. The activity might be due to the presence of the phytoconstituents including Alkaloids, Glycosides, Saponins, Flavonoids, Tannins, amino acids, phenol, Carbohydrates, Terpenoids (squalene) Starch, Vitamin C, Tryptophan and Quinones in the extract. Further studies are required to identify the active principle responsible for hepatoprotective activity.

Keywords: Hepatoprotective; Hemigraphiscolorata; Paracetamol; Silymarin.

INTRODUCTION

Traditional medicine is the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and the prevention, diagnosis, improvement or treatment of physical and mental illness [1]. The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners. The past decade has seen considerable change in opinion regarding ethnopharmacological therapeutic applications [2]. The presence of various life-sustaining constituents in plants has urged scientists to examine these plants to determine potential medicinal properties. Plants can be effectively used as medications to treat or prevent diseases. Modern medicine offers limited success in providing effective cures and there is a severe need to develop new drugs capable of healing toxic liver damages [3]. A large number of plants and purified natural substances have been screened for liver disorders [4]. *Hemigraphis colorata* is a tropical perennial herb mainly grown as an ornamental indoor and outdoor plant [5]. The major phytoconstituents present are carbohydrates, alkaloids, phenols, saponins, flavonoids (rutin), terpenoids (squalene), coumarins, carboxylic acid, xanthoproteins, tannins, phenols (gallic acid), steroids, chlorogenate, cinnamic acids, cinnamate and sterol [6]. It has many medicinal uses such as anti-diabetic activity,anti-oxidant/anti-inflammatory/cytotoxicity,anti-bacterial activity, wound healing activity, anti-ulcerogenic activity and anti-helminthic activity. In this study, we report the hepatoprotective activity of *Hemigarphiscolorata* against paracetamol-induced liver toxicity to provide the scientific basis for its use in traditional medicine.

MATERIALS AND METHODS

Plant Collection

Hemigraphis colorata the whole plant was collected in December 2020 from Changaramkulam and were authenticated (Specimen No: 005) by Dr. Kishore Kumar, Head of the Department Botany, Farook College, Kozhikode, Kerala, India. The voucher specimen was deposited in the Department of Botany, Farook College for future reference.

Preparation of Crude Extract

The whole part of the authenticated plant was dried under shade and finally pulverised into a coarse powder with the help of a mechanical grinder and subjected to successive solvent extraction with ethanol by continuous hot percolation method using the soxhlet apparatus [7]. The crude samples were subjected to qualitative chemical tests for the detection of various constituents like alkaloids, terpenoids, tannins, glycosides, flavonoids, saponins, amino acids, carbohydrates and phenolic compounds [8].

EXPERIMENTAL DESIGN

***In-vitro* methods**

DPPH Scavenging assay

0.3mM solution of DPPH in 100% ethanol (1ml) was prepared and allowed to stand at room temperature for 2-3 hours.3 ml of the plant extract dissolved in ethanol at different concentrations. Shaken and allowed to stand at room temperature for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer. The % scavenging activity at different concentrations with ascorbic acid as standard [9].

Percentage inhibition (% I) = (Abs control – Abs sample / Abs control)

Nitric oxide free radical scavenging activity

The method was assessed by the Griess reagent assay with some modifications. The reaction mixture consisted of 2 ml 10mM sodium nitroprusside, 0.5 ml phosphate buffered saline and 0.5 ml of various concentrations of extract/fractions. The mixture was incubated for 150 minutes at 250 C and 0.5 ml of the mixture was mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid). It was allowed to stand for 5 minutes followed by an addition of 1ml of 1% naphthylethylenediaminedihydrochloride. The mixture was incubated for 30 minutes and absorbance was measured at 540nm against the corresponding blank. Ascorbic acid was a positive control. The % scavenging was calculated [9].

Scavenging percentage (S %) = [(A control – A sample) / A control] × 100

***In-vivo* methods**

Animals

Wistar albino rats (150-200g) of either sex were selected for the study and maintained at a controlled temperature of 25 to 280 C with a 12 hours light/dark cycle and fed a standard diet (Amrut, India) and ad water *ad libitum.* These animals were purchased and maintained in the animal house of DevakiAmma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala, India. The protocols were approved by Institutional Animal Ethics Committee (DAMCOP/IAEC/066).

Acute oral toxicity study

The ethanolic extract of *Hemigraphis colorata* was subjected to an acute toxicity study as per OECD guidelines (No: 423). The test procedures minimize the number of animals required to estimate acute oral toxicity. A total of 6 female Swiss albino mice were used. The animals were fasted overnight before the acute toxicity studies. The starting dose was selected as 300 and 2000 mg/kg. After oral administration of a single dose of plant extract, animals were observed individually at least once during the first 30 minutes, followed with special attention given during the first 4 hours, periodically for 24 hours and daily thereafter for a total of 14 days [10].

Paracetamol induced hepatotoxicity

A total of 30 animals were equally divided into 5 groups each containing 6 animals. Extracts were given orally to the animals. Group I served as normal control which received 0.5% Carboxy methyl cellulose (CMC) solution (1 mL/kg) once daily for 14 days. Group II served as negative control which received Paracetamol (2 g/kg) once daily for 14 days. Group III served as reference control and received Silymarin (100 mg/kg) once daily for 14 days. Group IV and Group V received ethanolic extracts of *Hemigraphis colorata* (200 mg/kg and 400 mg/kg) once daily for 14 days. All groups except Group I received Paracetamol (2 g/kg) once daily for 14 days. On the 14th day animals were sacrificed by cardiac puncture with mild anaesthesia (Thiopentone Sodium 30 mg/kg i.p) and blood samples of animals were collected. Serum was separated for the assessment of liver function parameters. The liver was dissected for the assessment of biochemical parameters [11].

Preparation of liver samples

At the end of the experiment, after blood collection, the rats were sacrificed and the liver was dissected out, immediately washed with ice-cold saline, cleaned and weighed. Small pieces of liver were cut and transferred into a 10% neutral formalin solution for histological studies.

Estimation of biochemical parameters

Biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), gamma-glutamyltranspeptidase (GGTP), alkaline phosphatase(ALP) total bilirubin (TB), total protein (TP), glucose, lipid profile (triglycerides, total cholesterol, HDL, LDL, VLDL) were estimated by using commercial kits. [12, 13]

Statistical Analysis

The data were expressed as mean ± standard error of the mean (SEM). For paired comparisons, a student's t-test analysis was performed. Different groups were assessed by one-way analysis of variance (ANOVA), for multiple comparisons followed by Tukey- Kramer test (GraphPad software). The criterion for statistical significance was set at $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

***In-vitro* evaluation**

The DPPH assay was used to determine the level of antioxidant activity. The IC50 is defined as the concentration of plant extract that can decrease 50% of the initial absorbance of DPPH solution. The extract exhibited a concentration-dependent DPPH free radical scavenging effect.IC_{50} of EEHC is 101.1(µg/ml), comparable with IC_{50} of ascorbic acid 129.3 (µg/ml) (Table No.1). The results indicated that *Hemigraphis colorata* consist of a hydrophilic polyphenolic compound that causes the greater reducing power. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons etc. and is involved in various physiological processes. Incubation of solutions of sodium nitroprusside in PBS at 25±0ºC for 2 hours results in the nitrite production, which is reduced by the tested ethanolic extract of the plant. EEHC showed a concentration-dependent inhibition of NO-induced free radical IC_{50} -109.6µg/ml, compared to ascorbic acid IC_{50} -124.6µg/ml. The ethanolic extract of *Hemigraphis colorata* effectively reduced the generation of nitric oxide from sodium nitroprusside. The ethanolic extract of *Hemigraphis colorata* showed strong nitric oxide scavenging activity that of standard ascorbic acid.

***In-vivo* evaluation**

Acute oral toxicity

In the acute oral toxicity test, there was no lethality observed in any group of animals treated with the ethanolic extract of *Hemographis colorata*. There were no differences in body weight, behaviour, or sensory nervous system responses and no other abnormal activities were detected. The ethanolic extract of *Hemigraphis colorata* was found to be safe up to 2000 mg/kg.

Pharmacological screening

The effect of ethanolic extract of *Hemigraphis colorata* on various biochemical parameters was shown in Table No. 3.(a) and Fig 3. It was observed that administration of PCM (Negative) to rats significantly increased ($p<0.001$) the levels of serum transaminases like SGPT, SGOT, GGTP & ALP level when compared to the control group. The increased levels of these enzymes significantly decreased ($p<0.001$) by the treatment groups' ethanolic extract of *Hemigraphiscolorata* (200 mg/kg & 400 mg/kg) in a dose-dependent manner and were restored near to the normal levels in the Silymarin-treated group when compared to negative group. The effect of ethanolic extract of *Hemigraphis colorata* on various biochemical parameters was shown in Table No. 3.(b) and Fig 4. It was observed that administration of PCM to rats significantly increased ($p<0.001$) cholesterol, LDL & triglycerides levels and significantly decreased ($p<0.001$) HDL levels in the Paracetamol-treated groups when compared with the control

group. This is due to the damaged hepatic cell causing the leaking of all these parameters into the blood and which can be estimated in serum. Ethanolic extract *Hemigraphis colorata* treated group significantly decreased ($p<0.001$) the cholesterol, LDL & triglycerides levels as well as maintained the normal level of HDL when compared with the negative group in a dose-dependent manner. While the standard group restored near to the normal levels. The effect of ethanolic extract of *Hemigraphis colorata* on various biochemical parameters was shown in Table No.3.(c) and Fig 5. It was observed that administration of PCM to rats significantly decreased ($p<0.001$) total protein, and albumin levels and significantly increased ($p<0.001$) globulin levels in paracetamol-treated groups when compared with the control. Ethanolic extract of *Hemigraphis colorata* significantly increased ($p<0.001$) total protein and albumin levels and significantly decreased ($p<0.001$) globulin levels when compared with the negative group in a dose-dependent manner. While the standard group restored near to the normal.

The effect of ethanolic extract of *Hemigraphis colorata* on various biochemical parameters was shown in Table No. 3.(d) and Fig 6. It was observed that administration of PCM to rats significantly increased ($p<0.001$) Total bilirubin and Direct Bilirubin levels in Paracetamol treated groups when compared with the control. Ethanolic extract of *Hemigraphis colorata* significantly decreased ($p<0.001$) Total bilirubin level and Direct bilirubin level when compared with the negative group in a dose-dependent manner. While the standard Silymarin group was restored to normal levels. The effect of ethanolic extract of *Hemigraphis colorata* on various biochemical parameters was shown in Table No.3.(d) and Fig 7. It was observed that administration of PCM to rats significantly increased ($p<0.001$) Glucose levels in Paracetamol treated groups when compared with the control. Ethanolic extract of *Hemigraphis colorata* significantly decreased ($p<0.001$) Glucose level when compared with the negative group in a dose-dependent manner. While the standard group restored near to normal levels.

Organ index

The organ index was calculated to assess the hepatoprotective activity of ethanolic extract of *Hemigraphis colorata* was tabulated as shown in Table No. 4. The organ index of the negative group which was treated with Paracetamol was significantly increased ($p<0.001$) when compared to the control group. While the ethanolic extract-treated group significantly decreased ($p<0.001$) when compared with the negative group in a dose-dependent manner. The standard Silymarin maintained a normal organ weight when compared with the negative group. Histopathological examination of liver sections of the normal control group showed normal cellular architecture with sinusoids with intact hepatocytes and normal tissue pattern and density were the characteristic of the control group. The Paracetamol treated group were diffused with severe necrotic changes and vacuolation of hepatocytes. There showed swollen hepatocytes with degenerative nuclei. The standard group of Silymarin has shown moderate changes in tissue architecture with swollen hepatocytes and diffuse mild degenerative changes. The groups that were treated with ethanolic extract of *Hemigraphis colorata* 200 mg/kg showed severe diffuse necrotic changes. Severe vacuolation of hepatocytes and swollen hepatocytes. While EEHC with 400 mg/kg showed severe diffuse necrotic changes and complete loss of architecture and showed necrosis around the central vein with congestion. The destruction that caused to the hepatic cells made the unbalanced metabolism of hepatic enzymes which might have resulted in abnormal levels of biochemical parameters

DISCUSSION

The liver is the largest organ of great importance involved in vital body processes viz. maintenance of homeostasis, metabolic substances detoxification and disposition of endogenous substances like xenobiotics, drugs, etc., most importantly, the liver is considered to be the centre of metabolic transformation of drugs and other toxins entering from the gastrointestinal tract. Different homeostatic mechanisms get affected, if liver functions are impaired with potentially serious and adverse consequences [14]. Paracetamol is a widely used analgesic and antipyretic drug. It is believed that selective inhibition of the enzyme Cox-3 in the brain and spinal cord explains the effectiveness of Paracetamol in relieving pain and reducing fever. Paracetamol produces hepatic necrosis when ingested in large doses. It is metabolised in the liver primarily to glucuronide and sulphate conjugates. Paracetamol toxicity is due to

the formation of toxic metabolites when a part of it is metabolised by cytochrome P450. Induction of cytochrome P450 or depletion of glutathione is a prerequisite for paracetamol-induced hepatotoxicity [15]. Therefore the hepatoprotective activity of the EEHC against paracetamol-induced hepatotoxicity may be due to the inhibition of cytochrome P450; stimulation of hepatic regeneration or activation of the functions of reticuloendothelial systems. Thus the hepatoprotective activity of these extracts may be due to their ability to affect the cytochrome P450 mediated functions or stabilisation of the endoplasmic reticulum resulting in hepatic regeneration [16]. From the preliminary phytochemical examination and quantitative estimation of bioactive components in EEHC extract, it was observed that the extract was rich in phytoconstituents and showed the presence of Alkaloids, Flavanoids, Glycosides (Cardiac), Saponins, Flavonoids, Tannin, Protein and amino acids, Sterols, triterpenoids and Carbohydrates. The DPPH assay was used to determine the level of antioxidant activity. In the DPPH assay violet colour, DPPH solution is reduced to a yellow-colored product, diphenylpicrylhydrazine, by the addition of the extract in a concentration-dependent manner. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. Our results revealed that the ethanolic extract of *Hemigraphis colorata* had a similar free radical scavenging activity when compared with standard ascorbic acid. The extract exhibited a concentration-dependent DPPH free radical scavenging effect. IC_{50} of EEHC is 101.1 (μg/ml), comparable with IC_{50} of ascorbic acid 129.3(μg/ml).

The results indicated that *Hemigraphis colorata* consist of a hydrophilic polyphenolic compound that causes the greater reducing power. Incubation of solutions of sodium nitroprusside in PBS at 25ºC for 2 hours results of the nitrite production, which is reduced by the tested ethanolic extract of the plant. EEHC showed a concentration-dependent inhibition of NO-induced free radical IC_{50} - 109.6 μg/ml, compared to ascorbic acid IC_{50} - 124.6 μg/ml. The fraction of *Hemigraphis colorata* effectively reduced the generation of nitric oxide from sodium nitroprusside. The ethanolic extract of *Hemigraphiscolorata* showed strong nitric oxide scavenging activity that of standard ascorbic acid. The ethanolic extract of *Hemigraphiscolorata* was subjected to an acute toxicity study as per OECD guidelines (No 423). In acute toxicity, there was no lethality observed in any of the animals after the treatment with the ethanolic extract of *Hemigraphiscolorata*. In the present study, rats treated with Paracetamol alone developed significant hepatic damage and oxidative stress, Paracetamol is responsible for oxidative damage by enhancing hepatic cell membrane peroxidation by free radical formation. the fall in biochemical parameters like HDL, total protein and albumin by paracetamol was moderately maintained in the treatment group of EEHC and standard silymarin. While enzymes like AST, ALT, ALP, GGTP and cholesterol, LDL, triglycerides, globulin, Total bilirubin, Direct bilirubin and glucose were increased in paracetamol treated group due to hepatic cell injuries, which cause the leaking of cellular enzymes into the bloodstream and thus can be measured in serum. EEHC. Histopathological examination of liver sections of the normal control group showed normal cellular architecture with sinusoids with intact hepatocytes and normal tissue pattern and density were the characteristic of the control group [17] Theparacetamol-treated group were diffused with severe necrotic changes and vacuolation of hepatocytes. There showed swollen hepatocytes with degenerative nuclei. The groups that were treated with ethanolic extract of *Hemigraphis colorata* whole plant showed signs of reduced / absence of inflammatory cells, and severe diffuse necrotic changes. Severe vacuolation of hepatocytes in 200 mg/kg of EEHC and complete loss of architecture in 400 mg/kg of EEHC. Hence, the hepatoprotective activity of ethanolic extract of *Hemigraphis colorata* whole plant against paracetamol-induced hepatic toxicity was evaluated.

CONCLUSION

The present study was designed to evaluate the hepatoprotective activity of ethanolic extract of *Hemigraphis colorata* using Paracetamol-induced hepatotoxicity in Wistar albino rats. The results obtained from the present study suggest that the ethanolic extract obtained from *Hemigraphis colorata showed* significant hepatoprotective activity. The activity might be due to the presence of the phytoconstituents including phenols (gallic acid) flavonoids (rutin), glycosides triterpene (squalene), Alkaloids, Glycosides, Saponins, Flavonoids, Tannins, amino acids, Carbohydrates starch, vitamin C, Tryptophan and Quinones in the extract. Our results demonstrated that EEHC possessed

significant protection against paracetamol-induced hepatotoxicity, which might be associated with its antioxidant properties through scavenging free radicals to ameliorate oxidative stress and inhibit lipid peroxidation. The phytochemical analysis revealed the high content of phenolics and flavonoids in ethanol extract, which might be responsible for its stronger biological activities. These preliminary findings on antioxidant and hepatoprotective activities here reported lend support to the use of EEHC as a hepatoprotective agent. Further studies to identify and characterize the active principle and the mechanism.

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AUTHORS' CONTRIBUTION

VS and AAVV designed the study, and wrote the manuscript with an interpretation of the results. Asheena carried out the pharmacological study. SSS prepared the manuscript. All the authors read and approved the final version and agreed to publish it.

Disclosure of conflict of interest

All the authors declare that they have no conflict of interest.

Statement of ethical approval

All applicable International, National and /or Institutional guidelines for the care and use of animals were followed.

Statement of informed consent

Not applicable.

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Table No.1: Percentage inhibition and IC_{50} values of DPPH scavenging assay

S. No.	Concentration (µg/ml)	%Inhibition (mean±SD)	IC_{50} (µg/ml)
	Standard Ascorbic acid		
1	50	17.85±0.43	129.3
2	100	36.01±0.76	
3	150	52.16±0.80	
4	200	75.64±0.57	
	EEHC		
1	50	39.96±0.97	101.0
2	100	47.56±0.49	
3	150	55.76±0.31	
4	200	62.84±0.44	

Table No.2: Percentage inhibition and IC_{50} values of nitric oxide radical scavenging activity.

S. NO.	CONCENTRATION (µG/ML)	%INHIBITION (MEAN±SD)	IC_{50} (µG/ML)
	Standard Ascorbic acid		
1	50	21.85±1.43	124.6
2	100	42.01±0.46	
3	150	57.16±0.57	
4	200	76.86±2.19	
	EEHC		
1	50	39.96±0.97	109.6
2	100	47.56±0.49	
3	150	55.76±0.31	
4	200	62.84±0.44	

Table No.3.(a): Effect of *Hemigraphis colorata* extracts on biochemical parameters in Paracetamol induced hepatotoxicity in rats.

GROUP	PARAMETERS			
	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGTP (IU/L)
Control (0.5% CMC)	75.45± 0.05	68.31± 0.07	48.36± 0.060	85.51± 0.93
Negative (PCM 2 g/kg)	126.65± 0.54**	128.23± 0.09***	82.35± 0.08***	187.60± 0.35***
Standard Silymarin (100 mg/kg) + (PCM 2 g/kg)	88.75± 0.54*	82.36± 0.08***	52.35± 0.07ns	96.70± 1.39***
EEHC (200 mg/kg) + PCM (2 g/kg)	98.33± 0.50*	87.48± 0.08***	59.35± 0.07***	99.50± 0.93***
EEHC (400 mg/kg) + PCM (2 g/kg)	90.90± 0.49**	85.33± 0.04***	55.35± 1.03***	98.40± 1.87***

Values are expressed as Mean± SEM. One way ANOVA comparison between treatment groups with Negative control (Tukey kramer multiple comparison test). The data are considered significant as *** p<0.001, **p<0.01& *p<0.05, non-significant (ns)

Table No. 3.(b): Effect of *Hemigraphis colorata* extracts on biochemical parameters in Paracetamol induced hepatotoxicity in rats.

GROUP	PARAMETERS			
	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dL)	Triglycerides (mg/dL)
Control (0.5% CMC)	69.60± 2.23	37.46± 0.49	29.54± 0.32	58.48± 0.96
Negative (PCM 2 g/kg)	191.44± 3.44***	20.27± 0.93***	58.50± 0.03***	110.10± 0.71***
Standard Silymarin (100 mg/kg) + (PCM 2 g/kg)	77.14± 0.01**	30.80± 0.03***	32.35± 1.50***	62.98± 0.86***
EEHC (200 mg/kg) + PCM (2 g/kg)	82.45± 0.10***	32.87± 0.31***	38.64± 0.63***	72.10± 0.22***
EEHC (400 mg/kg) + PCM (2 g/kg)	79.57± 0.10***	33.32± 0.89***	35.63± 0.32***	65.80± 0.30***

Values are expressed as Mean± SEM. One way ANOVA comparison between treatment groups with Negative control (Tukey kramer multiple comparison test). The data are considered significant as *** p<0.001, **p<0.01& * p<0.05, non-significant (ns).

Table No. 3.(c): Effect of *Hemigraphis colorata* extracts on biochemical parameters in Paracetamol induced hepatotoxicity in rats.

GROUP	PARAMETERS		
	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control (0.5% CMC)	9.35±0.07	3.23±0.10	3.85±0.07
Negative (PCM 2 g/kg)	5.56±0.10***	0.56±0.08***	3.58±0.09***
Standard Silymarin (100 mg/kg) + (PCM 2 g/kg)	8.51±0.10***	3.43±0.10***	3.23±0.10***
EEHC (200 mg/kg) + PCM (2 mg/kg)	6.53±0.10***	2.61±1.13***	2.35±0.07***
EEHC (400 mg/kg) + PCM (2 g/kg)	8.78±0.10***	3.15±0.11***	2.68±0.08***

Values are expressed as Mean± SEM. One way ANOVA comparison between treatment groups with Negative control (Tukey kramer multiple comparison test). The data are considered significant as *** p<0.001, **p<0.01&* p<0.05, non-significant (ns).

Table No. 3.(d): Effect of *Hemigraphis colorata* extracts on biochemical parameters in Paracetamol induced hepatotoxicity in rats.

GROUP	PARAMETERS		
	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Glucose (mg/dL)
Control (0.5% CMC)	1.46 ±0.05	0.23 ±0.07	80.35±0.05
Negative (PCM 2 g/kg)	3.38±0.14***	3.84±0.08***	123.65±0.05***
Standard Silymarin (100 mg/kg) +(PCM 2 g/kg)	1.53±0.07***	0.33±0.07***	85.75±0.07***
EEHC (200 mg/kg) + PCM (2 g/kg)	1.03±0.11***	0.35±0.06***	99.48±0.13***
EEHC (400 mg/kg) + PCM (2 g/kg)	1.89±0.10***	0.26±0.01 ***	87.6±0.16***

Values are expressed as Mean± SEM. One way ANOVA comparison between treatment group with Negative control (Tukey kramer multiple comparison test). The data are considered significant as *** p<0.001, **p<0.01&* p<0.05, non-significant (ns).

Table No. 4: Effect of varying concentrations of *Hemigraphis colorata* ethanolic extract on the Organ index of Wistar Albino Rats.

S. NO.	ANIMAL GROUP	RELATIVE LIVER WEIGHT (MEAN ±SEM)
1.	CONTROL (0.5% CMC)	2.45±0.18
2.	NEGATIVE CONTROL (PCM 2 g/kg)	6.42±0.64***
3.	STANDARD (PCM (2 g/kg) + SILYMARIN (100 mg/kg)	3.37±1.34***
4.	EEHC (200 mg/kg) + PCM (2 g/kg)	4.87±0.56**
5.	EEHC (400 mg/kg) + PCM (2 g/kg)	3.02±0.36*

Values are expressed as Mean± SEM n=6. One way ANOVA comparison between treatment group with Negative control (Tukey kramer multiple comparison test). The data are considered significant as *** $p<0.001$ &**$p<0.01$&*$p<0.05$, non-significant ns.

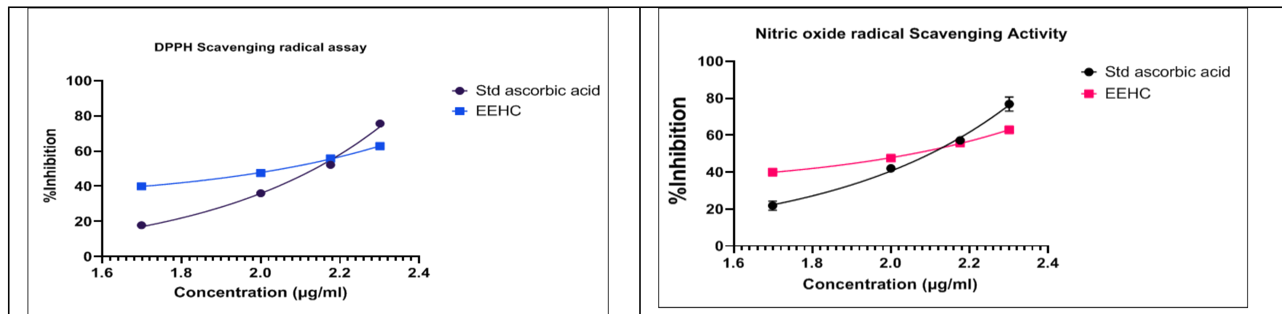

Fig.1: Concentration dependent inhibition of Ascorbic acid and EEHC by DPPH assay.

Fig. 2: Concentration dependent inhibition of ascorbic acid and EEHC by nitric oxide free radical scavenging activity.

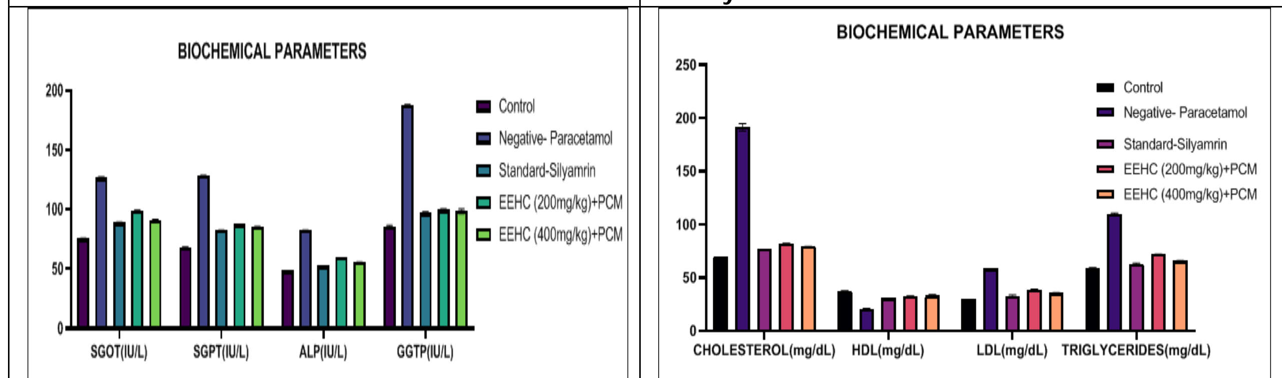

Fig. 3: SGOT, SGPT, ALP, GGTP Variation Observed in different group of rats administrated with EEHC.

Fig. 4: Cholesterol, HDL, LDL, Triglycerides variation Observed in different group of rats administrated with EEHC.

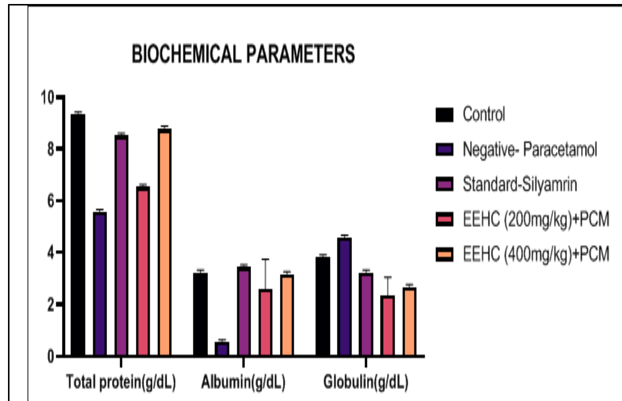

Fig.5: Total protein, Albumin, Globulin Variation Observed in different group of rats administrated with EEHC.

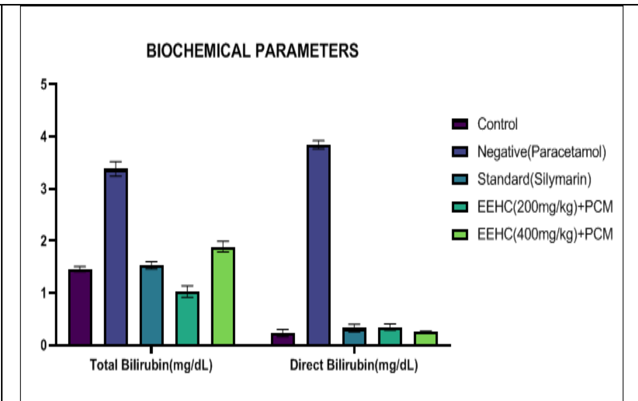

Fig.6: Total Bilirubin, Glucose Variation Observed in different group of rats administrated with EEHC

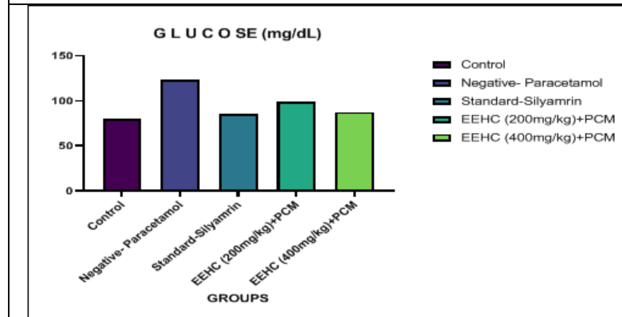

Fig. 7: Glucose Variation Observed in different group of rats administrated with EEHC

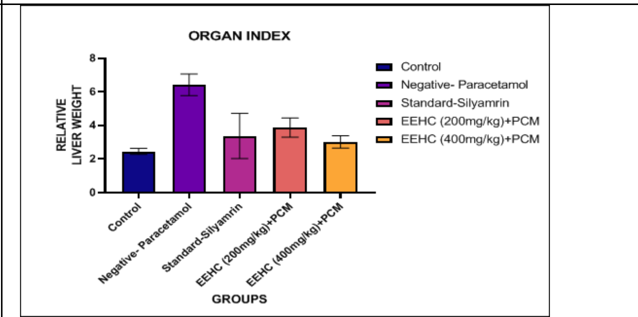

Fig. 8.Effect of EEHC on the relative weight of organ.

Fig. 9: Photograph of normal liver

Fig. 10: Photograph of liver treated with PCM only

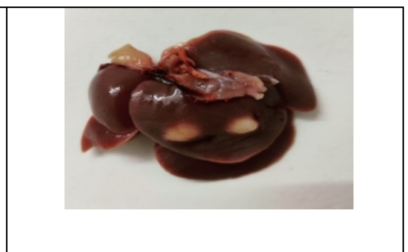

Fig. 11: Photograph of liver treated with PCM & Silymarin

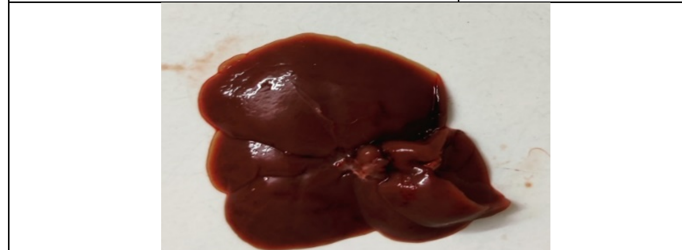

Fig. 12: Photograph of liver treated with Silymarin & PCM& EEHC 200 mg/kg

Fig. 13: Photograph of liver treated with PCM & EEHC 400 mg/kg

Fig. 14. Microphotographs showing histopathology of liver in normal group	**Fig. 15. Microphotographs showing histopathology of liver in PCM group**
Fig. 16. Microphotographs showing histopathology of liver in Standard group	**Fig. 17: Microphotograph of liver treated with PCM & EEHC 200 mg/kg**

Fig. 18: Microphotograph of liver treated with PCM & EEHC 400 mg/kg