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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *CAYRATIA PEDATA* VAR. *GLABRA* AGAINST PARACETAMOL-INDUCED LIVER DAMAGE IN ALBINO RATS

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ABSTRACT: The present pharmacological examination was purely focused on the assessment of the efficacy of ethanolic extract of *C. pedata* var. *glabra* for their protection against Paracetamol overdose-induced hepatotoxicity arbitrate with their phytoconstituents and also observe whether synergistic hepato-protection exists with Silymarin. For animal studies, the Albino rats (150–200 g) were divided into six groups viz., Group I served as control, Group II served as a toxic group, Group III received Silymarin and Group IV, V and VI received plant extracts at the rate of 100, 200 and 400 mg/kg. After group segregation, on 15th day, Paracetamol was administered to groups II, III, IV, V, and VI. The results indicate that compared with Group II the Group I are significant at $p<0.05$. However, Group III, IV, V, VI are significant at $p<0.05$ when compared with Group II. Pretreatment with ethanolic extract-treated groups significantly prevented the physical, biochemical, histological, and functional changes induced by Paracetamol in the liver. The extract showed significant hepatoprotective effects as evidenced by decreased serum enzyme activities like SGOT, SGPT, ALP, and B, whereas the total serum protein was significantly increased as compared with the induced group, which was supported by histopathological studies of the liver. The results concluded that the ethanol extract of the study plant showed significant hepatoprotective activity compared with standard drug and a hepatotoxin. Based on the above results, it is concluded that the *C. pedata* var. *glabra* has potential effectiveness in treating liver damage in a dose-dependent manner.

INTRODUCTION: The liver is considered to be one of the most important vital organs that functions as a center of the metabolism of nutrients such as carbohydrates, proteins, lipids, and excretion of waste metabolites. Furthermore, it is also managing the metabolism production, excretion of drugs, and other xenobiotics from the body, thereby providing protection against foreign substances by detoxifying and eliminating them.

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The bile secreted by the liver has, among other things, plays an important role in digestion [1]. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption, and microbes is well-studied [2].

Medicinal plants take part in a key role in the human health care system. About 80% of the world's population relies on traditional medicine to survive day-to-day life, which is predominantly based on plant materials [3]. Herbal treatments are the most accepted form of traditional medicine. Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients [4].

However, no scientific data regarding the identity and effectiveness of these herbal products were available, except in the treatise of Ayurveda and Unani medicine. The World Health Organization (WHO) has laid emphasis on promoting the use of traditional medicine for health care [5]. Nowadays, plants and natural products have been used traditionally worldwide to prevent and treat liver disease. Scientific research has supported the claims of the medicinal efficacy of several of these herbal compounds, as evidenced by the voluminous work on their hepatoprotective potentials [6]. Nowadays, more than 700 mono- and polyherbal formulations from over a hundred different plants are available for use[7]. Keeping this in view, the present study has been undertaken to identify the phytoconstituents present in ethanolic extract of *Cayratia pedata* var. *glabra* and also investigate the anti-hepatoprotective of active substances by serum enzymatic and antioxidant assays.

Cayratia pedata var. *glabra* belongs to the family Vitaceae, commonly known as "Kattuppirandai" is one such endemic and endangered species in Thaisholai, Nilgiris South Division, and Western Ghats. In the Indian traditional system of medicine, the studied genus is used as astringent, diarrhea, refrigerant, hysteria, liver disease, wound healing, and ulcer [8, 9, 10]. We wanted to build on these findings in relation to paracetamol-induced hepatotoxicity and observe for a synergistic or an additive effect with the combination of *C. pedate* var. *glabra* and a standard hepatoprotection.

MATERIALS AND METHODS:

Chemicals and Instruments: All the chemicals used for the study were of laboratory grade. Watch glass, glass slides, coverslips, conical flasks, and other common glasswares were used in this experiment. Histopathological photographs were taken using Nikon Lab phot 2 Microscopic Unit and Trinocular microscope. Acetone, ethanol solvents, and other reagents used for hepatotoxicity assay were procured from The Precision Scientific Co., Coimbatore, India.

Plant Collection: Aerial plant of *C. pedata* var. *glabra* was collected from Thiashola, Manjoor, Nilgiris South Division, the Western Ghats; before that, we got proper permission from the Principal Chief Conservator of Forests, Chennai, and the District Forest Officer, Ooty under Section 28 (i) of Wildlife Protection Act, 1972, in the month of October, and the voucher herbarium specimen was processed followed by standard methods [11]. The collected plants were identified with the help of the existing Floras [12, 13, 14] and the identity is authenticated with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore (No. BSI/SRC/5/23/2010-11/Tech. 1300), Tamil Nadu.

Shade Drying and Powdering of the Collected Plant Material: Freshly collected aerial plant parts were cleaned to remove the adhering dust and then shade dried at 31°C for 15 days. The shade dried plant materials were air-dried, mechanically ground to a coarse powder, passed through a Wiley Mill to get 60-Mesh size, and stored in airtight containers. The air-dried, powdered plant material was extracted in the Soxhlet apparatus successively with different solvents, and the extracts obtained were used for phytochemical quantification and hepatoprotective studies. Samples were stored in good-grade plastic containers, which are maintained at room temperature until analysis.

Successive Solvent Extraction: The air-dried, powdered plant material was extracted in the Soxhlet apparatus successively with different solvents in the increasing order of polarity [Acetone (56.5°C), Ethanol (78.5°C) and Water (99.98°C)]. Each time, before extracting with the next solvent, the powdered material was dried in a hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 hrs, and the water extract was filtered. The different solvent extracts were concentrated, vacuum dried, and weighed. The extracts were dried by over anhydrous sodium sulfate and stored in sealed vials in refrigerator (5-8°C) until analysis.

Preliminary Phyto-Chemical Screening: Phytochemical screening of different successive solvent extracts was carried out using the standard procedure [15].

Quantitative Phytochemical Studies: Determination of total phenolics and tannins were determined following the procedure [16]. Determination of total flavonoid contents was followed as per the method [17].

Animals: After approval of the Institutional Ethical Committee, pathogen-free Wistar strain Albino rats mass ranging from 150 to 200 g of the male breed were used for the hepatoprotective model. The rats were procured from the Small Animals Breeding Station, Mannuthy, Kerala, India. All *in-vivo* experiments were carried out as suggested by the Institutional Ethical Committee (CPCSEA) (Reg. No. 722/02/a/ CPCSEA). The animals were housed in polypropylene cages (38×23×10 cm) with not more than six animals per cage and maintained under standard environmental conditions (14 h dark/10 h light cycles; temp 25±2°C; 35–60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and freshwater ad libitum. The rats were acclimatized to the environment for 2 weeks before experimental use. Animals fasted overnight before the experimental schedule but have free access to water *ad libitum*.

***In-vivo* Toxicity Studies:**

Behavioural and Toxicological Effects: Toxicity studies of the extracts were carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD). Acute oral toxicity study was done according to OECD guidelines 423. In this experiment, animals were divided into 6 groups of 6 animals each. The first group served as a control and was treated with normal water. Group 2, 3, 4, 5, and 6 were treated with the single graded dose (1000, 2000, 3000, 4000 and 5000 mg/kg bw. orally) of ethanol extract of *C. pedata* var. *glabra,* respectively. Monitoring of the parameters commenced immediately after the administration of the sample. Animals were observed at 0 hr, 1 hr, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 24 hrs, and 72 hrs (with special attention given during the first four hour). Observation includes mortality and clinical signs, which includes changes in skin fur, eyes, and mucous membranes. The gross behaviours like body positions, locomotion, rearing, tremors, and gait were observed. The effect of plant extracts on passivity, grip strength, pain response, stereotypy, vocalization, righting reflex, body weight, and intake was also observed.

***In-vivo* Hepatoprotective and Antioxidant Activity:**

Treatment Regime: The therapeutic effect like hepatoprotective and antioxidant activities of the ethanolic extract of *C. pedata* var. *glabra* were evaluated against Paracetamol induced hepato-toxicity in healthy Wistar strain albino rat model. Rats were segregated into 6 groups of 6 animals each. The experiment was designed as follows.

Group I - Untreated control rats.

Group II - Paracetamol (750 mg/kg p.o.) treated rats.

Group III - Silymarin (25 mg/kg p.o.) + Paracetamol (750 mg/kg p.o.) treated rats.

Group IV- Ethanol extract of *C. pedata* var. glabra (100 mg/kg p.o.) + Paracetamol (750 mg/kg p.o.) treated rats.

Group V - Ethanol extract of *C. pedata* var. glabra (200 mg/kg p.o.) + Paracetamol (750 mg/kg p.o.) treated rats.

Group VI - Ethanol extract of *C. pedata* var. glabra (400 mg/kg p.o.) + Paracetamol (750 mg/kg p.o.) treated rats.

Group, I served as control and received normal saline (2 ml/kg), daily for 15 days. Group II served as a toxic (paracetamol) group, received normal saline daily for 15 days. Group III received Silymarin (Standard hepatoprotective herbal formulation from *Silybum marianum*) every day at the rate of 25 mg/kg p.o. for 15 days. Group IV, V and VI received plant extracts at the rate of 100, 200 and 400 mg/kg p.o. respectively, for 15 days. On 15th day paracetamol (750 mg/kg p.o.) was administered to groups II, III, IV, V, and VI.

Serum Biochemistry: Twenty-four hours after the last dosage of drug administration, all the animals were subjected to mild diethyl ether anesthesia. Blood samples were collected from all groups in a sterile centrifuge tube by cardiac puncture using sterile disposable syringes. The blood samples were allowed to clot for 20-30 min and centrifuged in a refrigerated centrifuge (4°C) at 3000 rpm for 10 min. Fresh serum samples were stored at -20°C for identifying biochemical parameters *viz*., Total protein, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), and total bilirubin.

Assessment of Liver Function: Immediately after blood withdrawal, all the animals were euthanized by cervical dislocation. Liver samples were carefully excised, perfused with ice-cold normal saline, plotted dry with blotting paper, weighed, cut into pieces, and milled (homogenized in 0.025 M Tris-HCl buffer of pH 7.5) to paste using a glass Teflon potter homogenizer with 20 up and down motions at half maximum speed to give a 10% (w/v) liver homogenate. After centrifugation at 10,000 rpm for 20 min at 4°C, the clear supernatant obtained was used to measure thiobarbituric acid reactive substances (TBARS). For the estimation of enzymic antioxidant levels, tissue was minced, homogenized (10% (w/v) in 0.l M phosphate buffer (pH 7.0), and centrifuged for 10 min. The resulting supernatant was used for enzymatic assays [18] *viz*., Estimation of Serum Glutamate Oxaloacetate Transaminase [SGOT] and Serum Glutamate Pyruvate Transaminase SGPT [19], Estimation of Alkaline Phosphatase ALP [20], Estimation of total bilirubin [21], Protein concentration in the liver supernatants was measured [22] using bovine serum albumin as a standard, Estimation of hepatic lipid peroxidation LPO [23], Estimation of superoxide dismutase activity SOD [24], Estimation of catalase activity CAT [25] and Estimation of glutathione peroxidase (GPx) activity [26]. GST activity was determined spectrophotometrically by method [27] with some modifications.

Histological Investigation: Liver slices fixed for 48 h in 10% formalin were processed for paraffin embedding following the standard microtechnique [28]. Sections (5 μm) of livers stained with hematoxylin and eosin were evaluated for histopathological changes under a light microscope.

RESULTS AND DISCUSSION:

Preliminary Phyto-Chemical Screening: A composite or a group of chemical substance present can serve as an "Active marker" and the presence of this chemicals and concentration of the same can be followed to fix on the genuineness of the drug formulation [29]. To examine the chemical constituents of plant powder of *C. pedata* var. *glabra*, the successive solvent extracts were subjected to qualitative and quantitative phytochemical screening. The preliminary phytochemical screening of the different extracts showed carbohydrates, proteins, amino acids, alkaloids, anthraquinones, flavonoids, glycosides, phenols and tannins, steroids, and sterols tri-terpenoids, and volatile oil. The acetone extraction was more efficient than ethanol and water extract **Table 1**. All the extracts showed a negative response to saponins. The results obtained from the preliminary phytochemical screening will reveal the useful findings of the chemical nature of the drug, and it is the base for quantification. This study provides referential information for the identification and characterization of *C. pedata* var. *glabra* and its extracts.

Quantitative Phytochemical Evaluation: As antioxidants, polyphenols protect cell constituents against oxidative damage and limit the risk of various degenerative diseases associated with oxidative stress by acting directly on ROS or by stimulating endogenous defense system [30]. Total phenolics and tannin content of different solvent extracts of *C. pedata* var. *glabra* were studied and expressed as tannic acid equivalent. As shown in **Table 2** the total phenolic content was maximum in ethanolic extract (131.7±3.6 mg/g) followed by acetone extract (56.8±0.8 mg/g). The minimum was recorded in water extract (54.1±1.8 mg/g). When compared with another solvent, ethanol extract registered higher levels of tannin content (52.8±12.9 mg/g) followed by acetone extract (24.1±4.5 mg/g). Total flavonoid content was studied and expressed as rutin equivalent. The total flavonoid content was maximum in ethanolic extract (72.1±0.9 mg/g) followed by water extracts (13.8±0.2 mg/g). Based on these results, ethanolic extract of *C. pedata* var. *glabra* was selected for antioxidant and antiulcer studies.

***In-vivo* studies:**

Acute Toxicity Studies: Assessment of the behavior of animals (Wistar albino rats) was carried out by general observations of each animal immediately after the administration of the drug at hourly basis (0 hr, 1 hr. 2 hrs, 4 hrs, 6 hrs, 8 hrs, 24 hrs, and 72 hrs). Any change or abnormalities recorded could be an indication of toxicity. The test animals at all dose levels showed no significant changes in behavior before and after administering an oral dose of ethanolic extract of *C. pedata* var. *glabra* **Table 3**.

***In-vivo* Hepatoprotective and Antioxidant Activities:** The liver is considered to be highly sensitive to toxic agents. The study of hepatic enzyme activity can be used as a valuable tool in the assessment of clinical and experimental liver damage. In the present study, oral administration of Paracetamol caused significant liver damage as evidenced by altered biochemical parameters. Administration of rats with ethanolic extracts of *C. pedata* var. *glabra* powder attenuated the elevated enzyme activities produced by the Paracetamol and led to subsequent recovery towards normalization of these enzymes **Table 4**. Paracetamol-induced hepatic injuries are commonly used in experimental models for the screening of hepatoprotective drugs [31]. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the bloodstream. The leakage of cellular enzymes into the plasma is an obvious sign of hepatic injury. It was observed that the size of the liver reduced in Paracetamol intoxicated rats, whereas it was normal in all drug tested groups **Table 5**. The significant increase ($p<0.05$) in liver weight when the drugs were administrated indicates their hepatoprotective role.

The hepatoprotective efficacy of the ethanolic extract of *C. pedata* var. *glabra* against Paracetamol-induced liver injury in Wistar albino rats is shown in **Table** 4, 5, and 6, Plate 1A to 1F. Treatment of the experimental rats with Paracetamol (750 mg/kg p.o.) markedly enhanced the activity of the marker enzymes such as SGOT, SGPT, ALP, and bilirubin significantly ($p<0.05$). Transaminases that play a vital role in protein metabolism [32] are located in the cytoplasm and in the mitochondria. The level of bilirubin in the blood serum indicated injury to the liver hepatocytes.

The total serum protein was significantly decreased as compared with the respective control value. However, pretreatment of the rats with ethanolic extract of *C. pedata* var. *glabra* (100, 200, and 400 mg/kg bw./day for 15 days) significantly restored the elevated enzyme levels to a normal level, exhibiting a clear dose-dependent response **Table 4**. An increase in serum bilirubin in Paracetamol-treated rats is an indication of hepatic damage [33] and might be due to the destruction of erythrocytes by toxic metabolites leading to overproduction or failure to excrete bilirubin. Co-administration of ethanol extract of *C. pedata* var. *glabra* offered hepatoprotection, which is evidenced by the inhibition of the rise in bilirubin level. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [34]. The antioxidant activity of ethanolic extract of *C. pedata* var. *glabra* against Paracetamol-induced hepatotoxicity is presented in **Table 6**.

Oral administration of Paracetamol (750 mg/kg p.o.) to the normal animals caused significantly ($p<0.05$) elevated lipid peroxidase level with a concomitant decrease in the activities of the antioxidant marker enzymes such as SOD, CAT, GPx, and GST when compared with the control. Contrarily, the increased level of lipid peroxidation in Paracetamol-treated animals was significantly ($p<0.05$) reduced by the administration of the plant extract **Table 6**.

Recently reported data to suggest that paracetamol hepatotoxicity is mediated by initial metabolic oxidation, covalent binding, and subsequent activation of macrophages to form reactive oxygen and nitrogen species [35]. Lipid peroxidation, an autocatalytic process resulting from oxidative stress, contributes to the initiation and progression of liver damage. Paracetamol-induced depletion of SOD, CAT, GPx and GST levels were also significantly ($p<0.05$) restored by the administration with ethanolic extract of *C. pedata* var. *glabra* in a dose-dependent manner. It is suggested to play a role in the defense mechanism against endogenously generated superoxide anion [36]. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and its highest activity is found in the red cells and in the liver [37]. Glutathione-S-transferase (GST) is a soluble protein located in cytosol. It plays an important role in the detoxification and excretion of xenobiotics by conjugating them with glutathione [38].

The Paracetamol-induced hepatotoxicity is already evident in literature, as indicated by increased levels of alanine-leucine-transaminase, 24 hrs after a single injection to rats of 400 - 500 mg paracetamol per kg body weight (3.3-4.0 mmol kg^{-}

[1]). Its toxicity is associated with the depletion of hepatic GSH, followed by covalent binding of NAPQI, produced by cytochrome P $_{450}$, to tissue proteins [39]. When GSH levels are low, NAPQI fails to be detoxified by conjugation; it accumulates and causes liver injury. Glutathione (GSH) is one of the most abundant tripeptide non-enzymatic biological antioxidants present in the liver. Its functions are concerned with removing free radical species such as hydrogen peroxide, superoxide radicals, and alkoxy radicals, maintaining membrane protein thiols and serving as a substrate for glutathione peroxidase GPx [40].

The hepatoprotective and antioxidant effects of *C. pedata* var. *glabra* were statistically compared with that of standard reference drug Silymarin. The percent hepatoprotection in terms of changes in certain biochemical parameters and antioxidant enzyme activities was calculated and depicted in **Fig. 1** and **2**.

Histopathological Studies: The hepatoprotective effect of the test drug against Paracetamol-induced injury was further confirmed by histopathological examination. The changes produced by the over dose of Paracetamol are illustrated in **Plates 1A** to **1F**. Liver sections from control rat showed normal hepatic architecture, where the hepatocytes are arranged around the central vein and altered with blood sinusoids. Each hepatic cell possesses a limiting membrane, centrally placed large nucleus and prominent nucleoli. No pathological changes were observed **Plate 1A**. Liver samples of Paracetamol (750 mg/kg p.o.) administrated rats showed extensive vascular degeneration, marked centrilobular necrosis, vacuolization with disappearance of nuclei. There is periportal inflammation by mononuclear cells of which lymphocytes predominate. Areas of piece-meal necrosis, apoptosis and steatosis are observed. In focal areas there is bile stasis **Plate 1B**. Silymarin (25 mg/kg p.o.) treated rat liver section showed no significant portal / periportal inflammation. Liver cell necrosis/apoptosis are not observed. A few central veins appear congested and dilated **Plate 1C**. Test drug at the rate of 100 mg/kg p.o. and 200 mg/kg p.o. produced only mild degenerative changes without any centrilobular necrosis of the hepatocytes. Steatosis is observed focally. There is patchy interstitial aggregation of lymphocytes without associated necrosis/apoptosis **Plate 1D** and **1E**. Liver section of the test drug (400 mg/kg p.o.) treated rats showed marked improvement with the normalcy of hepatic cells, central vein, and portal vein. There is no inflammatory cell in filtration. There is no steatosis either **Plate 1F**. The sample at 400 mg/kg p.o. recorded the significant result. Even at lower doses (200 mg/kg p.o. and 100 mg/kg p.o.) there is no evidence of liver cell necrosis/apoptosis, which is a significant improvement.

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF *C. PEDATA* VAR. *GLABRA*

S. no.	Constituents	Acetone	Ethanol	Water
1	Carbohydrates	-	-	+
2	Proteins and amino acids	-	+	+
3	Alkaloids	+	-	-
4	Anthroquinones	+	-	-
5	Flavonoids	+	+	+
6	Glycosides	+	+	+
7	Phenols and tannins	+	+	+
8	Saponins	-	-	-
9	Steroids and sterols	+	-	-
10	Triterpenoids	+	+	+
11	Volatile oil	+	-	-

Note: '+','-' indicates the presence / absence of compounds.

TABLE 2: ESTIMATION OF TOTAL PHENOLICS, TANNIN AND TOTAL FLAVONOID CONTENT OF DIFFERENT SOLVENT EXTRACTS OF *C. PEDATA* VAR. *GLABRA* PLANT POWDER

S. no.	Extraction Medium	Total phenolics (mg TAE/g extract) #	Tannin (mg TAE/g extract) #	Total flavonoid (mg RE/g extract) #
1	Acetone	56.8 ± 0.8	24.1 ± 4.5	13.5 ± 1.0
2	Ethanol	131.7 ± 3.6	52.8 ± 12.9	72.1 ± 0.9
3	Water	54.1 ± 1.8	10.5 ± 3.2	13.8 ± 0.2

Values are means of three independent analysis ± Standard Deviation TAE - Tannic acid equivalent; RE - Rutin equivalent.

TABLE 3: TOXICOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF STUDY PLANT ON ACUTE TOXICITY TEST IN WISTAR ALBINO RATS

S. no.	Response	Animals		S. no.	Response	Animals	
		Before treatment	After treatment			Before treatment	After treatment
1	Alertness	Normal	Normal	10	Gripping strength	Normal	Normal
2	Grooming	Absent	Absent	11	Pinna reflux	Present	Present
3	Restlessness	Absent	Absent	12	Corneal reflux	Present	Present
4	Touch response	Absent	Absent	13	Writhing	Absent	Absent
5	Torch response	Normal	Normal	14	Pupils	Normal	Normal
6	Pain response	Normal	Normal	15	Urination	Normal	Normal
7	Tremors	Absent	Absent	16	Salivation	Normal	Normal
8	Convulsion	Absent	Absent	17	Skin colour	Normal	Normal
9	Righting reflex	Normal	Normal	18	Lacrimation	Normal	Normal

TABLE 4: EFFECT OF ETHANOLIC EXTRACT OF *C. PEDATA* VAR. *GLABRA* ON SERUM BIOCHEMICAL PARAMETERS AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

S. no.	Treatment	Dose (mg/kg b.w)	SGOT (IU/L) #	SGPT (IU/L) #	ALP (IU/L) #	Bilirubin (mg/100 ml) #	Serum total protein (mg/100 ml) #
1	Control	--	36.41 ± 3.77	44.62 ± 2.30	142.51 ± 4.46	0.84 ± 0.05	11.66 ± 0.66
2	Paracetamol	750	83.42 ± 2.01[a]	87.99 ± 3.20[a]	316.53 ± 9.91[a]	3.86 ± 0.52[a]	5.37 ± 0.23[a]
3	Silymarin + PCM	25 + 750	43.11 ± 1.90[b]	49.43 ± 1.71[b]	159.53 ± 1.86[b]	1.00 ± 0.10[b]	10.82 ± 0.43[b]
4	CPE + PCM	100 + 750	69.26 ± 2.73[b]	72.97 ± 1.19[b]	212.29 ± 2.11[b]	1.87 ± 0.17[b]	8.16 ± 0.34[b]
5	CPE + PCM	200 + 750	58.01 ± 2.52[b]	64.23 ± 1.90[b]	187.95 ± 4.67[b]	1.19 ± 0.13[b]	9.06 ± 0.28[b]
6	CPE + PCM	400 + 750	44.26 ± 2.75[b]	50.11 ± 2.61[b]	166.46 ± 7.01[b]	1.05 ± 0.10[b]	10.38 ± 0.25[b]

Values are means of independent analysis ± Standard Deviation (n=6); a - Group II compared with Group I are significant at $p<0.05$; b - Group III, IV, V, VI compared with Group II are significant at $p<0.05$; CPE - Ethanolic extract of *C. pedata* var. *glabra* ; PCM – Paracetamol; SGOT - Serum Glutamate Oxaloacetate Transaminase (IU/L); SGPT - Serum Glutamate Pyruvate Transaminase (IU/L); ALP - Alkaline Phosphatase (IU/L)

TABLE 5: EFFECT OF ETHANOLIC EXTRACT OF *C. PEDATA* VAR. *GLABRA* ON LIVER WEIGHT AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

S. no.	Treatment	Dose (mg/kg b.w)	Liver weight g/100 g. b.w. #
1	Control	--	7.02 ± 0.50
2	Paracetamol	750	5.08 ± 0.44[a]
3	Silymarin + PCM	25 ± 750	6.28 ± 0.50[b]
4	CPE + PCM	100 ± 750	5.70 ± 0.35[b]
5	CPE + PCM	200 ± 750	6.45 ± 0.29[b]
6	CPE + PCM	400 ± 750	6.55 ± 0.42[b]

Values are means of independent analysis ± Standard Deviation (n=6); a - Group II compared with Group I are significant at $p<0.05$; b - Group III, IV, V, VI compared with Group II are significant at $p<0.05$; CPE - Ethanolic extract of *C. pedata* var. *glabra*; PCM – Paracetamol

TABLE 6: EFFECT OF ETHANOLIC EXTRACT OF *C. PEDATA* VAR. *GLABRA* ON CERTAIN ANTIOXIDANT ENZYMES OF LIVER CELLS AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

S. no.	Treatment	Dose (mg/kg b.w)	LPO #	SOD #	CAT #	GPx #	GST #	Liver total protein (gm/dl) #
1	Control	--	02.15±0.16	0.317±0.011	1.91±0.13	111.71±6.58	1.14±0.01	11.25±0.18
2	Paracetamol	750	11.04±0.92[a]	0.152±0.006[a]	0.74±0.05[a]	040.07±2.58[a]	0.55±0.04[a]	07.32±0.34[a]
3	Silymarin + PCM	25 + 750	02.72±0.25[b]	0.288±0.008[b]	1.72±0.10[b]	101.43±3.90[b]	1.06±0.04[b]	10.05±0.29[b]
4	CPE + PCM	100 + 750	04.92±0.51[b]	0.208±0.016[b]	1.13±0.04[b]	078.41±3.43[b]	0.71±0.02[b]	09.35±0.30[b]
5	CPE + PCM	200 + 750	03.96±0.31[b]	0.238±0.008[b]	1.36±0.07[b]	088.41±3.18[b]	0.87±0.04[b]	10.16±0.45[b]
6	CPE + PCM	400 + 750	02.74±0.21[b]	0.282±0.015[b]	1.68±0.06[b]	102.00±5.32[b]	1.09±0.03[b]	10.16±0.32[b]

Values are means of independent analysis ± Standard Deviation (n=6); a - Group II compared with Group I are significant at $p<0.05$; b - Group III, IV, V, VI compared with Group II are significant at $p<0.05$; CPE - Ethanolic extract of *C. pedata* var. *glabra*; PCM – Paracetamol; LPO – Lipid peroxidase (µ moles of TBARS / min/mg protein); SOD – Superoxide dismutase (Units/min / mg protein); CAT – Catalase (µ moles of H_2O_2 consumed / min/mg protein); GPx – Glutathione peroxidase (µ moles of GSH oxidized / min/mg protein); GST – Glutathione -S- transferase (µ moles of CDNB conjugation formed / min/mg protein)

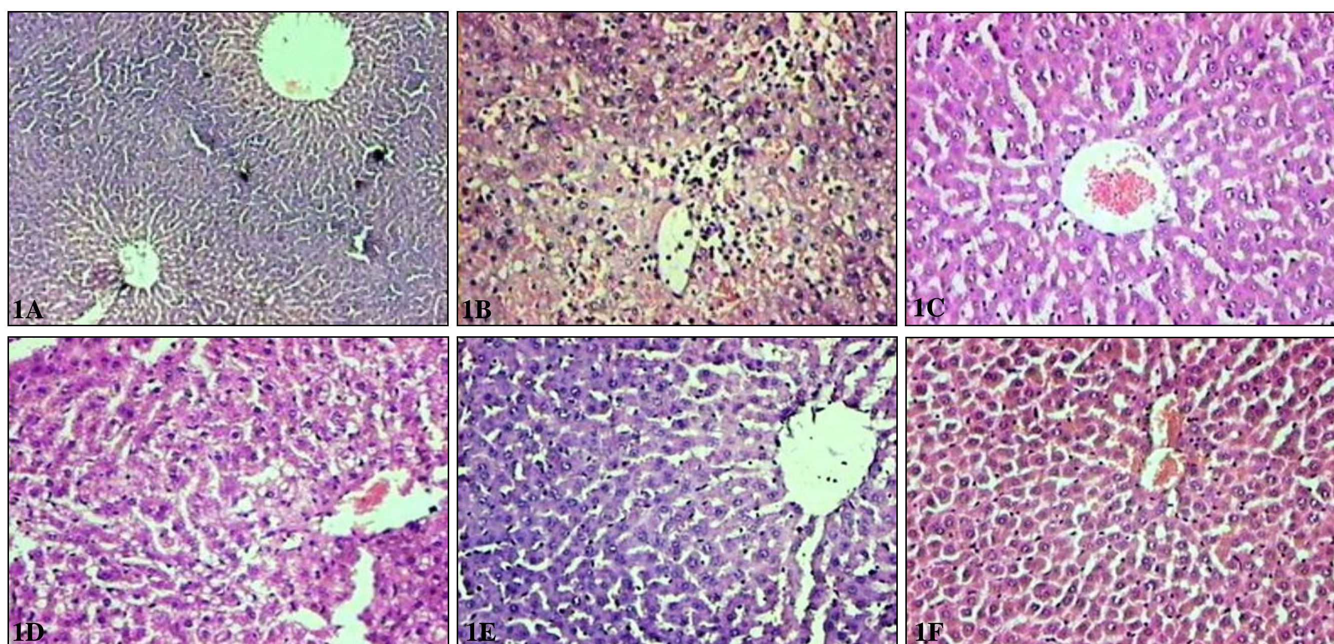
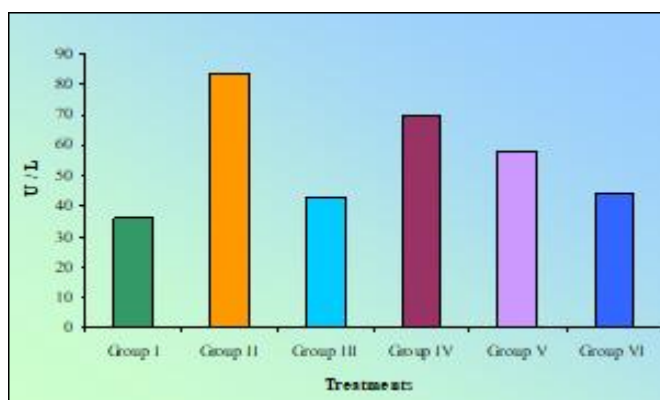
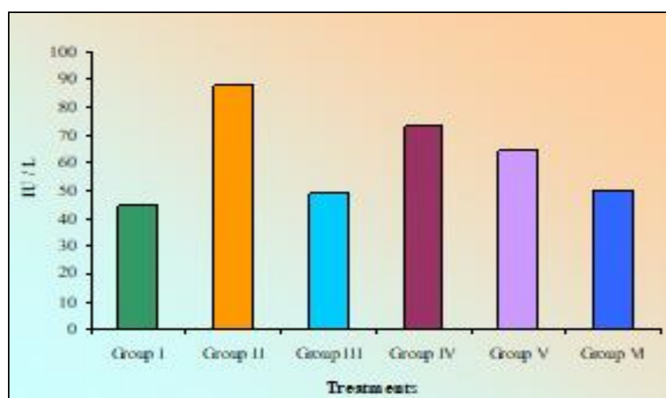


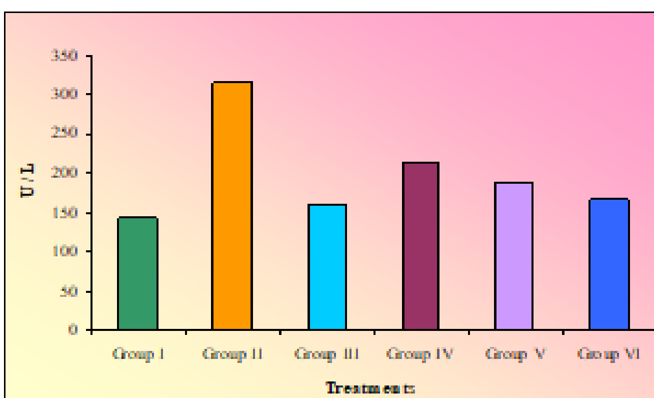
PLATE 1: REPRESENTATIVE PHOTOMICROGRAPHS OF HISTOPATHOLOGICAL CHANGES SHOWING THE EFFECT OF *C. PEDATA* VAR. *GLABRA* ON PARACETAMOL-INDUCED HEPATOTOXICITY INWISTAR ALBINO RATS1A. 1A: LIVER OF CONTROL GROUP: LIVER SHOWING NORMAL HISTOLOGY. 1B: LIVER OF RATS TREATED WITH PARACETAMOL (750 MG/KG P.O.) LIVER SHOWING PERIPORTAL INFLAMMATION BY LYMPHOCYTES AND NECROSIS. 1C: LIVER OF RATS TREATED WITH PARACETAMOL AND STANDARD DRUG SILYMARIN (25 MG/KG P.O.) LIVER SHOWING NORMAL HEPATOCYTES AND A CONGESTED CENTRAL VEIN. 1D: LIVER OF RATS TREATED WITH PARACETAMOL AND TEST DRUG AT THE RATE OF 100 MG/KG P.O. LIVER SHOWING FOCAL AREAS OF STEATOSIS. 1E: LIVER OF RATS TREATED WITH PARACETAMOL AND TEST DRUG AT THE RATE OF 200 MG/KG P.O. LIVER SHOWING NORMAL CENTRAL VEIN AND HEPATOCYTES. 1F: LIVER OF RATS TREATED WITH PARACETAMOL AND TEST DRUG AT THE RATE OF 400 MG/KG P.O. LIVER SHOWING COMPLETE REVERSAL OF PATHOLOGICAL CHANGES AND NORMAL HISTOLOGY



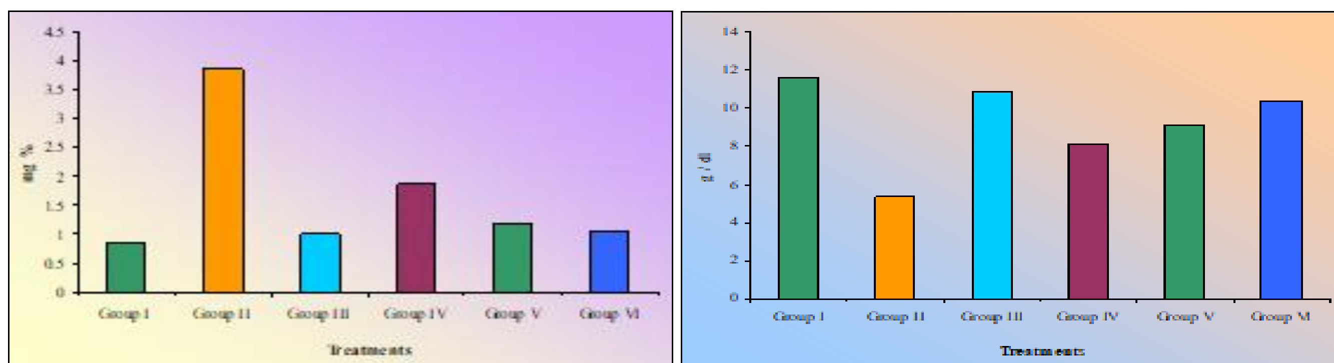
SGOT



SGPT



ALP



BILIRUBIN **TOTAL PROTEIN**

FIG. 1: EFFECT OF ETHANOLIC EXTRACT OF *C. PEDATA* VAR. *GLABRA* ON SERUM BIOCHEMICAL PARAMETER AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS. Group I-Control, Group II - Paracetamol 750 mg/kg b.w. Group III - Silymarin + PCM (25 + 750mg/kg b.w.), Group IV - CPE + PCM (100 + 750mg/kg b.w.), Group V- CPE + PCM (200 + 750mg/kg b.w.), Group VI - CPE + PCM (400 + 750mg/kg b.w.)

LPO **SOD**

CAT **GPx**

GST **TOTAL PROTEIN**

FIG. 2: EFFECT OF ETHANOLIC EXTRACT OF *C. PEDATA* VAR. *GLABRA* ON ANTIOXIDANT ENZYMEOF LIVER CELLS AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS. Group I-Control, Group II - Paracetamol 750 mg/kg b.w. Group III - Silymarin + PCM (25 + 750mg/kg b.w.), Group IV - CPE + PCM (100 + 750mg/kg b.w.), Group V- CPE + PCM (200 + 750mg/kg b.w.), Group VI - CPE + PCM (400 + 750mg/kg b.w.)

CONCLUSION: The result of the study points indicates that the ethanol extract of *C. pedata* var. *glabra* contains maximum phytochemical agents with antioxidant and free radical scavenging properties. The results of estimation of total phenolic and total flavonoid content point to the fact that the antioxidant and scavenging activities may be attributed to the presence of these secondary metabolites. From our results obtained, it can be concluded that this plant extract can be used as an antiulcer drug in combination with other drugs or alone. To use the aerial parts of *C. pedata* var. *glabra* for its potential antioxidant and antiulcer activity, further studies such as isolation, identification, chemical characterization, and *in-vivo* studies of these compounds may be carried in the future. In the future, it can be used as a bioactive source of natural antioxidants and are potential natural resources for the pharmacology of functional foods. The test animals at all dose levels showed no significant changes in behavior before and after the administration of an oral dose of ethanolic extract of *C. pedata* var. *glabra* denote the non-toxicity of the test plant studied.

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REFERENCES:

1. Dossing M and Sonne J: Drug induced hepatic disorders: Incidence, management and avoidance. Drug Safety. Pubmed 1993; 9: 441-9.
2. Agarwal SS: Development of hepatoprotective formulations from plant sources. In: Pharmacology and therapeutics in the New Millennium. Edited by Gupta SK, Narosa Publishing House, New Delhi, 2001: 357-58.
3. World Health Organization . Regional Office for the Western Pacific . Research guidelines for evaluating the safety and efficacy of herbal medicines . Manila: WHO Regional Office for the Western Pacific . 1993. https://apps.who.int/iris/handle/10665/207008
4. WHO Media Centre: Traditional Medicine Fact Sheet No. 134. 2008. Available from: http://www.who.int/media centre/factsheets/2003/fs134/en/
5. Jain SK: Ethnobotany and research on medicinal plants in India. Ciba Foundation Symposium 1994; 185: 153-64.
6. Handa SS, Chakraborty KK and Sharma A: Antihepatotoxic activity of some Indian herbal formulations as compared to silymarin. Fitoterapia 1986; 57: 307.
7. Ram VJ: Herbal preparations as a source of hepato-protective agents. Drug News Perspect 2001; 14(6): 353-63.
8. Sharmila S, Kalaichelvi K and Dhivya SM: Pharma-cognostical and Phytochemical analysis of Cayratiapedata var. glabra – A Vitaceae Member, International Journal of Pharmaceutical Sciences and Research 2018; 9(1): 218-26.
9. Ambasta SP: The Useful Plants of India. Publication & Information Directorate, CSIR, New Delhi 1986; 608-09.
10. Khare CP: Indian medicinal plants: An illustrated dictionary. Springer Science & Business Media 2008.
11. Jain SK and Rao RR: Hand Book of Field and Herbarium Methods. Today and Tomorrow's Printers and Publishers, New Delhi 1976.
12. Fyson PF: The flora of the Nilgiri and Pulney Hill Tops. Madras: Superintendent, Government Press. Vol. III, 1915-20.
13. Gamble JS and Fischer CEC: Flora of the Presidency of Madras. Calcutta. Vol. I, II and III, 1967.
14. Matthew KM: The Flora of the Tamilnadu Carnatic. Tiruchirapalli: The Rapinet Herbarium, St. Joseph's College 1983; 3: 278-79.
15. Kokate CK, Khandelwal KR, Pawar AP and Gohalz SB: Practical Pharmacognosy. New Delhi, India: Vallabh Prakashan. Fourth Edition 1995.
16. Siddhuraju P and Becker K: Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. Journal of Agricultural and Food Chemistry 2003; 51(8): 2144-55.
17. Zhishen J, Mengcheng T and Jianming W: The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 1999; 64(4): 555-59.
18. Murugan P and Pari L: Effect of tetrahydrocurcumin on lipid peroxidation and lipids in streptozotocin-nicotinamide-induced diabetic rats. Basic and Clinical Pharmacology and Toxicology 2006; 99: 122-27.
19. Reitman S. and Frankel S: A calorimetric method for the determination of serum oxaloacetic and glutamic-pyruvic transminase. American Journal of Clinical Pathology 1957; 28(1): 56-63.
20. King EJ and Armstrong AR: A convenient method for determining serum and bile phosphatase activity. Canadian Medical Association Journal 1934; 31(4): 376-81.
21. Malloy HT and Evelyn KA: Estimation of serum bilirubin. Journal of Biological Chemistry 1937; 119: 481.
22. Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976; 72: 248-54.
23. Niehius WG and Samuelson D: Formation of malondialdehyde from phospholipids arrachidonate during microsomal lipid peroxidation. European Journal of Biochemistry 1968; 6(1): 126-30.
24. Marklund S and Marklund G: Involvement of superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 1974; 47(3): 469-74.
25. Sinha AK: Calorimetric assay of catalase. Analytical Biochemistry 1972; 47: 389-94.

26. Rotruck J, Pope A, Ganther H and Swanson A: Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973; 179: 588-90.
27. Habig WH, Pablst MJ and Jakoby WB: Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry 1974; 49(22): 7130-9.
28. Galigher AE and Kozolff EN: In: Essentials of Practical Microtechnique. Lea and Fediger, Philadelphia, Edition 2, 1971: 77-210.
29. Pragasam A: Pharmacognostic studies on *Cissus vitiginea* L. International conference on medicinal plants and herbal drugs: Challenges and opportunities in cultivation, sustainable utilization and conservation (ICMPHD), 2010: 153-54.
30. Hagerman AE, Zhao Y and Johnson S: Methods for determination of condensed and hydrolysable tannins, In: Antinutrients and Phytochemicals in foods, American Chemical Society, Washington DC. 1997: 59-63.
31. Davis DC, Potter WZ, Jollow DJ and Mitchell JR: Species differences in hepatic necrosis after acetaminophen. Life Science 1974; 40: 2099-2109.
32. Bergmeyer HU: In: Methods of enzymatic analysis. Academic press, New York 1963: 3-5.
33. Singh B, Saxena AK, Chandan BK, Agarwal SG and Anand KK: *In-vivo* hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. Indian Journal of Physiology and Pharmacology 2001; 45(4): 435-41.
34. Thabrew MI, Joice PD and Rajatissa W: A comparative study the efficacy of *Pavetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. Planta Medica 1987; 53(3): 239-41.
35. Michael SL, Pumford NR, Mayeux PR, Niesman MR and Hinson JA: Pretreatment of mice with maxrophases inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatology 1999; 30(1): 186-95.
36. Prabhu HR: *In-vivo* inhibition of selenium dependent glutathione peroxidase and superoxide dismutase in rats by diethyl dithiocarbonate. Indian Journal of Experimental Biology 2002; 40(3): 258-61.
37. Chance B, Green Stein DS and Roughton RJW: The mechanism of catalase action-steady state analysis. Archives of Biochemistry and Biophysics 1992; 37: 301-39.
38. Boyer TD, Vessey DA, Holcomb C and Saley N: Studies of the relationship between the catalytic activity and binding on non-substrate ligands by the glutathione – s – transferase. Biochemical Journal 1984; 217: 179-85.
39. Ray SD, Kamandulis LM, Gurule MW, Yorkin RD and Corcoran GB: Ca^{2+} antagonists inhibit DNA fragmentation and toxic cell death induced by acetaminophen. American Journal Experimental Biology 1993; 7: 453-63.
40. Prakash D, Suri S, Upadhyay G and Singh BN: Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. International Journal of Food Sciences and Nutrition 2007; 58(1): 18-28.

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