

**HEPATOPROTECTIVE ACTIVITY OF PAEDERIA FOETIDA IN VITRO AND IN VIVO STUDIES***R. Arunkumar<sup>1</sup>, Arudyuti Chowdhury<sup>2</sup>, Bedabati Chowdhury<sup>3</sup>*<sup>1</sup>Associate Professor, Department of Anatomy, SRM Medical College, Chennai.<sup>2</sup>Associate Professor, Department of Anatomy, SRM Medical College, Chennai.<sup>3</sup>Assistant Professor, Department of Life Sciences, University of Science and Technology, Meghalaya.**ABSTRACT****INTRODUCTION**

To investigate the hepatoprotective activity of methanolic extract of *Paederia foetida* L. against freshly isolated rat hepatocytes and on animals. Freshly isolated rat hepatocytes were exposed to Carbon tetrachloride (1%) along with various concentrations of methanolic extract (MLE) of *Paederia foetida* L. (100, 200, 400 µg/ml) for in vitro studies and the levels of various liver enzymes (GOT, GPT, ALP, Total and Direct Bilirubin) were investigated. For in vivo studies, Wister rats of both sexes were used. The different groups of rats were administered with CCl<sub>4</sub> in olive oil (1:1, v/v) at a dose of 2ml/kg body wt. through subcutaneous route. The plant extract was given orally at a dose of (100, 200, 400 mg/kg.b.w.). The rats were monitored for biochemical changes of liver enzymes (GOT, GPT, ALP, Total and Direct Bilirubin) and histopathological changes. From the experimental results it was proved that the tested extract showed potent hepatoprotection in a dose dependent manner and the dose 400 mg/kg showed potency comparable to standard reference drug Silymarin (100mg/kg.b.w.). The methanolic extracts were able to restore the biochemical levels, histopathological status to normal which were altered due to Carbon tetrachloride or CCl<sub>4</sub> intoxication both in hepatocytes and in animals.

**KEYWORDS**

*Paederia foetida*, Hepatoprotective, Carbon tetrachloride, Hepatocytes, Silymarin.

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**INTRODUCTION:** Liver, the largest organ in vertebrate body, is the major site of intense metabolic activities. Liver injury caused by toxic chemicals and certain drugs has been recognised as a toxicological problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and cause serious adverse effects.<sup>1</sup> Therefore herbal drugs are playing an important role in healthcare programs worldwide, and there is resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. In Ayurveda, an indigenous system of medicine in India has a long tradition of treating liver disorders with plant drugs. Hepatoprotective effect of many plants has been well established,<sup>2</sup> yet there is paucity of information regarding hepatoprotective effects of *Paederia foetida*.

*Paederia foetida* locally called as *Bhedailota* belonging to Rubiaceae, is one the potential herb in North East India used for various medicinal practices. The leaves of the plant release a strong fetid odor when bruised. Methyl mercaptan was reported to be responsible for fetid odor of the plant.<sup>3</sup> It is used to treat enteromegaly, enterosis, flatulence, gastromegaly, rheumatism, rhinosis toothache, stomachache and sore in folk medicine.<sup>4</sup> The major classes of chemical constituents present in this plant are iridoid glycosides, sitosterol, stigmasterol, alkaloids, carbohydrates,

proteins, amino acids and volatile oils. This plant is used in the treatment of gout, diarrhoea, dysentery, piles, inflammation of the liver and as an emetic.<sup>5</sup> This plant also has anti-inflammatory effect.<sup>6</sup> In the present study the hepatoprotective activity of various concentrations of the Methanol extract (MLE) of this plant was evaluated both in vitro and in vivo experimental studies.

**MATERIALS AND METHODS:**

**Extract Preparation:** The plant was collected in the month of September from nearby areas of Guwahati, Assam. The plant was identified in the Department of Botany, Gauhati University, Guwahati, Assam, India. After proper identification the whole plant was shade dried, powdered and was exhaustively extracted in methanol. The extract was concentrated in rotary vacuum evaporator and kept in vacuum desiccator. The extract was thus made ready for use.

The extract was suspended in 5% Tween-80 in distilled water and used for in vivo biochemical activity.

For in vitro studies Carbon tetrachloride and silymarin, were dissolved in 0.5% dimethyl sulphoxide (DMSO) and their concentrations in the incubation medium were adjusted to reach a final concentration of 5mM CCl<sub>4</sub> and 0.5mM silymarin. The concentrations of plant extracts were selected according to the dose response experiment using 3 different concentrations from plant extract (1, 10, 100 µg/ml) dissolved in DMSO. Six replicates were used for each chemical and plant extracts. Cytotoxicity and Cytoprotection were determined by assessing of cell viability using trypan blue exclusion method and also by assessing the leakage of cytosolic enzymes such as GOT, GPT, ALP, Bilirubin. Control replicates were carried out simultaneously under the same

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conditions and at the same time intervals, using DMSO at a final concentration of 0.5%.

**Experimental Animals:** Male Wistar rats (180-210gm body wt.) were used for in vitro experiment and for in vivo studies Wistar rats (180-210gm body wt.) of both sexes were used. Animals were obtained from the animal house of Department of Zoology, Gauhati University for experimental purpose. They were maintained on a standard normal diet, provided with water ad libitum and maintained at ambient room temperature (25°C±2°C). The study was approved by the animal ethics committee and all the ethical norms was strictly followed during the experiment.

### Experimental Studies:

#### In Vitro Studies:

**Isolation of Hepatocytes:** Hepatocytes were isolated using a modified procedure of Seglen (1994).<sup>7</sup> The calcium free HEPES buffer and collagenase solutions were warmed in a water bath (37°C). The rat was anaesthetized by subcutaneous injection with 100mg ketamine/Kg, restrained, and an midline incision was made in the abdominal cavity and a loosely tied ligature was placed around the portal vein approximately 5mm from the liver and the cannula was inserted up to the liver and then the ligature was tightened and heparin was injected into the femoral vein (1000IU). The inferior venacava was cut below the renal vein. Perfusion was performed for 20min (37°C) with calcium free HEPES buffer (pH 7.4), containing 1% Bovine serum albumin at a flow rate of 30ml/min. The liver swells during this time, slowly changing its color from dark red to greyish white. The swollen liver was then perfused with TPVG (Trypsin Phosphate Versene Glucose) solution (50ml) followed by perfusion with calcium free HEPES buffer, which contained additional collagenase (0.075%) and calcium chloride (4mM) at a flow rate of 15ml/min for 20 min.

After the perfusion the lobes were removed and transferred into a sterile petri dish containing calcium free HEPES buffer and dispersed gently. It was transferred into a sterile conical flask and the crude cell suspension was stirred with the help of a magnetic stirrer for 5min to release hepatocytes into the solution. The cell suspension was filtered through a nylon mesh (250µ) and the cell suspension was centrifuged at 1000rpm for 15min. The supernatant was aspirated off and the loosely packed cell pellet was resuspended in calcium free HEPES buffer. This washing procedure was repeated three times. The isolated hepatocytes were counted using haemocytometer while the viability of the cells was assessed by 0.4% trypan blue exclusion technique.<sup>7</sup> Freshly prepared cell suspension had 90% or greater viability prior to each experiment. The isolated hepatocytes were cultured in Eagles MEM, supplemented with 10% inactivated serum at density of 0.5×10<sup>9</sup> cells/L in sterile disposable culture bottles and incubated in a humidified incubator at 37°C under 5%CO<sub>2</sub>.

#### Carbon Tetrachloride (CCl<sub>4</sub>) Induced in Vitro

**Hepatocytes Injury:** CCl<sub>4</sub> induced hepatic injury was carried out by the method determined by Yoshinobnu et al.<sup>8</sup> After an incubation of 24 hr, the hepatocytes were exposed to the fresh medium containing CCl<sub>4</sub> (1%) along with/without various concentrations (100, 200, 400µg/ml) of the methanol extract or the medium alone (as normal). The little percentage of DMSO present in the wells (maximum 0.2%) was found not to affect the experiment. After 60 min of CCl<sub>4</sub> exposure, concentrations of GOT, GPT, ALP, Bilirubin (Total and Direct) in the medium was measured as an indication of hepatocytes necrosis using Marck diagnostic kits.

**In Vivo Studies:** Rats were divided in six groups, with six animals per group (n=6). CCl<sub>4</sub> in olive oil (1:1, v/v) was administered (subcutaneous) at a dose of 2ml/kg body wt. Group-I (Normal control) Group received of 5% Tween-80 (5ml/kg, body weight., per oral) on each day for 4 days & (two doses of olive oil (1ml/kg, subcutaneous) on day-2 and 3. Group-II (CCl<sub>4</sub> control) received 5% Tween-80 like Group-I and given dose of CCl<sub>4</sub> suspension (2ml/kg, body weight, subcutaneous) on day-2 and 3. Group-III, IV and V (Test Groups) received extract suspension (100, 200 and 400mg/kg, per oral) on each day & CCl<sub>4</sub> suspension (2ml/kg, body weight., subcutaneous) on day-2, and day-3 and Group-VI (Reference Group) received Silymarin suspension (100mg kg, per oral) in distilled water daily & CCl<sub>4</sub> suspension (2ml/kg, body weight., subcutaneous) on day-2 & day-3.

Animals were sacrificed under mild ether anaesthesia on day-5, 48hrs after CCl<sub>4</sub> administration.<sup>9</sup> The body weight of each animal was recorded before starting the experiment and just before sacrificing the animals. After sacrifice blood was collected from the carotid artery. Blood samples were kept for 30 min, and then centrifuged at 3000rpm for 15 min. The serum was separated out for biochemical studies of GOT, GPT, ALP, Bilirubin (Total and Direct) using Merck diagnostic kits.

**Collection and Histological Study of Liver:** Liver of sacrificed animals were collected just after the sacrifice and thoroughly perfused in ice-cold saline. The livers were fixed in 10% formosaline (10% v/v formaldehyde in normal saline) for 48hrs. The livers were embedded in liquid paraffin following the standard micro technique (Galigher and Kozoff, 1976).<sup>10</sup> 5µ thick sections of paraffin embedded liver were used for staining (Delafield's haematoxylin and eosin stain) following routine histological procedure. The slides were examined under light microscope.

**Statistical Analysis of the Data:** Values are expressed as Mean±SEM & significance of inter-group differences of each parameter was analysed separately using the one way analysis of variance (ANOVA) and P<0.05 was considered to be significant. Significance within the group was analysed using Student's t test and P<0.01 and P<0.001 was considered to be significant.<sup>11</sup>

**RESULTS AND DISCUSSION:** Hepatoprotective effects on freshly isolated liver hepatocytes. The effects of the methanol extract (MLE) of *Paederia foetida* freshly isolated liver hepatocytes of rats intoxicated with Carbon tetrachloride were depicted in Table 1. The table shows significant increase in the levels of GOT, GPT, ALP, Total and Direct Bilirubin in hepatocytes exposed to CCl<sub>4</sub> in CCl<sub>4</sub> control

group when compared with normal group (P<0.01). The MLE significantly reduced the elevation in a dose dependent manner. Similar result was obtained when hepatocytes were treated with silymarin. Therefore the hepatoprotective effect of the MLE was comparable to the standard drug silymarin.

**Hepatoprotective effects on liver hepatocytes from in vitro studies:**

Groups	Concentration (µg/ml)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Control	-	12±0.42	17±0.16	29±0.27	0.927±0.03	0.084±0.003
CCl <sub>4</sub> Control	1%	78±0.5*	96±0.42*	97±0.3*	2.025±0.042*	0.394±0.02*
MLE	100	27±0.2**	30±0.39**	47±0.25**	1.695±0.016*#	0.206±0.007*#
	200	25±0.3**	32±0.23**	53±0.32**	1.462±0.016*#	0.18±0.004*#
	400	23±0.2**	35±0.26**	59±0.31**	1.268±0.018*#	0.144±0.003*#
Silymarin	1000	15±0.2**	23±0.23**	35±0.42**	1.027±0.033*#	0.092±0.003*#
One way ANOVA	F	4098.74	8690.046	5680.13	283.24	146.24
	Df	3, 20	3, 20	3, 20	3, 20	3, 20
	P	0.05	0.05	0.05	0.05	0.05

**Table 1: Effect of treatments of methanol extract of *Paederia foetida* on the biochemical parameters of freshly isolated rat hepatocytes Values are Mean±SEM, n=6**

P values \*<0.01 when compared with control group, \*# <0.01 when compared with CCl<sub>4</sub> Control group, \*\*<0.001 when compared with CCl<sub>4</sub> Control group using student's t test.

**In Vivo Hepatoprotective Effects on Biochemical Parameters:** The effects of MLE on biochemical parameters of Carbon tetrachloride toxicated rats were given in Table 2. The biochemical results showed a marked increase of all biochemical parameters i.e. GOT, GPT, ALP, Total and Direct Bilirubin after administration of the given dose of CCl<sub>4</sub>. As

shown in table 2 the concurrent treatment of MLE (400mg/Kg) significantly decreased (P<0.001) the elevation of enzymes by the toxin and thus provide satisfactory hepatoprotection in a dose dependent manner which was comparable to the effect of the reference drug Silymarin.

**Hepatoprotective effects on the biochemical parameters from in vivo studies:**

Groups	Concentration (mg/Kg)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Control	-	23.42±1.34	34.5±3.68	47.9±2.91	0.46±0.01	0.3±0.03
CCl <sub>4</sub> Control	2ml/Kg	112.28±5.07*	117.27±4.9*	126.2±8.4*	2.4±0.05*	1.36±0.05*
MLE	100	87±1.05**	87.9±1.69**	95.65±1.8**	1.43±0.14**	0.95±0.01**
	200	51.68±1.9**	57.3±2.58**	82.43±1.12**	0.85±0.02**	0.73±0.03**
	400	38.2±2.08**	39.7±1.2**	66±2.37**	0.63±0.02**	0.49±0.01**
Silymarin	100	37.45±1.23**	37.2±1.66**	65.53±2.23**	0.53±0.01**	0.41±0.02**
One way ANOVA	F	175.67	131.06	48.9	76.891	214.24
	Df	3, 20	3, 20	3, 20	3, 20	3, 20
	P	0.05	0.05	0.05	0.05	0.05

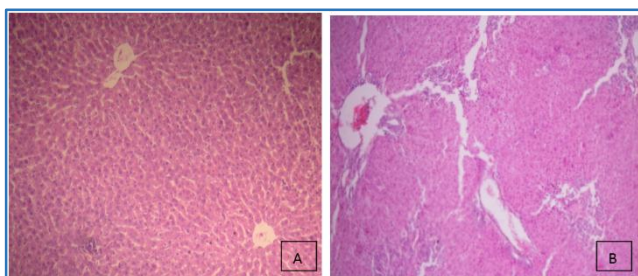
**Table 2: Effect of treatments of methanol extract of *Paederia foetida* on the biochemical parameters of CCl<sub>4</sub> treated rats Values are Mean±SEM, n=6**

P values \* <0.01 when compared with control group, \*\*<0.001 when compared with CCl<sub>4</sub> Control group using student's t test.

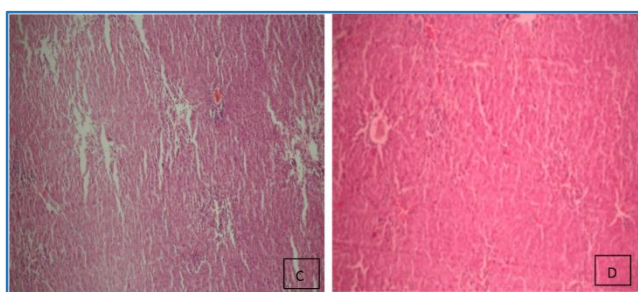
**Results of the Histopathological Studies:** Histopathological observations on the Carbon tetrachloride induced toxicity in liver showed hepatocytes necrosis, swollen hepatocytes and accumulation of fat lobules, necrosis of some of the centrizonal and lobular hepatocytes

perilobular cloudy swelling and cell vacuolization. Necrosis and steatosis are the peculiar characteristics of histopathological symptoms in CCl<sub>4</sub> induced toxicity.<sup>12,13</sup> In the microscopic picture of liver of rats treated with 400mg/Kg of MLE with Carbon tetrachloride, near to normal

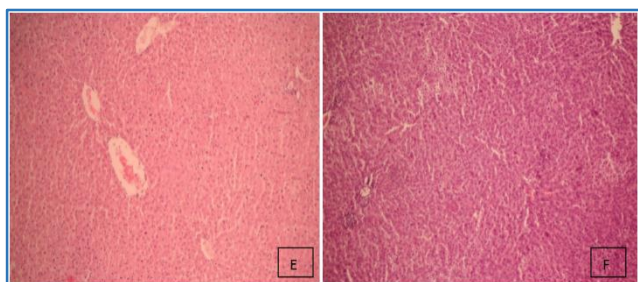
histology was seen. There was no inflammation or necrosis and fatty degeneration. The histopathological photograph of the reference drug silymarin treated liver presented mild necrosis in some areas (Figure 3F). Thus it is established that the 400mg/Kg dose of MLE offered very effective potency in restoring injurious state of liver in comparison to the reference drug and thus provided a very good hepatoprotection against CCl<sub>4</sub>.



**Fig. 1A, 1B**



**Fig. 1C, 1D**



**Fig. 1E, 1F**

**Figure 1:** Photomicrographs of liver slides of experimental animals (H&E, 100x). (A) Normal liver, (B) CCl<sub>4</sub> Control liver, (C) CCl<sub>4</sub> toxicated and MLE 100mg/Kg treated liver, (D) CCl<sub>4</sub> toxicated and MLE 200mg/Kg treated liver, (E) CCl<sub>4</sub> toxicated and MLE 400mg/Kg treated liver, (F) CCl<sub>4</sub> toxicated and silymarin treated liver.

Liver injuries by CCl<sub>4</sub> are commonly used models for the screening of hepatoprotective drugs.<sup>14</sup> The hepatotoxic effects of CCl<sub>4</sub> are largely due to its active metabolite, trichloromethyl radical.<sup>15</sup> These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. These lead to the formation of lipid peroxides which in turn give products like malondialdehyde (MDA) that cause damage to the membrane. This is evidenced by an elevation of the serum

marker enzymes. GOT, GPT, ALP, total and direct bilirubin in plasma has been reported to be sensitive indicators of liver injury.<sup>16</sup> The disturbances in the transport function of the hepatocytes, because of hepatic injury causes leakage of enzymes from cells due to altered permeability of membrane. This results in decreased level of GOT, GPT, ALP and bilirubin in the hepatic cells and a raised level in serum. The present study revealed a significant increase in the activities of GOT, GPT, ALP and bilirubin levels in serum after exposure to the CCl<sub>4</sub> indicating considerable hepatocellular injury. The increase in transaminases was the clear indication of cellular leakage and loss of functional integrity of cell. Decrease in serum GOT, GPT after treatment with the extract in liver damage induced by CCl<sub>4</sub> indicated the potency of the extract in normalizing the structural status of the liver and decrease in serum ALP indicated efficacy of the extract in recovering liver cholestasis whereas decrease in bilirubin indicated the effectiveness of the extract in regularizing functional status of the liver.

In the present study treatment with methanol extract of *Paederia foetida* significantly reduced the damage caused by CCl<sub>4</sub> both in vitro and in vivo studies. The histopathological results further proved the biochemical results and proved the hepatoprotective effect of this plant.

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