Advances in Biological Research 5 (5): 267-272, 2011
ISSN 1992-0067


# Paederia Foetida Protects Liver Against Hepatotoxin-Induced Oxidative Damage

*Borhan Uddin, Taslima Nahar, Mafroz Ahmed Basunia and Shahdat Hossain*

Department of Biochemistry and Molecular Biology,
Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

**Abstract:** The plant *Paederia foetida* has traditionally been used for medicinal purposes, though its mechanisms of beneficial effects have remained largely unknown. To understand the anti-hepatotoxic effects of this plant, we initially induced *in vitro* oxidative stress by Fenton reaction ($FeSO_4$+$H_2O_2$) in the rat liver whole homogenate. Co-incubation of rat liver homogenate with the ethanol extract of *Paederia foetida* leaves significantly decreased the oxidative stress, as indicated by the reduced lipid peroxide (LPO) levels (by 40%). This phenomenon was then confirmed by *in vivo* animal study where the extract was orally administered to rats for 21 successive days. We evaluated its potential effectiveness against *in vivo* carbon tetrachloride ($CCl_4$)-induced hepatic lesions and oxidative stress in male Sprague Dawley rats. As expected from the *in vitro* results, the oral administration of the extract ameliorated the *in vivo* $CCl_4$-induced hepatic LPO levels, determined by the thiobarbituric acid reactive substances (TBARs). The $CCl_4$-induced increase in serum enzyme activities (GPT, GOT, ALP) and bilirubin level were also significantly reduced. The beneficial effects were pronounced with dosages used. The results revealed that the ethanol extract of *Paederia foetida* afforded significant dose dependent protective and antioxidant effects in $CCl_4$-induced hepatic damage in rats.

**Key words:** Hepatoprotective activity · Antioxidant · *Paederia foetida* · Fenton reaction · $Ccl_4$

## INTRODUCTION

The liver is an organ of paramount importance as it plays a major role in metabolism and has numerous functions in the body, including glycogen storage, plasma protein synthesis, decomposition of red blood cells and detoxification of xenobiotics such as environmental pollutants and drugs. It is prone to oxidative stress because of its central role in xenobiotic metabolism, its portal location within the circulation and its anatomic and physiologic structure. The risk of liver intoxication has recently increased by consumption of contaminated foods and drinks as well as by higher exposure to environmental toxins, pesticides and frequent use of chemotherapeutics [1]. In spite of tremendous strides in modern medicine, there is hardly any drug that stimulates liver functions, offers protection to the liver from damage or helps regenerate hepatic cells. Consequently, there is a worldwide trend to go back to traditional medicinal plants to heal liver ailments. A large number of drugs are employed in traditional medicine for liver diseases. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem.

*Paederia foetida* is a perennial climbing shrub belonging to the family Rubiaceae. The leaves of the plant release a strong fetid odor when bruised. Methyl mercaptan was reported to be responsible for the fetid odor of the plant [2]. It is used to treat enteromegaly, enterosis, flatulence, gastromegaly, rheumatism, rhinosis, toothache, stomachache and sore in folk medicine [3]. *Paederia foetida* is also a popular shrub used as a remedy for diarrhea and dysentery in Bangladesh [4]. The present study was aimed to investigate the anti-hepatotoxic effects of the plant on liver injury both *in vitro* (Fenton reaction-induced) and *in vivo* ($CCl_4$-induced) experimental paradigms. In order to assess the beneficial effects, several serum biochemical markers related to hepatic functions, namely LPO, GPT, GOT, ALP and bilirubin were monitored.

**Corresponding Author:** Shahdat Hossain, Department of Biochemistry and Molecular Biology,
Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.
Tel: +88027791045-51 ext. 1379, Fax: +88027791052, E-mail: shahdat@dhaka.net.

## MATERIALS AND METHODS

**Extraction and Formulation of *Paederia foetida* Leaves:** *Paederia foetida* leaves were first cut and grinded and then subjected to mild hot continuous percolation for 12 hours using absolute ethanol as solvent in a soxhlet apparatus. Then the extract was concentrated under reduced pressure at 50°C using rotary evaporator. The concentrated extract was further dried by placing within a desiccator (approximate yield 10%, w/w). Dried ethanol-free extract of *Paederia foetida* was dissolved in physiological saline to make a homogenous solution of 15 mg/ml.

**Animals:** In-bred male SD rats (50-80 g b. wt.) reared in the animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, were used for the experiment. The rats were housed in plastic cages (bedding was hard wood chips) under controlled conditions of 12-h dark-light cycles. They all received standard pellet diet and water *ad libitum*. All the rats were cared for and sacrificed in accordance with the ethical norms approved by Bangladesh Association for Laboratory Animal Sciences.

**Experimental Protocol**

***In vitro* Anti-Oxidative Study:** Ten male SD rats were used for the assessment of *in vitro* anti-oxidative activity of the plant extract. The activity was monitored by inducing oxidative stress in liver tissue homogenate with the aid of Fenton reagent ($FeSO_4$ 0.5 mM: $H_2O_2$ 0.5 mM; 1: 1). The rats were sacrificed under deep anesthesia (ketamin HCL 100 mg/kg b. wt., I/M), the liver was immediately excised, washed and perfused with physiological saline to wash out blood cells. The washed liver was blotted, weighed, minced and homogenized with ice-cold phosphate buffer (25 mM, pH 7.4) in a Polytron homogenizer to make approximately 20% w/v liver homogenate. Liver homogenates (LH) from each rat were divided into three groups: Control group (0.2ml LH + 0.4 ml distilled Water); Positive Control group (0.2ml LH + 0.2 ml Fenton Reagent + 0.2 ml distilled Water); and Treatment group (0.2 ml LH + 0.2 ml Fenton Reagent + 0.2 ml plant extract) (Table 1). All the test tubes were vortex-mixed and incubated for 4 hours in a temperature-controlled water bath at 37°C. *In vitro* oxidative stress in terms of lipid peroxidation was determined by estimating the thiobarbituric acid reactive substances (TBARs) according to the method of Ohkawa *et al*. [5] with slight modification.

Table 1: Fenton reaction-induced *in vitro* lipid peroxidation study

| Groups | Treatment Received |
|---|---|
| Control | 0.2 ml LH + 0.4 ml distilled Water |
| Positive Control | 0.2 ml LH + 0.2 ml FR + 0.2 ml distilled Water |
| Treatment | 0.2 ml LH + 0.2 ml FR + 0.2 ml plant extract |

Here, LH = Liver homogenate; FR = Fenton reagent

***In vivo* Anti-Hepatotoxic Study:** In the present investigation, the ethanol extract of *Paederia foetida* (EPF) leaves was evaluated for its hepatoprotective and antioxidant activities against $CCl_4$-induced liver damage in male SD rats. The rats were divided into 4 groups: i) Vehicle control group [received saline (as vehicle of extract) for 21 successive days before paraffin (as vehicle of $Ccl_4$) injection prior to sacrifice]; ii) $CCl_4$-treated control group [received saline before $CCl_4$ injection prior to sacrifice]; iii) Extract Pre-administered group-1 [received 200 mg extract per Kg body weight per day instead of saline]; and iv) Extract Pre-administered group-2 [similarly received extract at a dose of 400 mg per kg body weight per day]. In this experiment 0.25 ml of $CCl_4$ was injected intraperitoneally using liquid paraffin as vehicle (1:3) per 100 g body weight [6].

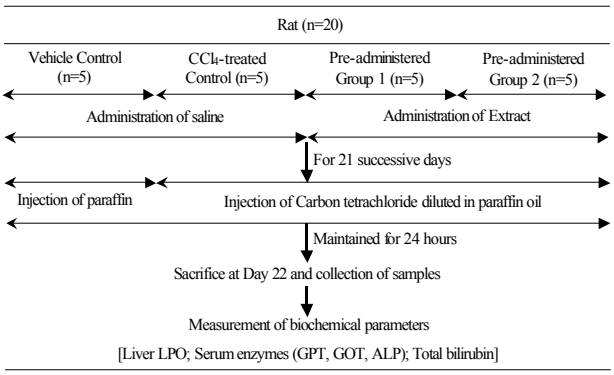


Fig. 1: Experimental design for *in vivo* anti-hepatotoxic study.

Here, Vehicle control group received saline (as vehicle of extract) for 21 successive days before paraffin (as vehicle of $CCl_4$) injection prior to sacrifice; $Ccl_4$-treated control group received saline before $CCl_4$ injection prior to sacrifice; Extract Pre-administered group-1 received 200 mg extract per Kg body weight per day instead of saline; and Extract Pre-administered group-2 similarly received extract at a dose of 400 mg per kg body weight per day.

**Biochemical Analyses**

**Lipid Peroxidation in Liver Tissue:** The malondialdehyde (MDA), formed during lipid peroxidation, reacts with thiobarbituric acid to form a pink colored complex. Briefly, 0.2 ml of 8.1% (w/v) sodium dodecylsulphate and 3 ml of 0.4% thiobarbituric acid in 20% acetic acid (pH 3.5) were added to the tubes. Each tube was tightly capped and heated in a boiling water bath at 95°C for one hour. After cooling the tubes with running tape water, 2 ml of n-butanol-pyridine (15:1, v/v) was added and shaken vigorously for about fifteen minutes. The tubes were then centrifuged and the absorbance of the supernatant fraction was measured at 532 nm using a UV-Visible Spectrophotometer (UV-1601 PC, Shimadzu, Japan). Linolenic acid was used as standard. Total protein in the tissue homogenate was estimated by the method of Lowry *et al*. [7]. The levels of thiobarbituric acid reactive substances were expressed as mM malondialdehyde/mg protein.

**Estimation of Serum Biochemical Markers:** All the parameters were measured by using commercially available reagent kits (Human GmbH, Germany). The activity of serum transaminases (sGPT and sGOT) were determined by the method of Reitman and Frankel as described by Bergmeyer and Bernt [8] and serum alkaline phosphatase activity was measured according to the method of Kind and King [9]. Total bilirubin level was estimated according to the method of Malloy and Evelyn [10].

**Statistical Analyses:** The results are expressed as mean ± SEM (Standard error of mean). For inter-group differences data were analyzed by one way ANOVA (analysis of variance). ANOVA was followed by Fisher's protected least square differences (PLSD) for post hoc comparisons. The statistical programs used were GBSTAT TM 6.5.4 (Dynamic Microsystems, Inc., Silver Spring, MD, USA) and StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA). A level of $P<0.05$ was considered statistically significant.

Table 2: Effects of *Paederia foetida* extract on *in vitro* LPO formation

| | LPO (mM/mg of protein) |
|---|---|
| Control | 1.56±0.12 [a] |
| Positive Control | 1.99±0.17 [b] |
| Treatment | 1.19±0.06 (186%) [c] |

## RESULTS

***Paederia foetida* Prevents Fenton Reaction-Induced *in vitro* Lipid Peroxidation:** Fenton reagent induced significant oxidative stress in rat liver homogenates, as indicated by 28% increase in lipid peroxide (LPO) level. Incubation of the liver homogenate with the extract prevented the rise in LPO levels significantly by 40% (Table 2).

Results are expressed in mean ± SEM (Standard error of mean), n = 10. Values in the same column that do not share common superscripts are significantly different at $P<0.05$. The value in parenthesis indicates percent of protection by the extract from Fenton reagent-induced elevation of lipid peroxide level. Percent of protection is calculated as 100 × (value of positive control – value of treatment) / (value of positive control – value of control). The data were analyzed by One-way ANOVA followed by Fisher's PLSD for post hoc comparisons.

**Effects on $CCl_4$-induced *in vivo* Hepatic LPO Formation:** LPO level in liver homogenate increased significantly in "$CCl_4$-treated control" group (2.46 mM/mg protein) compared to that of "Vehicle Control" group (1.25 mM/mg protein). Pre-administration of *Paederia foetida* extract caused inhibition of $CCl_4$-induced increase in the hepatic LPO level in a dose responsive way (Table 3).

**Effects on Liver-Function Specific Serum Enzymes and Total Bilirubin Levels:** Rats treated with a single dose of $Ccl_4$ developed significant hepatic damage as indicated by elevated serum levels of liver-function specific biochemical markers. The serum GPT, GOT and ALP activities ($P<0.05$) increased significantly in $CCl_4$-intoxicated control animals. Serum total bilirubin level increased also upon $CCl_4$ treatment. Pre-administration of *Paederia foetida* extract conferred a significant protection against $CCl_4$-induced increases in the serum total bilirubin and enzyme levels (Table 3).

Results are expressed as mean ± SEM (n = 5). Values in the same row that do not share common superscripts are significantly different at $P<0.05$. The values in parentheses indicate percent of protection from their $CCl_4$-induced altered values. Percent of protection is

Table 3: Pre-Administration effects of extract on biochemical markers

| Parameters | VCT | CTC | PRA 1 | PRA 2 |
|---|---|---|---|---|
| LPO (mM/mg of protein) | 1.25±0.12 [a] | 2.46±0.39 [b] | 1.22±0.15(103%) [a] | 1.12± 0.03(110%) [a] |
| sGPT (U/ml) | 55±4 [a] | 597±13 [b] | 243±10 (65%) [c] | 197±7 (74%) [d] |
| sGOT (U/ml) | 123±6 [a] | 503±20 [b] | 260±6 (64%) [c] | 193±12 (82%) [d] |
| sALP (U/L) | 125±1 [a] | 190±3 [b] | 138±2 (79%) [c] | 127±7 (96%) [a c] |
| Bilirubin (mg/dL) | 0.14±0.01 [a] | 0.38±0.01 [b] | 0.23±0.02 (62%) [c] | 0.16±0.01(95%) [a] |

calculated as 100 × (value of $CCl_4$-treated control– value of pre-administration 1 or 2) / (value of $CCl_4$-treated control- value of vehicle control). Here, VCT = Vehicle control; CTC = $CCl_4$- treated control; PRA 1 = Extract pre-administered group 1; PRA 2 = Extract pre-administered group 2.

## DISCUSSION

The aim of the study was to investigate whether the folkloric uses of the herbal medicines derived from the plant *Paederia foetida* in different diseases have any scientific ground; if so, then what they are. Our perspective was focused on whether the extract exhibits any anti-oxidative potential. With this view in mind, we first produced *in vitro* oxidative stress in the rat liver tissue homogenates by using Fenton reaction in order to reproduce the oxidative effects on liver tissues as encountered *in vivo*. The reaction generates highly reactive hydroxyl radicals ($^{\bullet}OH$) by utilizing hydrogen peroxide ($H_2O_2$).

Fenton reagent significantly increased the LPO levels in liver tissue homogenate (Table 2), suggesting a successful induction of *in vitro* oxidative insult. Interestingly, co-incubation with *Paederia foetida* extract significantly decreased the *in vitro* oxidative stress-induced increases of LPO levels. The results, thus, provide clear-cut evidence that *Paederia foetida* extract retains antioxidative agent(s) as one of its active ingredients. Therefore, two speculations are possible from the data: **i)** either the extract directly inhibits the production of reactive oxygen species (ROS) when they are derived from the Fenton reaction; or, **ii**) it induces or stimulates, somehow, the antioxidative enzymes of the liver tissues and assists to depress the elevations of the LPO levels. The latter possibility is less likely within the short period of *in vitro* incubation with the extract. Rather, the antioxidative substrates such as sulfhydryl compounds might come into action and/or the extract of the green *Paederia foetida* plant may contain polyphenolic compounds that act as natural antioxidants.

Detailed studies of these speculations are definitely essential to reveal the oxidation-defending properties. Whatever the mechanism(s) may be, the evidence of the *in vitro* anti-oxidative effects of the *Paederia foetida* led us to investigate its dietary effects on carbon tetrachloride ($CCl_4$) -induced *in vivo* hepatotoxicity in the rats. Carbon tetrachloride is frequently employed to study hepatotoxicity and anti-hepatotoxic activity of drugs in xenobiotics. It is used as a chemical inducer of experimental cirrhosis [11]. The mechanism(s) of $CCl_4$-induced hepatotoxicity is largely unknown. It is believed that $CCl_4$ accumulates in hepatic parenchymal cells and metabolically activated to trichloromethyl radical by the microsomal cytochrome P-450 dependent monooxygenases to induce hepatotoxicity [12]. The radical alkylates cellular proteins including its creator cytochrome P-450 and a variety of other macromolecules [13]. It may react very rapidly with oxygen to yield a more reactive trichloromethyl peroxy radical ($CCl_3OO^{\bullet}$). Therefore, under such a disseminated oxidative stress, bioactive molecules and polyunsaturated fatty acids of the biomembranes of the hepatic microsomes, mitochondria and nuclei are also impaired by lipid peroxide, with hepatocyes ultimately being destroyed [14, 15].

Thus the elevated level of lipid peroxidation could be considered as an oxidative damage marker after *in vivo* treatment of tissues with $CCl_4$. Chronic administration of *Paederia foetida* extract for 21 successive days significantly inhibited the $CCl_4$-induced hepatotoxicity with a concurrent significant suppression of the hepatic LPO levels. Thus the *in vivo* results again suggest the presence of antioxidative properties of the *Paederia foetida* extract. We here speculate that the chronic administration of *Paederia foetida* extract also increased the activities of cellular anti-oxidative enzymes. However, the activities of the anti-oxidative principle must be determined.

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane,


Fig. 2: Possible modulation of reactive oxygen species (ROS) metabolism by ethanol extract of *Paederia foetida* (EPF), Fenton reaction is shown in the pink shade.
Here, LPO = Lipid peroxide; CAT = Catalase; SOD = Superoxide dismutase; GPx = Glutathione peroxidase; GR = Glutathione reductase; GSH = Reduced glutathione; GSSG = Oxidized glutathione. (Here, "+" sign denotes acceleration and "–" sign denotes inhibition).

thereby causing an increased enzyme levels in the serum [16]. The damage to the structural integrity of the liver is also deduced from elevated lipid peroxide level in liver homogenate. Thus, in order to evaluate the anti-hepatotoxic activity in the present study, effects of *Paederia foetida* extract on elevated serum levels of hepatospecific enzymes (GOT, GPT and ALP) were monitored. Administration of *Paederia foetida* extract ameliorated the $CCl_4$-induced rise in serum parameters. A subsequent recovery towards normalization of these parameters strongly suggests the possibility of *Paederia foetida* being able to condition the hepatocytes so as to cause regeneration of hepatic homeostasis, thus acting against membrane fragility, decreasing the leakage of marker enzymes into the circulation.

Hyperbilirubinemia is a very sensitive test to substantiate the functional integrity of the liver. It indicates the severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate [17]. Stabilization of serum bilirubin and hepatic LPO levels through the administration of the extract is further a clear indication of the improvement of the functional status and structural integrity of the liver cells.

Thus, the results of the present investigation clearly demonstrated that various $CCl_4$-induced biochemical changes were protected by the oral administration of *Paederia foetida* extract. The probable mechanism by which the extract exhibited its hepatoprotective action might be the improvement of antioxidative status. Previous phytochemical investigations reported that *Paederia foetida* contains paederolone, paederone, β-sitosterol, paederoside, asperuloside and their related glucosides [18, 19]. The leaves of the plant are also rich in carotene, vitamin C, keto-alcohol and alkaloid [4]. These compounds may come into play (either individually or synergistically) to minimize the harmful effects of $CCl_4$-induced oxidative stress. Thus, further study is indeed necessary to find out the exact mechanism (s) through which *Paederia foetida* exhibits all its beneficial effects.

## REFERENCES

1. Jeena, K.J. and R. Kuttan, 2000. Hepatoprotective activity of *Emblica officinalis* and Chyavanaprash. J. Ethnopharmacol., 72: 135-140.

2. Bose, P.K., A.K. Baneriee and C. Ghosh, 1953. Chemical investigation of *Paederia foetida* L. Trans. Bose' Research Institute, 19: 77-78.
3. Johnson, T., 1999. CRC Ethnobotany Desk Reference. CRC Press, pp: 580.
4. Ghani, A., 1998. Monographs: Elaeocarpus serratus Linn. In: Medicinal plants of Bangladesh: Chemical constituents and Uses. Asiatic Society Bangladesh, pp: 253-254.
5. Ohkawa, H., N. Ohnishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Analytical Biochemistry, 95: 351-358.
6. Nishigaki, I., R. Kuttan, H. Oku, F. Ashoori, H. Abe and K. Yagi, 1992. Suppressive effect of curcumin on lipid peroxidation induced in rats by carbon tetrachloride or Co60 irradiation. J. Clinical Biochemistry and Nutrition, 13: 23-29.
7. Lowry, D.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biological Chemistry, 193: 265-275.
8. Bergmeyer, H.U. and E. Bernt, 1950. Calorimetric method for asparate and alanine aminotransferases. In: Varley, H., A.H. Gowenlock and M. Bell, (Eds.), Practical Clinical Biochemistry, fifth ed. William Heinemann Medical Books Ltd, pp: 741-742.
9. Kind, P.R.N. and E.J. King, 1954. Estimation of plasma Phosphatase by determination of hydrolysed phenol with antipyrine. J. Clinical Pathol., 7: 322-330.
10. Malloy, H.T. and K.A. Evelyn, 1937. The determination of bilirubin with the photometric colorimeter. J. Biological Chemistry, 119: 481-490.
11. Brattin, W.J., E.A. Glende Jr. and R.O. Recknagel, 1985. Pathological mechanisms in carbon tetrachloride hepatotoxicity. Free Radical Biology and Med., 1: 27-38.
12. McCay, P.B., E.K. Lai, J.L. Poyer, C.M. Dobose and E.G. Jamzen, 1984. Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. Observation of lipid radicals *in vivo* and *in vitro*. J. Biological Chemistry, 259: 2135-2143.
13. Recknagel, R.O., 1983. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci., 33: 401-408.
14. Compori, M., 1985. Lipid peroxidation and cellular damage in toxic liver injury. Lab. Clin. Invest., 53: 599-623.
15. Recknagel, R.O., E.A. Giende and A.M. Hruszkewycz, 1976. In: E.A. Pryor (Ed.), Free Radicals in Biology, Vol. III. Academic Press, pp: 97-132.
16. Zimmerman, H.J. and L.B. Seef, 1970. Enzymes in hepatic disease. In: Goodly, E.I. (Ed.), Diagnostic Enzymology. Lea and Febiger, pp: 1-38.
17. Sing, B., A.K. Saxena, B.K. Cahandan, K.K. Anand, O.P. Suri, K.A. Suri and N.K. Satti, 1998. Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia, 69: 135-140.
18. Shukla, Y.N., H.A. Lloyd, J.F. Mortons and G.J. Kapadia, 1976. Iridoid glycosides and other constituents of *Paederia foetida*. Phytochemistry, 15: 1989-1990.
19. Inouye, H., S. Inouye, N. Shimokawa and M. Okigawa, 1969. Studies on monoterpene glucosides. VII. Iridoid glucosides of P. scandens. Chemical Pharmaceutical Bulletin, 17: 1942-1948.