Investigation of hepatoprotective activity of aqueous and ethanolic extracts of Paederiafoetida on paracetamol induced hepatotoxicity in animal model

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Abstract-The plant *Paederiafoetida* belonging to family Rubiaceae though being used traditionally for long, the mechanism of action behind its beneficial activities are not yet well established. A wide range of activities namely antidiarrhoeal, anti-inflammatory, antinociceptive have been reported. In the present study, hepatoprotective activity of the above-mentioned plant was examined and results were found in accordance to the speculated activity. To examine the hepatoprotective activity of this plant, paracetamol (500mg/kg BW) induced adult wistar albino rats were subjected to oral administration of aqueous and ethanolic extracts of leaves of *P. foetida.* Phytochemical analysis was done using various phytochemical tests and HPTLC technique. Further, the activity was confirmed by evaluating the changes in biochemical indicators such as SGOT, SGPT, ALP, direct and total bilirubin. From experimental results, it was found out that the test extract showed potent hepatoprotection activity in case of ethanolic extract and the dose 200 mg/kg showed potency comparable to standard reference drug silymarin (150 mg/kg BW). The ethanolic extracts were able to restore the biochemical levels to normal which were altered due to paracetamol intoxication in the hepatocytes of adult wistar albino rats. The results indicate that there is some rationale behind the ethnomedical use of the plant for treating hepatic disorders.

***Index words*-adult wistar albino rats, bilirubin,*Paederiafoetida*, paracetamol,Rubiaceae, hepatoprotective activity,silymarin.**

1 INTRODUCTION

The liver is the largest gland and it is the main organ involved in the process of metabolism. It has a wide range of functions including detoxification of various metabolites, synthesis of proteins and production of biochemicals necessary for the process of digestion. It is an accessory digestive organ which is involved in the synthesis of bile which emulsifies fats to produce fatty acids [1]. In spite of conducting tremendous studies and research in modern medicines, there is hardly any drug that stimulates liver function and can help in regeneration of hepatic cells.

Paederiafoetida belonging to family Rubiaceae is a perennial climbing shrub found in most parts of India, also throughout the Malayan Archipelago, extending from Mauritius northward to China, Japan and Philippines [2], [3]. Extract from this plant is found to provide efficient antihyperlipidemic, antihyperglycemic, thrombolytic, cytotoxic and antioxidant properties [4], [5], [6]. Presence of methyl mercaptan gives this plant its fetid odour. Extracts from leaves of this plant has also shown to possess antihyperlipidemic, antihyperglycaemic and antioxidant property [4], [7], [8]. In traditional medicine, this plant has been used for treating enteromegaly, flatulence, rheumatism, stomachache and sores [9]. Phytochemical investigation provides evidence for the presence of iridiod glycosides, paederolone, paederone, paederine and paederenine in this plant [10], [11], [12]. Later studies on *P. foetida* confirmed the presence of phenolic compound which attributes to its anti-oxidant property [13]. The present study was undertaken to investigate the hepatoprotective activity of aqueous and ethanolic extracts of *P. foetida* on paracetamol induced hepatotoxicity model of adult wistar albino rats [14] using silymarin (150 mg/kg BW) as reference drug.

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2 MATERIALS AND METHODS

2.1 Plant materials

Paederiafoetida Linn. was collected from Durgapur, West Bengal and was identified by a taxonomist (V. P. Prasad) from Central National Herbarium, Shibpur (Reference

no.- CNH/7ECH.II2014/106). A voucher specimen (JDF-01) has been deposited at Dr. B.C. Roy College of Pharmacy and Allied Health Sciences, Durgapur, West Bengal. The whole plant was dried and leaves were separated and powdered. Plant contents were extracted by separately soaking certain quantity of the powder in distilled water and 90% ethanol respectively, for 7 days. The extract was then collected by filtration, concentrated in vacuo with rotavapor and dried under vacuum (yield obtained was 23.27% w/w for aqueous extract and 33.39% w/w for ethanolic extract).

2.2 Animals

Adult male and female wistar albino rats of 8-16 weeks (weighing 150-200 g) bred in the animal house of Dr. B.C. Roy College of Pharmacy and Allied Health Sciences, Durgapur, West Bengal were used for the experiments. The animals were provided with standard laboratory food and tap water and maintained at natural day/night cycle. The animals were grouped into five groups, each group comprising of six animals (n = 6) according to sex and body weight and were made to fast for 24 hours prior to their use. Acute oral toxicity studies were carried out as per OECD guidelines [15] and that no animals were found to be dead or showed any gross behavioural change even at a high dose (2000 mg/kg BW) of aqueous and ethanolic extract of the test drug. Following this, the grouped animals were subjected to the following treatment. Group I animals were subjected to oral administration of normal saline solution (2 ml/kg BW). Group II animals were subjected to oral administration of paracetamol (500 mg/kg BW). Group III animals were subjected to oral administration of paracetamol (500 mg/kg BW) 4-6 hours prior to oral administration of silymarin (150 mg/kg BW). Group IV animals were subjected to oral administration of paracetamol (500 mg/kg BW) 4-6 hours prior to oral administration of aqueous extract of *P. foetida* (200 mg/kg BW). Group V animals were subjected to oral administration of paracetamol (500 mg/kg BW) dissolved in water 4-6 hours prior to oral administration of ethanolic extract of *P. foetida* (200 mg/kg BW). The research was carried out in accordance to the rules of the institutional animal ethics committee governing the use of laboratory animals as acceptable internationally (CPCSEA).

3 Standardization of extract by HPTLC technique

3.1 Preparation of standard and sample solution

A stock solution of silymarin was prepared by dissolving 10 mg of standard silymarin in 10 mL of ethanol (1000 µg/mL) and used as standard. The sample solution was prepared by extracting 2.0 g of dried powdered crude drug with 50 mL of ethanol. Filtration of the ethanolic extract was carried out through the Whatman filter paper and evaporated to dryness under reduced pressure. The residue obtained was resuspended in 1.0 mL of ethanol and used for chromatography.

3.2 HPTLC instrumentation and procedure

5µL sample was spotted carefully in the Millipore Silica Gel *60 F254* Coated *Aluminium*-Backed TLC sheet (10 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a CamagLinomat V (Switzerland) in the form of the bands of width 4.0 mm with a Camag microliter syringe with a constant application rate of 200 nL/s and was dried using Camag TLC Plate Heater-III at 75° C for 70-80 seconds. The slit dimension was kept at 4.0 mm × 0.45 mm and scanning speed of 20 mm/s was employed. The mobile phase composed of chloroform: acetone: formic acid (9:2:1). The development of the HPTLC plate was carried out in ascending manner in glass chamber. After saturation of mobile phase, the chromatogram was developed up to a length of 80 mm of the HPTLC plate and dried in the room temperature. Scanning was done in absorbance mode at 296 nm wavelength.

4 Test for hepatoprotective activity by paracetamol induced hepatotoxicity model [15]

36 hours after last administration of paracetamol, blood was withdrawn from individual rats by recto-orbital puncture and allowed to clot at room temperature. Blood samples were marked and kept undisturbed for 30 minutes. Serum was separated by centrifugation at 5000 rpm for 10 minutes. The serum was used for the estimation of biochemical parameters to determine the functional state of liver.

4.1 Serum glutamic-oxaloacetic transaminase (SGOT) test[16]

Aspartate aminotransferase catalyses the transamination of L-aspartate and α-ketoglutarate to form oxaloacetate and L-glutamate. Oxaloacetate so formed is coupled with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form a

corresponding hydrazine, a brown coloured complex in alkaline medium which is measured colorimetrically at 505 nm (green filter). The test was carried out as per standard protocol.

AST(GOT) activity (IU/L) = $\frac{[O.D.(test)-O.D.(control)]}{[O.D.(standrd)-O.D.(blank)]}$ x concentration of standard

4.2 Serum glutamic pyruvic transaminase (SGPT) test[16]

Alanine aminotransferase catalyses the transamination of L-alanine and α-ketoglutarate to form pyruvate and L-glutamate. Oxaloacetate so formed is coupled with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown coloured complex in alkaline medium and which can be measured colorimetrically at 505 nm (green filter). The test was carried out as per standard protocol.

ALT (GPT) activity (IU/L) = $\frac{[O.D.(test)-O.D.(control)]}{[O.D.(standard)-O.D.(control)]}$ x 10

4.3 Alkaline phosphatase (ALP) test [17]

Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidising agent. Potassium ferricyanide forms an orange-red coloured complex, which can be measured colorimetrically. The intensity of colour is proportional to the enzyme activity. The test was carried out as per standard protocol.

Serum alkaline phosphatase activity (IU/L) = $\frac{[O.D.(test)-O.D.(control)]}{[O.D.(standard)-O.D.(control)]}$ x 10

4.4 Bilirubin test [18]

Direct: Conjugated bilirubin couples with diazotized sulfanilic acid forming azobilirubin, a red-purple coloured product in acidic medium which shows a single broad absorption band at 540 nm.

Indirect: Unconjugated bilirubin is diazotized only in the presence of methanol as dissolving solvent. Thus, the purple-red coloured azobilirubin produced in presence of methanol originates from both direct and indirect functions and thus represents total bilirubin concentration. The difference of total and direct bilirubin gives indirect (unconjugated) bilirubin. The intensity of red-purple colour so developed above is measured colorimetrically which is proportional to the concentration of the appropriate fraction of bilirubin.

Bilirubin concentration in mg/dl:

Total (A) = $\frac{[O.D.(T1)-O.D.(T2)]}{[O.D.(standard)]} X\ 10$

Direct (B) = $\frac{[O.D.(D1)-O.D.(D2)]}{[O.D.(standard)]} X\ 10$

Where T1 and T2 determine the value of total bilirubin, D1 and D2 determine the direct bilirubin value.

5 Statistical Analysis of the Data

Values are expressed as mean ± SD and significance of inter-group differences of each parameter was analysed separately using the one-way analysis of variance (ANOVA) and $p < 0.01$ are considered to be significant (Table 1). Significance within the group was analysed using Student's t-test where $p < 0.01$ and $p < 0.001$ are significant.

6 RESULTS AND DISCUSSIONS

Hepatoprotective activity of freshly extracted aqueous and ethanolic extracts of *Paederiafoetida* were investigated on paracetamol induced hepatotoxic model of adult wistar albino rats, in-vitro.

Preliminary phytochemical screening of aqueous and ethanol extracts of leaves of *P. foetida*were carried out for the detection of phytoconstituents, using standard chemical tests.

HPTLC chromatogram of standard silymarin (1000 µg/mL) at 296 nm generated a peak value of R_f of 0.44, while HPTLC chromatogram of ethanolic extract of *P. foetida* generated peaks with R_f values in range of 0.33 to 0.91. A peak value of $R_f$of 0.43 confirmed the presence of phenolic compounds responsible for the anti-oxidant property.

The effects of aqueous and ethanol extract of *Paederiafoetida* on various biochemical indicators such as SGOT, SGPT, ALP, direct and total bilirubin are represented precisely (Table 1). The table shows significant decrease in the levels of SGOT, SGPT, ALP, direct and total bilirubin when compared with normal group ($p < 0.01$).

A single dose of 500 mg/kg BW of paracetamol was able to produce diffuse areas of necrosis (50% of liver tissues) as revealed by standard histological procedures, while livers from paracetamol + silymarin treated rats showed a similar structure to normal (Group 1) (not shown).

Serum AST, ALT and ALP levels are elevated in viral and other forms of liver diseases associated with hepatic necrosis. From our experimental data, it was indicated that for Group II (paracetamol induced adult wistar albino rats), serum AST, ALT and ALP levels were the highest due to acute hepatocellular damage and biliary obstruction, where Group I was considered as normal (administered with vehicle alone). In case of Group III (silymarin treated adult wistar albino rats) serum AST, ALT and ALP levels were found to be lowest. In case of Group IV & V adult wistar albino rats, serum AST, ALT and ALP levels are found to have been lowered comparable to the effect of standard drug silymarin, as it is found to protect the liver against oxidative stress.

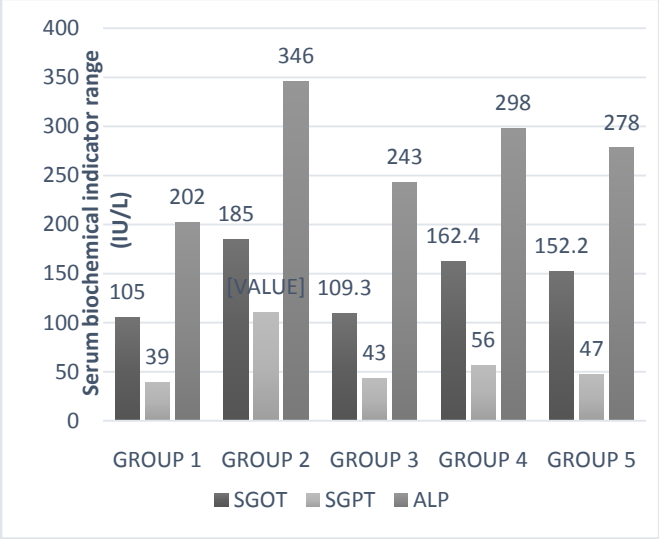
The present study revealed a significant increase in the activities of SGOT, SGPT, ALP and bilirubin levels in serum after exposure to paracetamol, indicating considerable hepatocellular injury. The increase in transaminases was a clear indication of cellular leakage and loss of functional integrity of cell. Extracts of *P. foetida* contains phenolic compounds which is responsible for its anti-oxidant activity, which was confirmed using standard phytochemical test and HPTLC technique.

Groups	Concentration	SGOT (IU/L)	GPT (IU/L)	ALP (IU/L)	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)
I.	2 ml/kg	105 ± 2.74	39 ± 3.23	202 ± 3.12	0.10 ± 0.03	0.25 ± 0.03
II.	500 mg/kg	185 ± 3.71	110 ± 3.46	346 ± 1.36	0.47 ± 0.02	1.05 ± 0.13
III.	150 mg/kg	109.3 ± 2.63	43 ± 2.71	243 ± 2.12	0.11 ± 0.07	0.27 ± 0.08
IV.	200 mg/kg	162.4 ± 2.21	56 ± 1.11	298 ± 3.10	0.27 ± 0.01	0.77 ± 0.03
V.	200 mg/kg	152.2 ± 3.36	47 ± 0.71	278 ± 2.11	0.21 ± 0.04	0.53 ± 0.04

Table 1: Biochemical levels of various serum enzymes for different groups.

The values obtained are expressed as mean ± S.E.M. of six rats in each group. (P <0.01) compared with the corresponding value for normal control animals (group I); (P <0.01) compared with the corresponding value for hepatotoxicity induced control animals (group II).

A two-tailed P values for all the groups when compared to hepatotoxic drug control (Group II) was calculated (p <0.001 is considered to be extremely statistically significant). Activities of the serum enzymes were found to be significantly lower ($p < 0.05$) in wistar albino rats treated with aqueous and ethanolic extracts of *P. foetida.* Figure 1a and 1b shows the effect of *P. foetida*on paracetamol induced hepatoxicity in adult wistar albino rats.



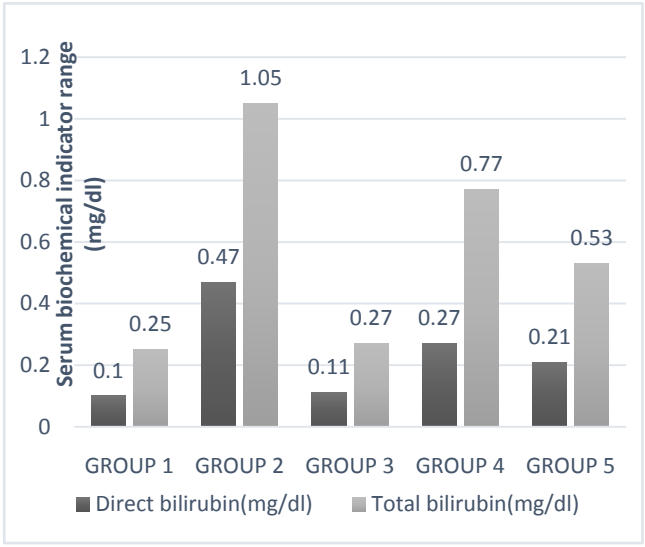


Fig. 1a: Graph showing comparison among SGPT, SGOTFig. 1b: Graph showing comparison between direct and ALP values for different groups. Each value represents bilirubin and total bilirubin for different groups.Each mean ± SEMof six animals per group. value represents mean ± SEM of six animals per group.

Biochemical analysis of the paracetamol administered group (Group II) showed significant increase ($p < 0.05$) in serum markers of liver damage (SGPT, SGOT, ALP, direct bilirubin and total bilirubin). The group (Group III) receiving paracetamol + silymarin showed normal levels of most of the markers of liver damage. This indicates that histological analysis correlated well with the biochemical findings. Groups IV and V showed near similar results in correlation to silymarin treated group (Group III). This protective action of silymarin is probably associated with its antioxidant properties, possibly acting as a free-radical scavenger even at low levels of reduced glutathione (GSH) [19]. Antioxidant property of *P. foetida*is well established [13]. Hepatoprotective activity of aqueous and ethanolic extract of this plant can be correlated with free radicle scavenging activity of the leaves of this plant and this is due to the presence of high levels of phenolic compounds [13].

7 CONCLUSION

Aqueous and ethanolic extracts of *Paederiafoetida*showed hepatoprotective activity in hepatotoxicity induced animal models. The obtained data thus give an experimental basis to understand the use of the extract of this plant in traditional medicine and as a hepatoprotective drug. Further isolation, quantification, purification and rounds of clinical trials is required to qualify it as a marketable product.

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9 CONFLICT OF INTEREST

The authors declare no conflict of interest in preparing this article

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