



Scholars Research Library

Central European Journal of Experimental  
Biology, 2012, 1 (4):145-154

(<http://scholarsresearchlibrary.com/archive.html>)



ISSN: 2278-7364

## Isolation of total alkaloid fraction from polyalthiya longifolia fruits and its evaluation for hepatoprotective and antioxidant potential

Jayaraman Rajangam<sup>a\*</sup>, A. J. M. Christina<sup>b</sup>

<sup>a</sup>Department of Pharmacy, Karpagam University, Coimbatore-641021, TamilNadu, India,

<sup>b</sup>KM College of Pharmacy, Madurai-625107. TamilNadu, India

### ABSTRACT

This study was aimed to evaluate the hepatoprotective and antioxidant potential of the total alkaloid fraction of *Polyalthiya longifolia* fruits (TAFPL) by in-vitro and in-vivo methods. In the in-vitro study, Primary rat hepatocytes and HepG2 cells were exposed with  $CCl_4$  along with/without various concentrations of TAFPL (50,100,200  $\mu\text{g}/\text{kg}$ ) and the effects were studied. In-vivo studies,  $CCl_4$  intoxication method was used and aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total bilirubin and total proteins were estimated and supported with histopathological studies. TAFPL (50,100,200  $\mu\text{g}/\text{kg}$ ) treated animals showed dose dependant protective activity in terms of increased percentage of viability against  $CCl_4$  intoxication in both primary hepatocytes ( $p < 0.001$ ) and in HepG2 cells ( $p < 0.01$ ). TAFPL at the doses of (100 and 200  $\text{mg}/\text{kg}$ ) produced significant hepatoprotective effect by decreasing the elevated serum enzymes, bilirubin, LPO and significantly increased the levels of glutathione (GSH), catalyse (CAT) and super oxide dismutase (SOD). Additionally, in-vitro antioxidant studies revealed that TAFPL scavenged free radicals and maximum percentage inhibition of DPPH was 57% at 200 $\mu\text{g}/\text{mL}$  whereas in nitric oxide radical scavenging model, inhibition is about 51% at 200 $\mu\text{g}/\text{mL}$  and in reducing power method, TAFPL demonstrated dose dependent antioxidant activity comparable with ascorbic acid. Additionally, treatment with TAFPL fruits showed a dose dependent reversal of histopathological changes induced by  $CCl_4$ . Based on the results, it was concluded that, TAFPL possesses hepatoprotective and antioxidant activity against  $CCl_4$  intoxication in both in-vitro and in-vivo methods through its free radical scavenging and antioxidant properties on hepatocytes.

**Keywords:** *Polyalthiyalongifolia*, Hepatocytes, Hepatoprotective, antioxidant, Carbon tetrachloride, HepG2.

### INTRODUCTION

Injury to liver and damage to the hepatic parenchyma cells were always proved to be associated with distortion of different metabolic functions of liver [1,2,3]. Recent research in free radicals suggested the pathophysiological role of free radicals and oxidative stress in liver damage, acute and chronic hepatic injury [4,5]. The mechanism of actions of potent hepatotoxins such as  $CCl_4$ , Paracetamol etc, also indicated the role of oxidative stress and free radicals in the pathophysiology of hepatic injury. Excess production of reactive oxygen species (ROS) along with significant decrease of antioxidant defence in these pathological conditions impairs and alters various cellular functions through the processes of lipid peroxidation. Accordingly, effects of antioxidants or free radical scavengers have been widely tested for the prevention and treatment of acute and chronic liver injuries [6,7,8,9]

Available data from earlier studies also indicated the beneficial effects of antioxidants specifically, for prevention and treatment of acute and chronic liver injury [10]. Therefore, antioxidants can be used to reverse the harmful and pathological actions caused by free radicals. The antioxidants in use are either derived from plants as natural origin or prepared synthetically. Due to possibility of carcinogenic effect, synthetic antioxidants are not preferred

much[11]. Therefore, many folklore remedies from natural origin are tested for its antioxidant and hepatoprotective potential on liver damage by using various animal models using Carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity to screen the hepatoprotective effects of drugs and plant extracts [12,13].

*Polyalthia longifolia* (Family: *Annonaceae*) is a lofty evergreen tree found in India and Sri Lanka, commonly planted for its effectiveness in alleviating noise pollution [14]. The extract of stem bark of the plant and the alkaloids isolated from it has been reported for anti-bacterial and anti-fungal activities[15].Its aqueous extract stimulates the isolated ileum and uterus, depresses heart rate, decreases blood pressure and respiration rate in experimental animals [16]. The crude extracts of the seeds of the plant have also showed remarkable anti-bacterial activities [17] and plants of *annonaceae* family are said to contain antitumor and anticancer active principles[18,19].In this study, we have evaluated the hepatoprotective and antioxidant potential of total alkaloid fraction of *polyalthiya longifolia* fruits (TAFPL) against CCl<sub>4</sub> intoxication by *in-vitro* by using isolated rat primary hepatocytes and *in-vivo* methods in wistar albino rats.

## MATERIALS AND METHODS

### Chemicals

All chemicals and solvents used were obtained from S.D. Fine Chemicals, Mumbai, Loba Chemie Indo Austral Co., Mumbai, Ranbaxy laboratories Ltd., Sigma Fine Chemicals, Mumbai and Hi media Laboratories, Mumbai, India. For various biochemical estimations, kits were procured from E. Merck Ltd., M.I.D.C., Talaja. The human liver derived HepG2 cell line was obtained from, cell culture department, KMCH, Coimbatore, India.

### Plant material

*Polyalthiya longifolia* fruits were collected from Irumbulikulurichi, Perambalur district, Tamilnadu, India and authenticated by G.V.S Murthy, botanical survey of India (BSI), southern circle, Coimbatore, Tamilnadu, India (BSI/SC/5/23/11-12/Tech-1759).

### Preparation of Polyalthiya longifolia fruit extract and isolation of total alkaloid fraction

The chopped and shade dried fruits were powdered and passed through a 40-mesh sieve which was then subjected to single extraction with methanol in a Soxhlet apparatus. The solvent from the methanolic extract was completely removed and concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. The *Polyalthiya longifolia* fruits yielded brown semisolid residue of methanolic extract, weighing 9.0% w/w with respect to the dried starting material. The total alkaloid fraction was (TAFPL) isolated from this extract using conventional methods and confirmed by TLC using butanone / xylene /methanol / diethyl amine solvent system, spots were visualized by UV light at 254 nm[20].

### Preparation of Suspensions

The total alkaloid fraction of *polyalthiya longifolia* (TAFPL) was dissolved in DMSO and the volume was made upto 10 ml with Ham's F-12/MEM to obtain a stock solution of 1mg/ml and stored at -20°C prior to use. Further dilutions were made to obtain different concentrations (125,250,500µg/kg).

### Experimental animals

The experiments were carried out on adult albino rats (150-180g). They were housed in a quite environment with a temperature of 25±1°C and fed with standard rat feed with water ad libitum, except during the test period. The experimental protocols were approved by institutional animal ethics committee (IAEC KMCRET/Pharm/06/2012) and conducted according to the CPCSEA guidelines for the use and care of experimental animals, New Delhi, India.

### Acute toxicity studies

An acute oral toxicity study was performed according to the OECD guidelines for the testing of chemicals, Test No. 423 (OECD, 2001; acute oral toxicity acute toxic class method. Wistar rats (n= 3) of either sex were selected by a random sampling technique for the acute toxicity study. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. Extract was administered orally in increasing dose up to 2000mg/kg.

### Preparation of drug solution

#### Hepatotoxin and test substances

For *in-vitro* studies, CCl<sub>4</sub> (1%), was used to produce submaximal toxicity in isolated rat hepatocytes. The test solution (TAFPL) was administered at dose levels of 50,100 and 200 µg/mL and Sylimarin was used as a positive control at a dose level of 250µg/mL. During *in-vivo* studies, liver damage was induced by administration of CCl<sub>4</sub>

(30%) suspended in olive oil (1ml/kg/b.w) and TAFPL (100,200mg/kg) was suspended in 1% carboxy methyl cellulose, for oral administration whereas Sylimarin was administered at 250 mg/kg i.p. [21,27].

### **In-vitro hepatoprotective activity**

#### **Isolation and culture of rat hepatocytes**

The livers were isolated under aseptic conditions and placed in HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid) buffer I containing HEPES (0.01 mol/L), NaCl (0.142 mol/L) and KCl (0.0067 mol/L), pH 7.4. The isolated livers were cut into small pieces and incubated with a second buffer containing HEPES (0.1 mol/L), NaCl (0.0667 mol/L), KCl (0.0067 mol/L) and 0.5% Collagenase type IV, pH 7.6, for about 45 min at 37°C with constant shaking in an incubator. Hepatocytes were obtained after filtration through a nylon mesh (250  $\mu$ ) followed by cold centrifugation (4°C, 200 rpm for 2 min, thrice) and suspended in HEPES buffer I. The viability of the hepatocytes was assessed by trypan blue (0.2%) exclusion method. The isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% newborn calf serum, antibiotics,  $10^{-6}$  dexamethasone and  $10^{-8}$  bovine insulin. The cell suspension was incubated at 37°C for 30 min in a humidified incubator under 5% CO<sub>2</sub>. Twenty four hours after the establishment of the monolayer of hepatocytes, the medium was decanted and the culture was washed with HEPES buffer-I and finally the hepatocytes were suspended in Buffer-I. The hepatic cytotoxicity was induced with CCl<sub>4</sub> (0.1 mol/L). Triplicate hepatocyte suspensions (0.1mL) from different cultures were distributed into various culture tubes and labelled as per the study design[22].

#### **Study design**

- |              |   |  |
|--------------|---|--|
| 1. Groups I  | - | Control-Treated with DMSO 30%                      |
| 2. Group II  | - | Toxicant CCl <sub>4</sub> (1%)                     |
| 3. Group III | - | TAFPL 50 $\mu$ g/mL + CCl <sub>4</sub> (1%)        |
| 4. Group IV  | - | TAFPL 100 $\mu$ g/mL + CCl <sub>4</sub> (1%)       |
| 5. Group V   | - | TAFPL 200 $\mu$ g/mL + CCl <sub>4</sub> (1%)       |
| 6. Group VI  | - | Sylimarin (250 $\mu$ g/mL) + CCl <sub>4</sub> (1%) |

#### **Assessment of hepatoprotective activity**

The effect of TAFPL on liver protection was determined by measuring an increase in the percentage of viable cells in that group of cells incubated with extracts, compared with the control and toxicant groups. Reversal of toxin induced elevations in the level of enzymes was also considered to assess hepatoprotective activity. After 1 hr of CCl<sub>4</sub> challenge, the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) [23] and alkaline phosphatase (ALP) [24], total bilirubin[25], total proteins [26,27] in the medium were measured as an indication of hepatocytes necrosis using diagnostic Kits procured from Ecoline, E. Merck Ltd, using an auto analyser (Table 1).

#### **Hepatoprotective study in HepG2 Cell line**

HepG2 Cell lines are suitable for *in-vitro* model system for the study of polarized human hepatocytes. HepG2 Cell line with proper culture conditions displays robust morphological and functional differentiation with a controlled formation of apical and basolateral cell surface domain in models. In this method, the basic principle involved is based on the protection of human liver derived HepG2 cells against CCl<sub>4</sub> induced damage and this was determined by estimating mitochondrial synthesis using tetrazolium assay. The HepG2 cells were exposed to toxicant containing 1% CCl<sub>4</sub> along with /without plant extracts of different concentrations or the medium alone is considered as control. At the end of the period, cytotoxicity was assessed by estimating the viability of the HepG2 cells by the MTT reduction assay[28].

#### **In-vivo hepatoprotective activity**

Thirty albino wistar rats of either sex, weighing about 150-180 g were divided into 5 groups of six animals each.

Group I animals received CMC (0.3%) and served as control.

Group II animals received CCl<sub>4</sub> (30%) suspended in olive oil (1ml/kg i.p)

Group III and IV received TAFPL at a dose of 100 and 200 mg/kg respectively.

Group V animals received standard Sylimarin (250 mg/kg)

The animals were treated for 7 days as per the study design mentioned above and on the 7th day after one hour of dosing, the toxicant 30% of CCl<sub>4</sub> (1 ml/kg i.p.) was administered to all the groups except Group I. After 24 h, the animals were anesthetized and blood was collected from retro orbital plexus for the assessment of various enzyme activities. The blood was centrifuged at 2000 rpm for 10 min. The serum was separated and analyzed for various biochemical estimations such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) [23] and alkaline phosphatase (ALP) [24], total bilirubin[25], total proteins [26,27] by using diagnostic kits. (Table 1).

The animals were sacrificed later and the liver was perfused and excised. Part of the liver was stored in 10% formalin saline for histopathological studies. The remaining portion was rinsed in ice cold normal saline, followed

by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation. A part of homogenate after precipitating proteins with Trichloro acetic acid (TCA) was used for estimation of glutathione. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of SOD and CAT activity.

#### **Determination of Reduced Glutathione (GSH)**

To measure the reduced glutathione (GSH) 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml precipitating reagent (1.67 g of met phosphoric acid, 0.2 g of EDTA disodium salt, 30 g sodium chloride in 1 L of distilled water) was added, mixed thoroughly and kept for 5 min before centrifugation. To 2.0 ml of the supernatant, 4.0 ml of 0.3-M disodium hydrogen phosphate solution and 1.0 ml of DTNB (5,5-dithio bis 2-nitro benzoic acid) reagent were added and was read at 412 nm[29].

#### **Assay of Super Oxide Dismutase (SOD)**

The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1ml phenazine methosulphate (186 mM), 0.3 ml NBT (300 mM), 0.2 ml NADH (780 mM), diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol and concentration of SOD was expressed as units/mg protein. Absorbance values were compared with a standard curve generated from known SOD [30].

#### **Assay of Catalase (CAT)**

The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1–4°C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H<sub>2</sub>O<sub>2</sub> and the enzyme extract. The specific activity of catalase was expressed in terms of units/mg protein. Absorbance values were compared with a standard curve generated from known CAT[31].

#### **In-vitro Antioxidant activity**

##### **DPPH radical scavenging activity**

The free radical scavenging activity of TAFPL fruits was observed spectrophotometrically by measuring the decrease in the absorbance of methanolic solution of DPPH. 1mL extract of TAFPL at various concentrations ((25, 50,100,200,400,800µg/mL) were mixed with 1mL of methanolic solution of DPPH (100µM). Similarly a 1mL methanolic solution of ascorbic acid (100 µg/mL) was mixed with 1mL of DPPH solution. A mixture of 1mL of methanol and 1mL of methnolic solution of DPPH (100 µM) served as control. After mixing, all the solutions were incubated in dark for 20 minutes and absorbance was measured at 517 nm [32].The experiments were performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition.

$$\text{Scavenging \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

##### **Nitric oxide radical scavenging activity**

The nitric oxide radical scavenging activity was measured by using Griess' reagent. 5ml of each extract solutions of different concentrations (25,50,100,200 µg/mL) in standard phosphate buffer solution (pH 7.4) were incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4) at 25°C for 5 hours. In an identical manner 5mL of ascorbic acid solution (100µg/mL) in standard phosphate buffer solution (pH 7.4) was also incubated with 5mL of sodium nitroprusside solution (5mM) in standard phosphate buffer (pH 7.4). Control experiments with equivalent amount of buffer and without the extract were also conducted. After incubation, 0.5mL of the incubation mixture was mixed with 0.5 mL of Griess' reagent (Sulphanilamide 1%, O-phosphoric acid 2% and naphthyl ethylene diamine dihydrochloride 0.1%) and the absorbance was measured at 546nm[33].The experiments were performed in triplicate and scavenging activity was expressed as percentage of inhibition.

##### **Reducing power**

2.5mL of solutions of different concentrations of the extract (25,50,100,200µg/mL) in standard phosphate buffer solution (pH 6.6) were incubated with 2.5mL of potassium ferricyanide solution (1% w/v) at 50°C for 20 min. Similarly ascorbic acid solution (100 µg/mL) was also incubated. After incubation, 2.5mL of 10% trichloro acetic acid solution was added to each tube and the mixture was centrifuged at 650 rpm for 10 minutes. 5mL of the upper layer solution was mixed with 5mL of deionised water and 1mL of ferric chloride solution (1%w/v) and the absorbance was measured at 700 nm[34].

**Statistical analysis**

The statistical analysis was carried out by one-way analysis of variance (ANOVA). The values are represented as mean  $\pm$  SEM. Comparison of mean values of different groups treated with different dose levels of extracts and positive controls were estimated by Turkey's Multiple Comparison Test.  $P < 0.05$  was considered significant.

**RESULTS****Hepatoprotective effects in freshly prepared rat hepatocytes**

The effects of total alkaloid fraction on freshly isolated rat hepatocytes intoxicated with  $\text{CCl}_4$  were shown in Table 1. Rat hepatocytes incubated with  $\text{CCl}_4$  resulted in 69% depletion in viability, elevation of AST, ALT, ALP, total bilirubin ( $P < 0.001$ ) and a significant reduction in the level of total proteins ( $P < 0.001$ ) when compared to normal and standard drug received groups. Hepatocytes upon treatment with TAFPL showed a significant restoration of the altered biochemical parameters when compared with normal groups while the same trend was observed in Sylimarin treated animals as shown in Table 1 and Table 2.

**Table 1: Effect of TAFPL on the biochemical parameters of  $\text{CCl}_4$  induced toxicity in isolated rat hepatocytes.**

Treatment	Viable cells (%)	ALT (IU/dl)	AST (IU/dl)
Control	92.56 $\pm$ 0.73	7.38 $\pm$ 0.52	18.42 $\pm$ 0.22
$\text{CCl}_4$	31.32 $\pm$ 1.41	61.34 $\pm$ 1.73 <sup>a</sup>	76.37 $\pm$ 1.65 <sup>a</sup>
Std Sylimarin	85.71 $\pm$ 2.11 <sup>b</sup>	18.63 $\pm$ 0.46 <sup>b</sup>	23.56 $\pm$ 0.27 <sup>b</sup>
TAFPL 50 $\mu\text{g}/\text{mL}$	32.43 $\pm$ 2.34 <sup>b</sup>	49.25 $\pm$ 0.52 <sup>b</sup>	47.22 $\pm$ 0.59 <sup>b</sup>
TAFPL 100 $\mu\text{g}/\text{mL}$	45.45 $\pm$ 1.12 <sup>b</sup>	41.47 $\pm$ 0.41 <sup>b</sup>	42.44 $\pm$ 0.41 <sup>b</sup>
TAFPL 200 $\mu\text{g}/\text{mL}$	59.41 $\pm$ 1.10 <sup>b</sup>	38.33 $\pm$ 0.53 <sup>b</sup>	36.64 $\pm$ 0.45 <sup>b</sup>

Values are mean  $\pm$  SEM: <sup>a</sup> $p < 0.001$  When compared to normal group, <sup>b</sup> $p < 0.001$  When compared to  $\text{CCl}_4$  groups.

**Table 2: Effect of TAFPL on the biochemical parameters of  $\text{CCl}_4$  induced toxicity in isolated rat hepatocytes.**

Treatment	ALP (IU/dl)	Total Bilirubin (mg/dl)	Total protein (mg/dl)
Control	36.22 $\pm$ 0.67	0.24 $\pm$ 0.002	1.24 $\pm$ 0.03
$\text{CCl}_4$	96.34 $\pm$ 2.54 <sup>a</sup>	0.65 $\pm$ 0.03 <sup>a</sup>	0.62 $\pm$ 0.03 <sup>a</sup>
Std Sylimarin	34.33 $\pm$ 0.56 <sup>b</sup>	0.28 $\pm$ 0.002 <sup>b</sup>	1.17 $\pm$ 0.02 <sup>b</sup>
TAFPL 50 $\mu\text{g}/\text{mL}$	51.21 $\pm$ 0.39 <sup>b</sup>	0.40 $\pm$ 0.002 <sup>b</sup>	1.02 $\pm$ 0.03 <sup>b</sup>
TAFPL 100 $\mu\text{g}/\text{mL}$	49.48 $\pm$ 0.69 <sup>b</sup>	0.38 $\pm$ 0.003 <sup>b</sup>	1.04 $\pm$ 0.02 <sup>b</sup>
TAFPL 200 $\mu\text{g}/\text{mL}$	46.22 $\pm$ 0.51 <sup>b</sup>	0.33 $\pm$ 0.002 <sup>b</sup>	1.10 $\pm$ 0.03 <sup>b</sup>

Values are mean  $\pm$  SEM: <sup>a</sup> $p < 0.001$  When compared to normal group, <sup>b</sup> $p < 0.001$  When compared to  $\text{CCl}_4$  groups.

**Hepatoprotective effects in the HepG2 Cell line**

HepG2 cells exposed with  $\text{CCl}_4$  showed depletion of viability by 81% whereas TAFPL received HepG2 cell after exposure to  $\text{CCl}_4$  showed a dose dependant increased percentage viability ( $P < 0.001$ ) when compared to  $\text{CCl}_4$  intoxicated groups. TAFPL 50  $\mu\text{g}/\text{mL}$  treated groups showed 56.47 %, TAFPL 100  $\mu\text{g}/\text{mL}$  showed 60.49% and TAFPL 200  $\mu\text{g}/\text{mL}$  showed 62.56% of viability as shown in Table 3.

**Table 3 :Hepatoprotective activity of TAFPL on  $\text{CCl}_4$  intoxicated HepG2 cells.**

Treatment	% Viability
Control	100
$\text{CCl}_4$	19.43 $\pm$ 2.12 <sup>a</sup>
Std Sylimarin + $\text{CCl}_4$	89.23 $\pm$ 3.24 <sup>b</sup>
TAFPL 50 ( $\mu\text{g}/\text{mL}$ ) + $\text{CCl}_4$	56.47 $\pm$ 3.63 <sup>b</sup>
TAFPL 100 ( $\mu\text{g}/\text{mL}$ ) + $\text{CCl}_4$	60.49 $\pm$ 3.43 <sup>b</sup>
TAFPL 200 ( $\mu\text{g}/\text{mL}$ ) + C	62.56 $\pm$ 3.45 <sup>b</sup>
$\text{CCl}_4$	

Average of 5 determinations (n=5), 4 replicates:

<sup>a</sup>  $p < 0.001$ , When compared to normal; <sup>b</sup>  $p < 0.01$ , when compared to  $\text{CCl}_4$  intoxicated cells.

**In-vivo hepatoprotective activity**

Administration of  $\text{CCl}_4$  showed significant increases in ALT (226.31  $\pm$  8.65 IU/mL,  $P < 0.001$ ), AST (261.31  $\pm$  8.73 IU/ mL,  $P < 0.001$ ) and ALP (326.24  $\pm$  9.54 IU/mL,  $P < 0.01$ ) levels when compared to normal control rats (48.40 $\pm$ 0.22 IU/mL, 87.34 $\pm$ 0.42, and 246.26 $\pm$ 0.67 IU/mL, respectively) as shown in Table 4 and Table 5. Treatment with TAFPL showed a significant restoration of the altered biochemical parameters in a dose dependant manner when compared with normal groups ( $P < 0.01$ ) and the same effect was also observed in standard Sylimarin treated animals.

**Table 4: Effect of TAFPL on the biochemical parameters of CCl<sub>4</sub> intoxicated rats.**

Treatment	Liver Weight (g)	AST (IU/dl)	ALT (IU/dl)
Control	3.8±0.15	87.34±0.42	48.40±0.22
CCl <sub>4</sub>	6.7±0.13 <sup>c</sup>	261.31±8.73 <sup>c</sup>	226.31±8.65 <sup>c</sup>
Std Sylimarin	5.9±0.17 <sup>b</sup>	118.53±3.45 <sup>b</sup>	73.46±4.27 <sup>b</sup>
TAFPL 100 mg/mL	3.2±0.13 <sup>a</sup>	145.25±3.52 <sup>b</sup>	74.25±4.67 <sup>b</sup>
TAFPL 200 mg/mL	4.4±0.15 <sup>b</sup>	141.47±3.47 <sup>b</sup>	73.47±5.36 <sup>b</sup>

Number of animals (n=6): Values are mean ± SEM: <sup>a</sup> p<0.01, <sup>c</sup> p<0.001 When compared to normal group, <sup>b</sup> p<0.01 When compared to CCl<sub>4</sub> groups.

**Table 5: Effect of TAFPL on the biochemical parameters of CCl<sub>4</sub> intoxicated rats**

Treatment	ALP (IU/dl)	Total Bilirubin (mg/dl)	Total protein (mg/dl)
Control	246.26±0.67	0.44±0.002	7.37±0.03
CCl <sub>4</sub>	326.24±9.54 <sup>a</sup>	0.88±0.03 <sup>a</sup>	4.49±0.03 <sup>c</sup>
Std Sylimarin	234.37±7.56 <sup>b</sup>	0.40±0.002 <sup>b</sup>	6.76±0.02 <sup>b</sup>
TAFPL 100 mg/mL	227.11±9.27 <sup>b</sup>	0.39±0.003 <sup>b</sup>	6.28±0.01 <sup>b</sup>
TAFPL 200 mg/mL	221.42±9.62 <sup>b</sup>	0.35±0.003 <sup>b</sup>	6.37±0.04 <sup>b</sup>

Number of animals (n=6): Values are mean ± SEM: <sup>a</sup> p<0.01, <sup>c</sup> p<0.001 When compared to normal group, <sup>b</sup> p<0.01 When compared to CCl<sub>4</sub> groups.

### Effect of TAFPL Liver weight

It was observed that the size of the liver was enlarged in CCl<sub>4</sub> intoxicated rats but it was normal in drug treated group (Table 4.). A significant reduction (P<0.01) in liver weight supports this finding.

### Effect of TAFPL on antioxidant status.

As depicted in Table 4. administration of CCl<sub>4</sub> elevated LPO as revealed by elevated MDA levels (15.7 ± 1.2 nmol, P < 0.001) when compared to normal control (5.2 ± 0.4 nmol) (Table 4). Treatment with TAFPL at a dose of 100, 200 mg/kg, resulted in significant (P < 0.01) reductions in lipid peroxidation (9.8±0.4 and 8.7±0.6 nmol) when compared to toxicant control. Sylimarin at 2 mL/kg showed significant (P < 0.01) reductions in lipid peroxidation (6.3 ± 0.7 nmol) when compared to CCl<sub>4</sub> administered rats. Glutathione, SOD, and CAT levels were significantly (p<0.05) improved in total alkaloid fraction treated groups in comparison to control and Sylimarin treated groups as shown in Table 6.

**Table 6: Effect TAFPL on the biochemical parameters of CCl<sub>4</sub> intoxicated rats.**

Treatment	LPO nmol of MDA/mg of Protein	GSH µg/mg protein	SOD U/mg protein	CAT U/ mg protein
Control	5.2±0.4	7.49±0.29	146.22±6.41	4.21±0.26
CCl <sub>4</sub>	15.7±1.2 <sup>c</sup>	2.59±0.22 <sup>c</sup>	67.29±5.62 <sup>c</sup>	1.89±0.15 <sup>c</sup>
Std Sylimarin	6.3±0.7 <sup>b</sup>	5.64±0.25 <sup>b</sup>	118.34±6.12 <sup>b</sup>	2.24±0.27 <sup>b</sup>
TAFPL 100 mg/mL	9.8±0.4 <sup>b</sup>	3.35±0.28 <sup>a</sup>	58.44±5.66 <sup>b</sup>	2.59±0.31 <sup>b</sup>
TAFPL 200 mg/mL	8.7±0.6 <sup>b</sup>	4.36±0.13 <sup>b</sup>	69.23±4.31 <sup>b</sup>	2.89±0.34 <sup>b</sup>

Number of animals (n=6): Values are mean ± SEM: <sup>a</sup> P<0.01, <sup>c</sup> P<0.001 When compared to normal group, <sup>b</sup> P<0.01 When compared to CCl<sub>4</sub> groups.

**Table 7: In-vitro Antioxidant activity of TAFPL**

Sample (µg/mL <sup>-1</sup> )	% Inhibition (Mean± SEM)		Absorbance
	DPPH	NO	Reducing Power
TAFPL 200	57.59±1.63	49.63±0.49	0.72±0.05
TAFPL 100	46.48±1.22	31.65±0.31	0.66±0.08
TAFPL 50	24.67±1.31	19.52±0.68	0.36±0.06
TAFPL 25	19.51±1.49	09.37±0.19	0.14±0.005
Ascorbic acid 200	82.39±1.61	56.23±0.63	1.25±0.37
Ascorbic acid 100	68.37±2.34	39.36±0.22	0.79±0.06
Ascorbic acid 50	48.57±2.48	29.11±0.71	0.65±0.09
Ascorbic acid 25	35.34±2.56	19.72±0.83	0.59±0.02

Values represent the mean ± SEM: Number of readings in each group = 3

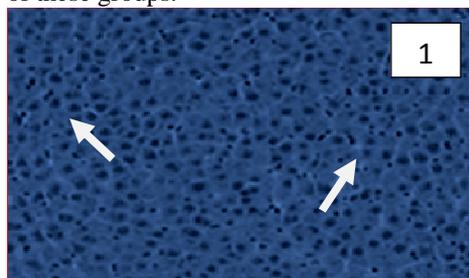
### In-vitro Antioxidant activity

Results from our *in-vitro* antioxidant studies revealed that TAFPL scavenged free radicals in a concentration dependent manner in the models studied. Maximum percentage inhibition of DPPH radicals by the extract was about

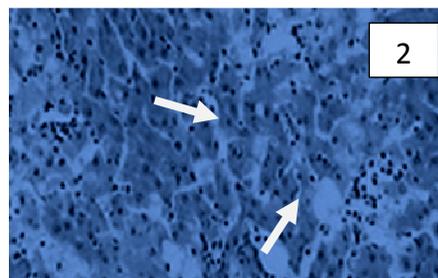
62% at 800  $\mu\text{g}/\text{mL}$  concentration whereas standard drug ascorbic acid showed maximum percentage of inhibition of DPPH radicals is about 82% at 200 $\mu\text{g}/\text{mL}$  (Table.5). In the nitric oxide radical scavenging model, the maximum percentage inhibition of nitric oxide radicals by TAFPL is about 51% at 200 $\mu\text{g}/\text{mL}$  (Table.5) whereas Ascorbic acid at 200 $\mu\text{g}$  caused about 56% inhibition and in reducing power method, TAFPL demonstrated dose dependent antioxidant activity comparable with ascorbic acid.(Table.7)

### Toxicity test

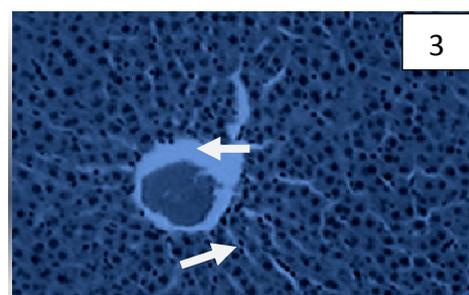
Experiment was carried out on normal healthy rats. The behaviour of the treated rats appeared normal. No toxic effect was reported at doses up to 7-10 times of effective dose of the methanol extract and there was no death in any of these groups.



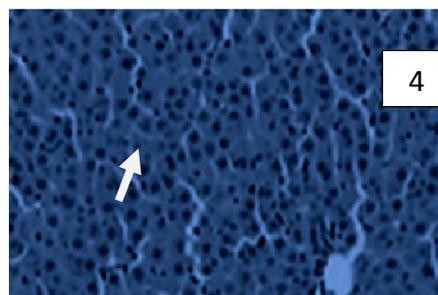
**Fig.1.** Shows the liver section of normal control rats with normal histology and normal architecture. (H & E, 100 xs).



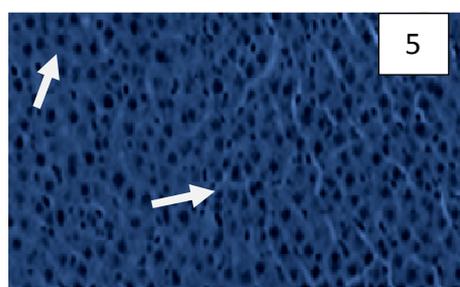
**Fig.2.** Shows the liver treated with  $\text{CCl}_4$  showing necrosis and ballooning like structure in the hepatocytes and fatty vacuole (H & E, 100 x).



**Fig.3.**Section of liver tissue treated with Sylimarin, showing normal histology with little ballooning, degeneration of hepatocytes. (H & E,100x).



**Fig.4.**Liver tissue treated with TAFPL showing a normal histology lobular pattern with mild changes (H & E,100x).



**Fig.5.**Liver section treated with TAFPL rat showing a normal histology lobular pattern with when compared to normal and Sylimarin received groups. (H & E,100x).

### Histopathological studies

In histopathological studies, the normal histological architecture of liver was lost in rats exposed to CCl<sub>4</sub> with the appearance of vacuolated hepatocytes and degenerated nuclei (Figure 2) were observed, while groups that received TAFPL 100 mg/kg and 200 mg/kg (Figure 4,5) shows normal hepatocytes with mild feathery changes with little ballooning. Liv 52 (Figure.3) shows a normal lobular pattern with minimal pooling of blood in the sinusoidal spaces. This histopathological findings further supports the hepatoprotective activity of total alkaloid fraction of *Polyalthiya longifolia* fruits against CCl<sub>4</sub> induced hepatic injury.

### DISCUSSION

The present study demonstrated the hepatoprotective and antioxidant effects of total alkaloid fraction of *polyalthiya longifolia* fruits against CCl<sub>4</sub> induced liver injury in rats. Treatment with TAFPL exhibited significant increase in percentage viability on rat hepatocytes (Table.1) and Hep G2 cells (Table 2) in the *in-vitro* study and *in-vivo* study exhibited restoration of the altered biochemical parameters towards normal and Sylimarin standard groups against CCl<sub>4</sub> intoxication.

The liver mainly detoxifies toxic chemicals, drugs and becomes the main target organ for all possible toxic xenobiotics. CCl<sub>4</sub> is one of the most commonly used hepatotoxin in experimental study of liver diseases [35]. CCl<sub>4</sub> is biotransformed by cytochrome p-450 in liver to produce highly reactive trichloromethyl free radical. This, in presence of oxygen generated by metabolic leakage from mitochondria produces lipid peroxidation of membrane lipid leading to loss of integrity of cell membranes and damage of hepatic tissue[36]. This effect was evidenced by increased levels of serum marker enzymes, namely AST, ALT and ALP and total bilirubin. Serum ALP and bilirubin are related to the function of hepatic cell [37,38].

In the present study, acute administration of CCl<sub>4</sub> produced a marked elevation of the serum levels of AST, ALT, ALP, serum bilirubin and total proteins in animals (Group II) when compared with normal control (Group I) confirming cellular breakage and loss of functional integrity of cell membranes in liver. Treatment with TAFPL at a dose of 100 mg/kg and 200 mg/kg significantly reduced the elevated levels of ALT, AST, and ALP towards the respective normal value which is an indication of plasma membrane stabilization as well as repair of hepatic tissue damage caused by carbon tetrachloride. Available data from earlier studies states that, stimulation of protein synthesis accelerates regeneration process and production of liver cells[39]. TAFPL received groups showed increased levels of total protein which indicates its hepatoprotective activity comparable with standard drug Sylimarin. These alterations can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.

Serum bilirubin is a breakdown product of haem in red blood cells and regarded as one of the most important clinical and pathophysiological indicator of necrosis of liver tissues. Treatment with TAFPL resulted in a significant decrease in total bilirubin levels as compared to CCl<sub>4</sub> treated group (Group II) and this effect was possibly due to stabilization of hepatic cellular membranes by the extract. The results were further confirmed by the histopathological observations. The effective control of alkaline phosphatase (ALP) and bilirubin levels towards normal is good indication suggesting improvement in the secretory mechanism of the hepatic cell.

An increase in LPO level in liver homogenate suggests enhanced lipid peroxidation which leads to tissue damage and failure of antioxidant defense mechanisms resulting in excessive free radicals. Inhibition of lipid peroxidation to a significant degree is also a predominant mechanism of hepatoprotection. Treatment with TAFPL at a dose of 100,200 mg/kg, produced significant (P<0.01) reductions in lipid peroxidation when compared to CCl<sub>4</sub> administered rats which further supports the above said hepatoprotective mechanism. Glutathione is known to protect the cellular system against toxic effects of lipid peroxidation [40]. Decreased level of GSH in the liver tissue on CCl<sub>4</sub> exposure represents its increased utilization due to oxidative stress[41]. In the present study, a significant elevation of GSH level was observed in the TAFPL treated CCl<sub>4</sub> rats which indicate that TAFPL can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH.

Superoxide dismutase is also an important indicator of hepatocellular damage in both acute and chronic conditions. SOD has potential role in scavenging the superoxide anion to form hydrogen peroxide and thus diminishes the toxic effect caused by this radical [42]. In the present study, it was observed that the TAFPL caused a significantly increased in the hepatic SOD activity of the CCl<sub>4</sub> intoxicated rats. This shows TAFPL can reduce reactive free radicals that might cause oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme. Increase in the SOD level portrays the repairing mechanism of antioxidant defense system, which plays an important role in hepatoprotection. CAT is a heme protein, which catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals[43]. This decrease in CAT activity could result from

inactivation by glycation of the enzyme[44]. Further, an increase in the SOD activity may protect CAT against enzyme inactivation by superoxide radical as these radicals have been shown to inactivate CAT [45]. Thus, the increase in SOD activity may indirectly play an important protective role in preserving the activity of CAT.

In the present study, observed results from the DPPH radical scavenging and nitric oxide radical scavenging method indicates, the definite scavenging activity of the TAFPL towards DPPH radicals in comparison with ascorbic acid. Nitric oxide is a free radical produced in the mammalian cells and is involved in regulation of various physiological processes. However excess production of nitric oxide is associated with several diseases [46,47]. Administration of TAFPL has demonstrated dose dependent radical scavenging activity against NO free radicals. TAFPL also has good radical quenching activity against both DPPH and the nitric oxide radicals. In reducing power method, TAFPL demonstrated dose dependent antioxidant activity comparable with Ascorbic acid. In all the methods, scavenging effect of TAFPL increases with increasing concentration and maximum antioxidant activity was found at the dose of 800 µg/ml and above of TAFPL. Additionally, histopathological studies confirmed that CCl<sub>4</sub> caused fatty degeneration, necrosis and fibrosis of liver tissue in rats (Fig 2.). Treatment with TAFPL showed dose dependent protection of liver tissue, which supports the findings of biochemical studies. The ability of a hepatoprotective drug to reduce the injury or to preserve the normal hepatic physiological mechanisms against hepatotoxin, is an index of its protective effects.

### CONCLUSION

Total alkaloid fraction of *Polyalthia longifolia* fruits (TAFPL) inhibited the degree of hepatic necrosis and concomitantly decreased the leakage of intracellular enzymes by stabilizing hepatic cellular membranes. Inhibition of lipid peroxidation to a significant degree is also a predominant mechanism of hepatoprotection as suggested by the significant decrease in MDA levels. Increase in the SOD level was also suggestive of repairment of antioxidant defense system, which plays an important role in hepatoprotection. Based upon the results, we can conclude that TAFPL has proven itself as a significant hepatoprotective as well as a considerable antioxidant against CCl<sub>4</sub> intoxication which is further supported by the histopathological results. The *in-vitro* and histopathological studies clearly indicates and supports the hepatoprotective and antioxidant potential of total alkaloid fraction of *polyalthia longifolia* fruits against CCl<sub>4</sub> intoxication through its free radical scavenging and antioxidant properties both *in vitro* and *in vivo* models.

### Acknowledgements

We are very grateful to Dr. K. T. Manisenthil Kumar, HOD, Department of Pharmacology, KMCH, Dr.N.Arun kumar, Department of Pharmaceutics for their valuable suggestions and support and Dr.N.Adhirajan Department of Biotechnology, KMCH, for providing cell lines to carry out this research work.

### REFERENCES

- [1] FM Ward;MJ Daly; Hepatic Disease, In: Clinical Pharmacy and Therapeutics, Churchill Livingstone press, New York, 1999, 195-212.
- [2] K Law; E Brunt; *Clinics. Liver. Dis.* 2010, 14, 591-604.
- [3] F Stickel; D Schuppan ; *Digestive Liver Dis .* 2007, 39, 293-304.
- [4] M Feijoo; I Túnez ; A Ruiz ; Tasset ; E Muñoz ; E Collantes; *Clínica.* 2010, 6, 91-94.
- [5] BK Chandan; AK Saxena; S Shukla; N Sharma; DK Gupta; K Singh; *J. Ethnopharmacol.* 2008, 119, 218-224.
- [6] WE Stehbens; *Exp .Mol .Pathol.* 2003, 75, 265-276.
- [7] H Jaeschke; TR Knight; ML Bajt; *Toxicol Lett.* 2003, 144, 279-288.
- [8] KH McDonough ; *Toxicology.* 2003, 189, 89-97.
- [9] H Jaeschke; CV Smith; JR Mitchell; *J Clin Invest.* 1998, 81, 1240-1246.
- [10] M Gupta; UK Mazumder; KR Sambath.; *European. Bull .Drug Res.* 2004, 12, 13-22.
- [11] V Kukongviriyapan; T Janyacharoen; U Kukongviriyapan; P Laupattarakasaem; S Kanokmedhakul; P Chantaranonthai; *Phytother. Res.* 2003, 17, 717-721.
- [12] D Rubinstein; *American. J. of Physiology.* 1962, 203, 1033-1037.
- [13] SR Suja; PG Latha; P Pushpangadan; S Rajasekharan; *J. Tropical Medicinal Plants.* 2002, 3, 191-195.
- [14] K Raghunathan; MK Mitra; Pharmacognosy of indigenous drugs, Central council for research in Ayurveda and Siddha, New Delhi 1982, 1, 127-139.
- [15] CM Hasan; SN Islam; M Ahsan; Antibacterial activity of stem bark of *Polyalthia longifolia*. Dhaka Univ. Stud, 1988, 4, 63-66.
- [16] G Achari *Ind Pharm.* 1952, 8, 538-545.
- [17] A Sayeed; A Islam; MY Mia; MA Bhuyan; G Khan; *J. Biol. Sci.* 1995, 3, 165-169.
- [18] SK Chakrabarti; B Mukherjee; *J. Res. Ind. Med.* 1968, 3, 84- 85.

- [19] C Chen; F Chang; Y Shih; *J. Nat. Prod.* **2000**, 63, 1475–1478.
- [20] GW Trease; WC Evans; *Alkaloids, Pharmacognosy*, Elsevier Saunders, India: **2005**, 333-338.
- [21] SA Tasaduq; K Singh; S Sethi; SC Sharma; KL Bedi; J Singh; BS Jaggi; RK Johri; *Hum Exp. Toxicol.* **2003**, 22, 639-645.
- [22] PO Seglen; *Cell. Biol* **1976**, 13, 29-83.
- [23] HU Bergmeyer ; P Scelibe; *Clin. Chem.* **1978**, 24, 58-61.
- [24] K King; *The hydrolases-acid and alkaline phosphatase*, Practical Clinical Enzymology, Van, London, Nostr and company Lt: **1965**, 191-208.
- [25] HJ Malloy; KA Evelyn; *J. Biol. Chem.* **1937**, 119, 481.
- [26] OH Lowry; NJ Rosebrough; AL Farr; RJ Randall; *J. Biol. Chem.* **1951**, 193, 265-75.
- [27] Y Kiso; M Tohkin; H Hikino; *planta. Med.* **1983**, 49, 222-5.
- [28] K Hu ; H Kobayashi; A Dong; Y Jin; S Iwasaki; X Yao; *Planta. Med.* **1999**; 65; 35-8.
- [29] GL Ellman; *Arch. Biochem. Biophys.* **1959**, 82, 70-77.
- [30] P Kakkar; B Das; Viswanathan PN. *Ind. J. Biochem. Biophys.* **1984**, 21, 131-2.
- [31] A Aebi; *Methods in enzymatic analysis*, Academic Press, New York: **1974**, 2, 674-84.
- [32] MS Blois; *Nature* **1958**, 181, 1199-1200.
- [33] LC Green; DA Wagner; J Glogowski; PL Skipper; Wishnok; *Anal. Biochem.* **1982**, 126, 131-138.
- [34] M Oyaizu; *. Japanese J. Nutr* **1986**, 44, 307-315.
- [35] DE Johnson; C Kroening; *Pharmacol. Toxicol.* **1998**, 83, 231-39.
- [36] RO Recknagel; EA Glende; AM Hruszkewycz; *Free radicals in biology*. Academic press: **1976**, 3, 97.
- [37] P Muriel; T Garciapina; V Perez Alvarez; M Murelle; *J. Appl. Toxicol* **1992**, 12, 439-42.
- [38] M Uma Maheswari; PGM Rao; *Indian. J. Pharmacol.* **2005**, 37, 179-82.
- [39] P Nicotera; S Orrenius; *Adv. Exp. Med. Biol.* **1986**, 197, 41–49.
- [40] CV Anuradha; R Selvam; *J. Nutr. Biochem.* **1993**, 4, 212–217.
- [41] SJ Curtis; M Moritz; PJ Snodgrass; *Gastroenterology.* **1972**, 62, 84-92.
- [42] B Chance; DS Greenstein; RJW Roughton; *Arch. Biochem. Biophys.* **1952**, 37, 301–339.
- [43] H Yan; JJ Harding; *Biochem. J.* **1997**, 328, 599–605.
- [44] Y Kono; I Fridovich; *. J. Biol. Chem* **1982**, 257, 5751–5754.
- [45] HC Wu; HM Chen; CYC Shia; *Food. Res. Int* **2003**, 36, 949-957.
- [46] R Gibanananda; AH Sayed ; *Indian J. Exp. Biol.* **2002**, 40, 1213-1232.
- [47] A Ialenti; S Moncada; M Di Rosa; *Brit. J. Pharmacol.* **1993**, 110, 701-706.