International Journal of Biomedical and Pharmaceutical Sciences ©2009 Global Science Books

Isolation, Characterization and Sub-acute Toxicity Studies of a New Compound PITC-2 Isolated from Tissue-Cultured Medicinal Plant, *Pluchea indica* (L.) Less

Kartick Chandra Pramanik • Tapan Kumar Chatterjee*

Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India

Corresponding author: * **tkchatterjee_81@rediffmail.com; tkchatterjee81@yahoo.co.in**

ABSTRACT

The present study focused on the chemical characterization of PITC-2 (2-(prop-1-ynyl)-5(5,6-dihydroxyhexa-1, 3-diynyl)-thiophene, a thiophen derivative) isolated from the root extract of tissue-cultured medicinal plant *Pluchea indica* (L.) Less. Evaluation of sub-acute toxicity study of the new compound was also done *in vivo*. Methanolic root extract of tissue-cultured *P. indica* was separated by solvent-solvent partition into three fractions (water, *n*-butanol, and ethyl-acetate). The ethyl-acetate soluble fraction was subjected to column chromatography using silica gel and PITC-2 was isolated and characterized by NMR IR, and MS. Sub-acute toxicity study of PITC-2 was studied on Swiss Wistar rats. The studies included the gross observation such as changes in body weight, hematological profiles [total count of red blood cell (RBC), white blood cell (WBC) and differential count of white blood cell and haemoglobin (Hb) percentage], biochemical parameters of serum [total protein, serum glutamate oxaloacetate transminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum bilirubin, creatinine, urea, triglyceride, cholesterol, glucose], liver enzymes level [thiobarbituric acid reactive substance (TBARS), glutathione (GSH), super oxide dismutage (SOD)] and histopathology of the liver, kidney, heart, spleen and lung of both the control and experimental groups of rats. The changes of body weight, hematological profiles, biochemical parameter, and liver enzymes were not significant compared to control groups of animals. However, a change of liver weight but not of hepatic enzymes was observed.

Keywords: biochemical parameter, hematological profiles, root, spectroscopy

INTRODUCTION

Natural product chemistry can be defined as the exploration of nature in the search of novel drug or drug leads. Drugs were isolated from natural sources, many of which have been used by various cultures throughout history. The World Health Organization (WHO) estimates that approximately 80% of the world relies on natural sources for primary medical treatment and that the health care systems for the remaining 20% of the population also incorporate natural sources in their medical treatments (Cragg 2002). Some well known and important drugs have originated from natural sources. In a study of the Pharmaceuticals on the market from 1981-2002, only 43% of the drugs were purely synthetic, while the remaining 57% were derived from a natural source (Newman *et al.* 2003). In order to develop and establish the safety and efficacy level of a drug, toxicological studies are very essential experiments in animals like rats, guinea pigs, dogs, monkey etc under various conditions. Toxicological data help to make decision whether a new drug is adopted for clinical use or not.

Pluchea indica (L.) Less. (Asteraceae) is a large, evergreen shrub found abundantly in salt marshes and mangrove swamps in Sunderbans (India), Bangladesh, Myanmar, China, Philippines, Malaysia, Tropical Asia and Australia. In Indo-China the a decoction of roots is prescribed for fevers as a diaphoretic and an infusion of the leaves is given internally to treat lumbago while the roots and leaves are used as an astringent and antipyretic (Kirtikar and Basu 1999). The plant is also used to cure rheumatoid arthritis (Chatterjee 1996). The root extract has anti-inflammatory (Sen and Nag Chowdhury 1991), antiulcer (Sen *et al.* 1993), and neuropharmacological (Thongpraditchote *et al.* 1996) properties. The hypoglycemic and antihyperglycemic effects of *P. indica* leaves (Pramanik *et al.* 2006) and the anti-oxidant activity (Sen *et al.* 2002) of roots have already been reported. Four of the pure compounds (R/J/1-stigmasterol (+ β-sitosterol), R/J/2-stigmasterol glucoside (+ β-sitosterol glucoside), R/J/3-2-(prop-1-ynyl)-5 (5,6-dihydroxyhexa-1,3 -diynyl)-thiophene, and R/J/4-(-) catechin) isolated from wild *P. indica* roots have been reported to possess potent antimicrobial activities (Biswas *et al.* 2005). A eudesmane derivative from the leaves (Mukhopadhyay *et al.* 1983), five terpenic glycosides from aerial parts (Uchiyama *et al.* 1989), three eudesmane-type sesquiterpenes and three lignan glycosides, together with a eudesmane-type sesquiterpene from roots (Uchiyama *et al.* 1991) and two thiophene derivatives, besides two pentacyclic triterpenes of rare occurrence from roots (Chakravarty and Mukhopadhyay 1994) have been isolated from this plant. A pure compound (R/J/3) isolated from the roots of *P. indica* was found to be very effective against the protozoa *Entamoeba histolytica* (Biswas *et al.* 2006). *P. indica* plant was tissue cultured with the purpose of obtaining more secondary metabolites. Tissue-cultured *P. indica* leaves have a diuretic effect (Pramanik *et al.* 2007). The root extract of tissue-cultured *P. indica* has antibacterial activity (Pramanik *et al.* 2008), *in vitro* and *in vivo* antibacterial activities against bacillary dysentery (Pramanik *et al.* 2008) and antitumor activity (Pramanik *et al.* 2008). We report here for the first time the isolation of a thiophene derivative from the root of tissue-cultured medicinal plant *P. indica* along with its sub-acute toxicity studies in rats.

Received: 3 January, 2009. Accepted: 31 March, 2009.

Original Research Paper

ISOLATION, PURIFICATION AND CHARACTERIZATION OF PITC-2

General

^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively on a Bruker-300 NMR spectrometer. Mass spectra were taken on a JEOL JMS 600 mass spectrometer. IR spectra were recorded on a JASCO IR-700 spectrophotometer. Optical rotations were measured on a JASCO P-1020 polarimeter.

Chemicals

Chemicals and reagents that were used for the study of sub-acute toxicity study were purchased from Sigma-Aldrich Co. while other chemicals, solvents and reagents used in chromatography were purchased from Merck.

Plant material

The roots of tissue-cultured *P. indica* (8 months-old, matured, with a voucher specimen maintained in the laboratory) based on the protocol by Pramanik *et al.* (2007), were separated, washed, oven-dried at 60°C for 2-3 h (depending on moisture content), powdered by a micro pulverizer and sieved through 100 meshes (0.0254 cm diameter; Wire Netting Stores, Kolkata, India). Fibers and unwanted debris were discarded after sieving. The powder was preserved in an airtight container for further use. The plant was taxonomically identified and authenticated by the Botanical Survey of India, Sibpur, India.

Extraction and isolation

The pulverized powder (500 g) was soaked overnight with petroleum ether (60-80°C for synthesis, Merck) dried it at room temperature and extracted with methanol (AR Grade, Purity 99.8%, Sisco Research Laboratories Pvt. Ltd.) using a Soxhlet extractor to obtain the methanolic root extract of tissue-cultured *P. indica*. The solvent was then evaporated under reduced pressure using a rotary evaporator (Model no. HS-2005V) to obtain a semi-solid residue. The yield of the extract was 8.7% (w/w). A portion (43.5 g) of the crude methanolic extract was partitioned between *n*-butanol and water. The butanol and water fractions were separately evaporated. The butanol fraction was then shaken with ethyl acetate to obtain an ethyl acetate soluble and insoluble part. The former part was concentrated in a rotary vacuum evaporator and then dried to obtain a crude residue (10 g) which was subjected to column chromatography over silica gel (60-20 mesh, 300 g) as an adsorbent using petroleum ether-ethyl acetate mixtures of increasing polarity as eluants. 10 ml fractions were collected and mixed on the basis of their TLC behavior. Elution with pet-ether: ethyl acetate mixture (6: 4, 4: 6) afforded one fraction A (1.5 g) with trace material in the TLC chamber I (benzene: chloform: ethyl acetate = 6: 3: 1). Fraction A was further chromatographed over silica gel (45 g) yielded a yellowish solid B (0.8 g) in the chamber I when eluting with pet-ether:ethyl acetate (8:2, 7:3). This B fraction was further chromatographed over silica gel (24 g) by elution with pet-ether: ethyl acetate (8.5: 1.5, 8: 2) to yield a single prominent spot which was further studied by NMR, IR, and MASS spectra to give PITC-2 (**Fig. 1**), a light yellow solid (80 mg; relative amount = 0.000016 mg w/w).

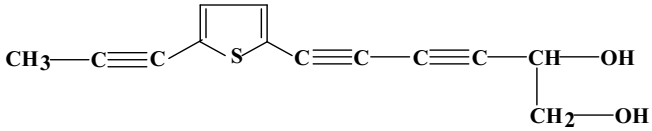

Fig. 1 PITC-2, 2-(prop-1-ynyl)-5-(5,6-dihydroxy-hexa-1,3-diynyl)-thiophene.

The chambers used for TLC

Five chambers were use for TCL:
Chamber I: benzene: chloroform: ethyl acetate = 6: 3: 1
Chamber II: pet ether: ethyl acetate = 7.5: 2.5
Chamber III: benzene: ethyl acetate = 3: 7
Chamber IV: chloroform: methanol = 8.5: 1.5
Chamber V: toluene: ethyl acetate: formic acid = 5: 4: 1.

Sub-acute toxicity study of PITC-2 in rats

Animals

Healthy inbred gender-matched Swiss Wistar rats weighing 200-220 g (supplied by m/s Reeta Ghosh) were used for the study. The rats were housed in iron cages (considering group) under controlled temperature and light (Khanna *et al.* 1989) and fed with standard laboratory diet (Hawks *et al.* 1954) and water *ad libitum.* Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced. The study was approved by the University Animal Ethical Committee.

Administration of sample

The animals were divided into three groups of 10 each. The animals of group I were left untreated and considered as the control and administered with isotonic vehicle daily. Animals of group II were treated with 2.5 mg/kg bw/day of PITC-2 and animals of group III were treated with PITC-2 (5 mg/kg bw/day) intraperitonially for 14 consecutive days.

Blood collection

For the hematological study (total and differential blood cell count and percent hemoglobin determination), blood was drawn from the retro orbital puncture of three groups before drug administration, at the 7th day and after completion of treatment. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis. All rats were sacrificed after blood collection. The internal organs and some tissues (liver, kidney, heart, and lung) were weighed to determine relative organ weights. Liver tissues were preserved in 10% buffered formaldehyde solution for histopathological examination and enzyme assay.

Gross general observation

During the whole experimental period their behavior, central nervous system (CNS) excitation, CNS depression, muscular weakness, salivation, diarrhea and food intake were observed. Toxic manifestations such as sign of mortality and body weight changes were monitored daily.

Investigation of hematological profile

The hematological parameters like total count (TC) of red blood cells (RBC), white blood cells (WBC), and differential count (DC) of WBC, and haemoglobin percentage were performed just before drug intake and 7th day of treatment and after completion of treatment. For TC, DC blood smears were made on glass slides followed by staining with Leishman reagent. Heamoglobin percentage was measured by Van Kampen-Ziftra's method.

Biochemical parameters of blood

SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase), SALP (Serum Alkaline Phosphates), serum bilurubine, creatinine, total protein, burn urea (uric acid), triglyceride, and cholesterol were determined after 14 days of drug administration using an automatic analyzer by the use of a Span diagnostic Kit.

Histopathological investigation

The tissues of liver, kidney, heart, and lung were obtained from

Table 1 Effect of PITC-2 on body weight of rats after intraperitoneal administration.

Groups	Body weight (g)						% Changes
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 14	
Normal (only NS)	195.00 ± 5.40	197.50 ± 3.22	199.50 ± 5.40	203.00 ± 3.50	205.00 ± 5.20	208.00 ± 3.50	6.66% weight gain
PITC-2 (2.5 mg/kg)	196.00 ± 4.50	197.50 ± 3.50	198.50 ± 2.50	199.00 ± 5.30	201.50 ± 3.50	205.00 ± 3.50	4.59% weight gain
PITC-2 (5 mg/kg)	195.25 ± 4.38	196.83 ± 5.20	197.25 ± 2.50	198.00 ± 3.50	198.56 ± 2.50	202.25 ± 5.60	3.58% weight gain

$P > 0.05$ vs. normal (received only saline). Values are expressed as mean± SEM (n = 10). Results were analyzed by ANOVA followed by Dunnett's test.

both experimental and control groups after 14 days of drug administration for histopathological studies. Pieces of tissues of two groups of animals were fixed in 4% formalin (10 ml of 40% formaldehyde made up to 100 ml with normal saline) overnight. Then the tissues were dehydrated with a graded concentration of alcohol, cleared with benzene and embedded with paraffin bath. The paraffin blocks were cut at 5 µm in a rotary microtome (Model: MT-1090A, WESWDX, OPTIK) and the section were stained with vangeison and mounted in Canada balsam on slides. Thus the slides were prepared for microscopical observation under power microscope (AXIOSTAR Plus, Zeiss, Japan, 400X).

Liver enzyme assay

Estimation of lipid peroxidation

According to the methods of Niehius and Samuelsson (1968) and Jiang *et al.* (1992) lipid peroxidation in liver was estimated calorimetrically by thiobarbituric acid reactive substances (TBARS). In brief, 0.1 ml of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 ml of (1: 1: 1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in a water bath at 97°C for 45 min, then cooled. The absorbance of clear supernatant was measured against a reference blank at 535 nm.

Reduce glutathione

Reduced glutathione (GSH) in liver was assayed by the method of Ellman (1959). 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. Reduced glutathione was expressed as µg/mg of protein.

Super oxide dismutase

Super oxide dismutase (SOD) activity was assayed by the method of Kakkar *et al.* (1984). The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1 ml PMS (186 mmol/L), 0.3 ml NBT (300 mmol/L), 0.2 ml NADH (780 mmol/L) and approximately diluted enzyme preparation and water in a total of 3ml. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml *n*-butanol. The color intensity of chromogen in the butanol layer was measured at 560 nm against *n*-butanol.

Statistical analysis

The results were expressed as mean ± SEM (standard error of mean). The data were subjected to one way ANOVA followed by Dunnett's test and values with $P \leq 0.001$ were considered statistically significant.

RESULTS AND DISCUSSION

PITC-2 was crystallized from C_6H_6 as light yellow needles (80 mg) and it appeared green with Liebermann-Burchard reagent. The melting point of the compound was 106–108°C. PITC-2 in IR showed ν_{max}^{KBr} cm^{-1}: 3328 (br), 3104, 2956, 2923, 2872, 2150, 1778, 1451, 1322, 1186, 1080 (s), 1022, 946, 864, 805 (m), 688. The compound showed IR absorptions for alcoholic groups (strong bonds at 3328 and 1080 cm^{-1}). Small but significant peaks at 3104 and 2150 cm^{-1} were indicative of an unsaturated system with a triple bond. EI-MS showed signals at *m/z* (rel. abundance %): 230 (M^+, 90), 199 (100), 171 (33), 170 (32), 169 (33), 145 (22), 139 (20), 127 (50). The mass spectrum showed a strong molecular ion peak at *m/z* 230, the base peak at *m/z* 199 and another strong peak at *m/z* 169 which agreed with the presence of a CHOH-CH_2OH moiety. In ^{1}H NMR δ^{TMS} ($CDCl_3$, 300 MHz): 1.64 (D_2O exchangeable, OH merged with solvent H_2O), 2.04 (3H, s), 3.78 (^{1}H, dd, J=11.4, 6.3 Hz, H_A of CH-C$\underline{H}_2$OH), 3.82 (^{1}H, dd, J=11.4, 3.9 Hz, H_B of CH-C$\underline{H}_2$OH), 4.69 (^{1}H, dd, J=6.3, 3.9 Hz, C$\underline{H}$OH-CH_2OH), 7.04, 7.18 (^{2}H, m, thiophene-H). The ^{1}H NMR spectrum showed peaks for a methyl attached to unsaturation (δ 2.04, s), a CHX-CH_2Y unit and an aromatic system. Finally the compound was identified as 2-(prop-1-ynyl)-5-(5,6-dihydroxy-hexa-1,3-diynyl)-thiophene (**Fig. 1**).

Table 2 Hematological profile of control group rats.

Hematological parameter	Normal rats	Treated with vehicle	
	Day 1	Day 7	Day 14
Total RBC count (million/cc)	4.87 ± 0.08	5.17 ± 0.04 a	4.97 ± 0.04 b
Total WBC count (thousand/cc)	5.00 ± 0.33	5.66 ± 0.18 b	6.05 ± 0.06 a
Hemoglobin/g%	12.36 ± 0.17	12.80 ± 0.17 b	12.91 ± 0.09 b
Differential count of WBC in %			
Monocyte	1.50 ± 0.28	1.55 ± 0.28 b	1.75 ± 0.25 b
Lymphocyte	45.5 ± 2.02	57.50 ± 0.86 b	49.25 ± 0.47 b
Neytrophil	43.0 ± 1.78	45.00 ± 2.04 b	42.50 ± 2.39 b
Eosinophil	2.50 ± 0.28	2.62 ± 0.23 b	3.00 ± 0.40 b

a, $P < 0.05$, b, $P > 0.05$ vs. normal (received only saline). Values are expressed as mean ± SEM (n = 10). Results were analyzed by ANOVA followed by Dunnett's test.

In the present study, we found that the average body weight of all rats during 14 days treatment with intraperitoneal administration of PITC-2 (2.5 mg/kg bw and 5 mg/kg bw) increased. After 14 days the control group gained 6.66% weight and the experimental groups gained 4.59% weight (2.5 mg/kg bw of PITC-2) and 3.58% (5 mg/kg bw of PITC-2) (**Table 1**). Changes in body weight for both control and experiment groups were insignificant. During the whole experimental period their behavior, central nervous system (CNS) excitation, CNS depression, muscular weakness, salivation, diarrhea and food intake were observed and there is no physical abnormality was observed in both experimental groups and control. **Tables 2** and **3** showed a haematological profile that was determined before treatment, at the 7th day of treatment and after treatment and data was compared against control to check the haematological disorders. No significant changes in the values of RBC and WBC count, differential count of WBC and haemoglobin percentage of the experimental group were observed when compared to the control group. The organ weight of liver, kidney, lung, spleen and heart were obtained from both experimental and control groups after 14 days of drug administration. As shown in **Table 4**, the liver weight decreased slightly compared to the control but this is not an affected liver function or liver enzymes level. In contrast, the weight of heart, kidney, spleen and lung of experimental group were significantly higher than in the control. All of the increases and decreases in organ weight were minor changes and the difference may have been due to the variation in size of internal organ or body weight of the animals (Bailey *et al.* 2004; Carol 1995). Necropsy and histopathology examinations were performed to further confirm whether or not the organs or tissue had been damaged. There were no macroscopic or microscopic changes in the internal organs

Table 3 Hematological profile treated with PITC-2 (2.5 mg/kg bw and 5 mg/kg bw) group rats.

Haematological parameter	Normal rats	PITC-2 (2.5 mg/kg bw)		PITC-2 (5 mg/kg bw)	
	1st day	7th day	14th day	7th day	14th day
Total RBC count (Million/ml)	4.87 ± 0.08	5.22 ± 0.07 a	4.98 ± 0.65 a	5.27 ± 0.08 a	5.40 ± 0.10 b
Total WBC count (Thousand/ml)	5.00 ± 0.33	5.23 ± 0.13 a	5.68 ± 0.29 a	5.52 ± 0.18 a	5.72 ± 0.08 a
Hemoglobin/g%	12.36 ± 0.17	12.05 ± 0.28 a	12.38 ± 0.33 a	11.33 ± 0.61 a	12.10 ± 0.30 a
Differential count of WBC in %					
Monocyte	1.50 ± 0.28	1.80 ± 0.25 a	2.10 ± 0.29 a	2.50 ± 0.45 a	3.75 ± 0.47 b
Lymphocyte	45.50 ± 2.02	41.02 ± 0.005 a	38.45 ± 0.25 c	36.50 ± 1.19 b	37.75 ± 1.31 c
Neytrophil	43.00 ± 1.78	43.25 ± 2.50 a	47.41 ± 0.45 a	45.00 ± 2.04 a	47.00 ± 0.92 a
Eosinophil	2.50 ± 0.28	2.65 ± 0.37 a	3.25 ± 0.14 a	3.75 ± 0.47 a	4.62 ± 0.23 b

a, $P > 0.05$, b, $P < 0.01$, c, $P < 0.05$ vs. normal (received only saline). Values are expressed as mean± SEM (n = 10). Results were analyzed by ANOVA followed by Dunnett's test.

Table 4 Weight of organs after 14 days treatment with PITC-2.

Organs	Normal group After 14 days treatment	treated with PITC-2 for 14 consecutive days	
		PITC-2 (2.5 mg/kgbw)	PITC-2 (5 mg/kgbw)
Liver weight (g)	7.73 ± 0.05	6.91 ± 0.03 a	6.38 ± 0.07 a
Kidney weight (g)	0.85 ± 0.03	0.88 ± 0.01 b	0.87 ± 0.01 a
Lung weight (g)	1.29 ± 0.01	1.30 ± 0.02 c	1.31 ± 0.02 c
Spleen weight (g)	0.36 ± 0.00	0.37 ± 0.01 c	0.37 ± 0.01 c
Heart weight (g)	0.76 ± 0.01	0.77 ± 0.01 c	0.77 ± 0.01 c

a, $P > 0.05$, b, $P < 0.01$, c, $P < 0.05$ vs. normal (received only saline). Values are expressed as mean± SEM (n = 10). The results were analyzed by ANOVA followed by Dunnett's test.

Table 5 Effect of PITC-2 on biochemical parameters of rats blood after intraperitoneal (i.p) administration for 14 consecutive days.

Biochemical parameters	Normal (treated with only NS)	treated with PITC-2 for 14 consecutive days	
		PITC-2 (2.5 mg/kgbw)	PITC-2 (5 mg/kgbw)
Total Protein (mg/dl)	7.26± 0.13	7.58 ± 0.03 a	7.78 ± 0.03 b
SGOT (IU/L)	97.66 ± 0.10	103.26 ± 1.22 b	133.00 ± 0.80 b
SGPT (IU/L)	63.50 ± 0.18	73.73 ± 1.08 b	74.29 ± 0.27 b
SALP (IU/L)	190.41 ± 0.96	215.5 ± 3.11 b	226.66 ± 0.88 b
Serum bilirubin (IU/g)	0.53 ± 0.01	0.87 ± 0.01 b	0.90 ± 0.02 b
Creatinine (mg/dl)	0.79 ± 0.02	0.89 ± 0.02 c	0.92 ± 0.02 c
Urea (mg/dl)	22.08 ± 0.54	29.41 ± 0.79 b	31.41 ± 0.74 b
Triglyceride (mg/dl)	126.91 ± 0.22	136.75 ± 0.38 b	148.50 ± 0.28 b
Cholesterol (mg/dl)	113.58 ± 0.30	83.66 ± 0.22 b	99.50 ± 1.25 b
Glucose (mg/dl)	124.41 ± 0.22	122.70 ± 0.50 a	120.83 ± 0.49 b

a, $P > 0.05$, b, $P < 0.01$, c, $P < 0.05$ vs. normal (received only saline). Values are expressed as mean± SEM (n = 10). The results were analyzed by ANOVA followed by Dunnett's test.

of any of the treated rats. Biochemical parameters of blood (total protein, SGOT, SGPT, alkaline phosphatase, serum bilirubin, creatinine, urea, triglyceride, cholesterol, glucose) were determined after treatment of PITC-2 and compared to that of control group of rats to check any changes of these parameters. Most of the parameters changed slightly with respect to control group of rats but remain within the normal level (**Table 5**). After 14 days treatment the animals of both control and experimental groups were sacrificed and the liver, heart, lungs, kidney and spleen were isolated and examined under a microscope. The effect of PITC-2 on liver enzyme such as GSH, SOD was checked and no mentionable change of experimental rats was observed when compared to that of control group rats (**Table 6**). Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity and carcinogenicity of many xenobiotics (Anane *et al.* 2001). However, in our study, the MDA level of experimental groups was normal compared to control. No abnormality was detected in the organs of both control and experimental animals, indicating that PITC-2 possessed no significant adverse effect on cellular structures. The results of sub-acute toxicity studies have shown no abnormalities on body weight, gross general behavior, haematological and biochemical parameters of blood and liver enzymes level and histopathological study. The biological effect of PITC-2 and its present toxicological studies suggest that PITC-2 can be safely subjected to chronic toxicological studies and clinical trials.

ACKNOWLEDGEMENTS

The authors wish to thank All India Council for Technical Education (AICTE), New Delhi for the financial assistance rendered [F. No: 8023/BOR/RPS-184/2006-07 revalidated 2007-08 dt. 13.07.2007].

REFERENCES

Anane R, Creppy EE (2001) Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase + catalase and vitamins E and C. *Human and Experimental Toxicology* **20**, 477-481

Bailey SA, Zidell RH, Perry RW (2004) Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? *Toxicologic Pathology* **32 (4)**, 448-466

Biswas R, Dasgupta A, Mitra A, Roy SK, Dutta PK, Achari B, Dastidar SG, Chatterjee TK (2005) Isolation, purification and characterization of four pure compounds from the root extract of *Pluchea indica* (L.) Less. and the potentiality of the root extract and the pure compounds for antimicrobial activity. *European Bulletin of Drug Research* **13**, 63-70

Biswas R, Dutta PK, Achari B, Bandyopadhyay D, Mishra M, Pramanik KC, Chatterjee TK (2006) Isolation of pure compound R/J/3 from *Pluchea indica* (L.) Less and its anti-amoebic activities against *Entamoeba histolytica. Phytomedicine* **14**, 534-537

Carol SA (1995) Acute, subchronic and chronic toxicology. In: Derelanko MJ, Hollinger MA (Eds) *CRC Handbook of Toxicology*, CRC Press, Inc., FLA, pp 51-104

Chakravarty AK, Mukhopadhyay S (1994) New thiophene derivatives from *Pluchea indica. Indian Journal of Chemistry* **33**, 978-980

Chatterjee TK (1996) *Herbal Options*, M/s Eastern Traders, Calcutta, India, pp 183, 215

Cragg GM (2002) Natural product drug discovery and development: The United States National Cancer Institute role. *Puerto Rico Health Sciences Journal* **21**, 97-111

Ellman GL (1959) Tissue sulfhydryl groups. *Archives in Biochemistry and Biophysics* **82**, 70-77

Hawks PB, Oser L, Summerson WH (1954) *Practical Physiological Chemistry* (13th Edn), McGraw Hill Book Co., US, pp 394-395

Jiang ZY, Hunt JV, Wolff SP (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low-density lipoprotein. *Analytical Biochemistry* **202**, 384-387

Table 6 Effect of PITC-2 on the GSH, SOD, and MDA in rat after intraperitoneal (i.p) administration for 14 consecutive days.

Groups	Protein (mg/ml)	GSH (µg/mg of protein)	MDA (nmol/mg of protein)	SOD (U/mg of protein)
Normal	37.20 ± 0.10	11.63 ± 0.19	0.1085 ± 0.0008	32.95 ± 0.29
PITC-2 (2.5 mg/kgbw)	35.49 ± 0.18 a	11.41 ± 0.10 b	0.114 ± 0.001 c	31.70 ± 0.10 a
PITC-2 (5 mg/kgbw)	34.33 ± 0.22 a	9.40 ± 0.25 a	0.117 ± 0.0007 a	29.68 ± 0.09 a

[a]$p<0.01$, [b]$p<0.05$ [c]$p>0.05$ vs. Normal (received only saline). Values are expressed as mean± SEM (n = 10). The results were analyzed by ANOVA followed by Dunnett's test.

Kakkar P, Das B, Viswanathan PN (1984) A modified spectroscopic assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics* **21**, 130-132

Khanna N, Ray A, Alkondon M, Sen P (1989) Effect of β-adrenoceptor antagonists and some related drugs on maximal electroshock seizures in mice. *Indian Journal of Experimental Biology* **27**, 128-130

Kirtikar KR, Basu BD (1999) *Indian Medicinal Plants* (2nd Edn), International Book Distributors, Dehradun, India, pp 1344-1345

Mukhopadhyay S, Cordell GA, Ruangrungsi N, Rodkird S, Tantivatana P, Hylands PJ (1983) Traditional medicinal plants of Thailand. IV. 3-(2', 3'-Diacetoxy-2'-methyl butyryl)- cuauhtemone from *Pluchea indica*. *Journal of Natural Products* **46**, 671-674

Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products* **66**, 1022-1037

Niehius WG, Samuelsson D (1968) Formation of Malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry* **6**, 126-130

Pramanik KC, Bhattacharya P, Biswas R, Bandyopadhyay D, Mishra M, Chatterjee TK (2006) Hypoglycemic and antihyperglycemic activity of leaf extract of *Pluchea indica* Less. *Oriental Pharmacy of Experimental Medicine* **6**, 232-236

Pramanik KC, Biswas R, Mitra A, Bandyopadhyay D, Mishra M, Chatterjee TK (2007) Tissue culture of the plant *Pluchea indica* (L.) Less. and evaluation of diuretic potential of its leaves. *Oriental Pharmacy of Experimental Medicine* **7**, 197-204

Pramanik KC, Chatterjee TK (2008) *In vitro* and *in vivo* Antibacterial Activities of root extract of tissue cultured *Pluchea indica* (L.) Less. against bacillary dysentery. *Pharmacognosy Magazine* **14**, 66-72

Pramanik KC, Chatterjee TK (2008) *In vitro* and *in vivo* Antibacterial Activities of root extract of tissue cultured *Pluchea indica* (L.) Less. *Oriental Pharmacy of Experimental Medicine* **8**, 295-301

Pramanik KC, Ghosh S, Midya DK, Chatterjee TK (2008) Antitumor activity and antioxidant role of tissue-cultured *Pluchea indica* root against Herlich Ascites carcinoma in Swiss albino mice. *International Journal of Biomedical and Pharmaceutical Sciences* **2**, 47-50

Sen T, Chaudhuri Nag AK (1991) Anti-inflammatory evaluation of a *Pluchea indica* root extract. *Journal of Ethnopharmacology* **33**, 135-141

Sen T, Ghosh TK, Chaudhuri AK (1993) Studies on the mechanism of anti-inflammatory and anti-ulcer activity of *Pluchea indica*-probable involvement of 5-lipooxygenase pathway. *Life Science* **52**, 737-743

Sen T, Dhara AK, Bhattacharjee S, Pal S, Chaudhuri Nag AK (2002) Antioxidant activity of the methanol fraction of *Pluchea indica* root extract. *Phytotherapy Research* **16**, 331-335

Thongpraditchote S, Matsumoto K, Temsiririrkkul R, Tohda M, Murakami Y, Watanabe H (1996) Neuropharmacological actions of *Pluchea indica* Less. root extract in socially isolated mice. *Biological Pharmaceutical Bulletin* **19**, 379-383

Uchiyama T, Miyase T, Ueno A, Khan U (1989) Terpenic glycosides from *Pluchea indica. Phytochemistry* **28**, 3369-3372

Uchiyama T, Miyase T, Ueno A, Khan U (1991) Terpene and lignan glycolsides from *Pluchea indica*. *Phytochemistry* **36**, 655-657