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Evaluation of Hepatic Microsomal Enzyme Functional Integrity on Picroliv Pretreatment Against CCl_4 Induced Hepatotoxicity

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Abstract: The effect of picroliv isolated from *Picrorrhiza kurroa* was evaluated at different dose ranges to establish its ED_{50} against carbon tetrachloride (CCl_4) induced hepatotoxicity. The CCl_4 was given orally (1 mL kg^{-1} b.wt.) in liquid paraffin (1:1). Picroliv at different doses and silymarin (20 mg kg^{-1} b.wt.) standard were administered orally for 14 days. Body weight, biochemical parameters like Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and Alkaline Phosphatase (ALP) were estimated. Hepatic microsomal drug metabolizing enzyme functional integrity was accessed by *in vitro* bromosulphalein (BSP) uptake test of liver slices and thiopental (40 mg kg^{-1} b.wt.) induced sleeping time. The results showed dose related hepatoprotective efficacy of picroliv. The ED_{50} values for effectiveness of liver enzyme metabolizing activity on BSP uptake test and sleeping time were 11.94 and 17.49 mg kg^{-1} b.wt., respectively.

Key words: Picroliv, carbon tetrachloride, hepatoprotective, cytochrome P_{450}, bromosulphalein

INTRODUCTION

Liver disorders can result from a wide variety of insults, including infections, drugs, toxins, ischemia and autoimmune disorders. Most liver disorders produce some degree of hepatocellular injury and necrosis, resulting in various abnormal laboratory test results and symptoms. Symptoms may be due to liver disease itself (e.g., jaundice due to acute hepatitis) or to complications of liver disease (e.g., acute GI bleeding due to cirrhosis and portal hypertension). The liver performs an astonishingly large number of tasks including vascular, secretory, excretory functions and metabolic achievements in control of synthesis and utilization of carbohydrates, lipids and proteins that impact all body systems (Mitra and Metcalf, 2009). The main enzymes involved in metabolism belong to the cytochrome P_{450} group (Smith *et al*., 1998; Werck-Reichhart and Feyereisen, 2000).

Unique gene encodes in each P_{450} protein increase the risk of hepatic injury in different races, as blacks and Hispanics may be more susceptible to isoniazid toxicity on alcohol ingestion. Long acting drugs, host factors, AIDS, malnutrition and fasting can provoke a person to become more susceptible to hepatic injury because of low glutathione stores (Lemke *et al*., 2007). Elderly persons have increased risk of hepatic injury because of decreased clearance, drug to drug interactions and reduced hepatic blood flow. Besides that chemicals and toxicants, aging, diseases, genetic deficiency can reduce hepatic blood flow, causing decrease in numbers of hepatocytes and decline in metabolic enzyme potential of liver. Also, the use of drugs as well as dietary and environmental factors can influence liver metabolic function.

The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver diseases by carefully synergizing the strengths of the traditional systems of medicine with the modern concept of evidence based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy. The most commonly used herbs for liver problems include milk thistle, dandelion root, licorice root, chicory root (Zafar and Ali, 1998) and kutki rhizome.

Kutki is the common trade and vernacular name of the herb *Picrorrhiza kurroa* Benth. (family: Scrophulariaceae). Kutki is a medicinal plant found in the Himalayas from Kashmir to Sikkim at altitude of 2700-4500 m. The main iridoid glycoside reported from rhizomes of *P. kurroa* is picroliv (Chander *et al*., 1990) or kutkin which is a mixture of picroside I and kutkoside and is responsible for hepatoprotective activity (Dwivedi *et al*., 1992; Visen *et al*., 1991; Shukla *et al*., 1991). Until now, the nitric acid scavenging activity (Jagetia and Baliga, 2004), cardio protective effect (Subramaniam *et al*., 2001), anti-cancer effect (Joy *et al*., 2000; Jeena *et al*., 1999), anti-diabetic activity (Joy and Kutton, 1999) and anti-viral effect

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(Mehrotra, 1990) of *P. kurroa* extracts have been evaluated. Pharmacological studies revealed anti-inflammatory (Singh *et al.*, 1993; Pandey and Das, 1989), anti-asthma (Dorsch *et al.*, 1991) and immunostimulant activity (Puri *et al.*, 1992). Hepatoprotective activity was accessed on basis of biochemical parameters (SGOT, SGPT and ALP) against CCl_4 induced hepatic damage. The present study was design to standardize medium effective (ED_{50}) dose level of picroliv capable of maintaining metabolizing hepatic enzyme integrity on CCl_4 intoxicated rat liver along with evaluation of hepatoprotective parameters on different dose levels on chronic treatment basis. Hepatocyte structural and functional integrity governs the rate of liver biotransformation. Activation of hepatocellular function restores the metabolic activity of liver and reduces barbiturate induced sleeping time in intoxicated animals. The recovery in BSP clearance rate signifies restoration of microsomal enzyme functional integrity by improving excretory capacity of hepatocytes suggesting hepatoprotective efficacy of drug under test. Hepatic microsomal drug metabolizing enzyme functional integrity of picroliv pretreatment was evaluated in CCl_4 induced hepatotoxic animal model by *in vitro* BSP uptake and barbiturate sleeping time accessment to establish the regenerating as well as liver stimulant activity of picroliv.

MATERIALS AND METHODS

Plant material: *Picrorrhiza kurroa* rhizomes were procured from Khari Bawli market, Delhi, India in the month of November 2008. The plant was identified with the aid of available literature and authenticated by taxonomist Dr. H.B. Singh of Herbarium Department, NISCAIR, New Delhi (voucher specimen no. NISCAIR/RHMD/consult/-2009-10/1233/37).

Preparation of methanolic extract: The plant material was dried in tray dryer at temperature 55°C±2°C, milled into coarse powder and passed through the sieve 40/60. The coarse powder (250 g) was soaked in 1.0 L of 95% ethanol for four days with intermittent shaking. On 5th day, the whole material was filtered through muslin cloth. The filtrate was collected and concentrated. The residual solvent was removed under vacuum in a rotatory vacuum evaporator under 40°C at 650 mm vacuum pressure and a solid blackish-brown mass was obtained (yield: 9.30% w/w).

Isolation of picroliv: The solid mass was pulverized to a fine powder of mesh 80-100, dissolved in acetone and warmed at 50°C. This mixture was poured in 80% acetone with continuous stirring for 1 h and set aside for 2 h. The clear supernatant liquid was filtered, the process was repeated for six times. The different fractions of acetone were mixed and evaporated under reduced pressure at 50°C and the dried residue obtained was picroliv (yield: 7.09% w/w). This picroliv was used for the experimental studies.

Authentication of isolated picroliv by analytical HPLC technique: The HPLC system (Shimadzu, Japan), equipped with CAT-228-39001-38 pump, 228-393000-38 photodiode array detector, LC solution integrated software and a Rheodyne injection valve fitted with a 20 µL injection loop, was used for the analysis. Baseline resolution of picroliv was obtained at 25±2°C using stainless steel column (15 cm×4.6 mm), packed with octadecylsilane bonded to porous silica (5 µm). An isocratic solvent system consisting of 1% v/v of orthophosphoric acid: acetonitrile in the ratio of 83:17 (v/v) was used. The mobile phase was passed through 0.45 PVDF filter, degassed before use. The flow rate was kept constant at 1 mL min^{-1} and effluents were monitored at 280 nm. The test solution was prepared by dissolving 100 mg of substance under examination in 25 mL of methanol and filtered.

Test animals: Laboratory bred Wistar albino rats of either sexes weighing between 150-200 g were maintained under standard laboratory conditions at 25±2°C, relative humidity 50±15% and photoperiod (12 h-dark and light). Commercial pellet diet (Hindustan Lever, India) and water were provided *ad-libitum*. Animals were allowed to free access of water and food during the experiment but no water and food were allowed before and after 1 h of dosing. In order to avoid diurnal variation all the experiments were carried out at same time of day i.e., between 10:00 am to 05:00 pm. Ethical committee approval was obtained from institutional animal ethical committee (approved body of Committee for the purpose of control and supervision of experiments on animals Chennai, India) of Radharaman College of Pharmacy, (Reg. No. 1169/ac/08/CPCSEA), Bhopal, before carrying out the experiments.

Treatment protocol: Animals were randomly divided into 9 groups with 12 rats in each. Group I and III-IX was treated with vehicle control, positive control (silymarin) and different doses of picroliv continuously for 14 days. On 7th day all the groups including group II (negative CCl_4 control) were treated with CCl_4 (1 mL kg^{-1} b.wt.) 2 h after drug treatment and afterwards on alternate days for a week. From each group 6 animals were used to access hepatoprotective activity by estimating biochemical parameters and *in vitro* BSP uptake by treated liver slices and remaining 6 animals were used to determine thiopental induced sleeping time.

Vehicle control group animals were treated with normal saline (0.2 mL/100 g). Standard drug silymarin was prepared freshly in 1% gum-accacia in normal saline. Picroliv was dissolve in normal saline as per the required quantity. All the treatments were given by orogastric intubation. Treatment plan was as following:

- **Group I:** Vehicle control group: normal saline for fourteen days
- **Group II:** CCl_4 control group (1 mL kg^{-1} b.wt.) on seventh day afterwards alternate days for one week
- **Group III:** Silymarin (20 mg kg^{-1} b.wt.) + CCl_4 (1 mL kg^{-1} b.wt.)
- **Group IV:** Picroliv (5 mg kg^{-1} b.wt.) + CCl_4 (1 mL kg^{-1} b.wt.)
- **Group V:** Picroliv (10 mg kg^{-1} b.wt.) + CCl_4 (1 mL kg^{-1} b.wt.)
- **Group VI:** Picroliv (15 mg kg^{-1} b.wt.) + CCl_4 (1 mL kg^{-1} b.wt.)
- **Group VII:** Picroliv (20 mg kg^{-1} b.wt.) + CCl_4 (1 mL kg^{-1} b.wt.)
- **Group VIII:** Picroliv (25 mg kg^{-1} b.wt.) + CCl_4 (1 mL kg^{-1} b.wt.)
- **Group IX:** Picroliv (30 mg kg^{-1} b.wt.) + CCl_4 (1 mL kg^{-1} b.wt.)

Accessment of hepatoprotective activity: Body weights of all the animals were recorded on 1st, 7th and on 14th day before sacrifice. On the 14th day, 2 h after drug treatment six animals of each group were given thiopental sodium (40 mg kg^{-1} b.wt.) intraperitonially and the effects of drug on CCl_4 induced prolongation of thiopental sodium sleeping time were studied. Remaining six animals of each group were anaesthetized by light ether anaesthesia and blood was withdrawn by intracardiac puncture. Blood was allowed to coagulate for 30 min at room temperature and serum was separated by centrifugation at 3000 rpm for 5 min (Remi Centrifuge, Model RM 12 C). The serum was used to estimate serum SGPT, serum SGOT (Reitman and Frankel, 1957) and ALP (Kind and King, 1954). The liver was harvested, washed in normal saline, blotted in filter paper and weighed. Each liver was cut into three slices of 60 mg weight and used for BSP uptake. Percentage hepatoprotection was calculated with the method described by Ranjan and Subramanyan (1965).

Statistical analysis: The results were expressed in term of Mean±SEM. Experimental data of various physical and biochemical parameters were analyzed using one way ANOVA followed by Turkey-Kramer multiple comparisons using InStat graph pad version. $p<0.05$ were considered statistically significant.

RESULTS

Picroliv (mp 210°C) was obtained as brown crystalline powder, bitter in taste. It exhibited a red purple colour with Godin reagent (MacLennan *et al.*, 1959). The isolated picroliv was subjected to chromatography on silica gel G plates using ethyl acetate: methanol (92:8), which showed two spots with R_f value 0.25 and 0.42. These two spots are designated as kutkoside and picroside I, compared with the standard picroliv (Rajpal, 2002).

The isolated picroliv was further authenticated by HPLC analysis. The HPLC method described herein provides a good separation of picroliv. Chromatogram of picroliv reference standard (Indian Pharmacopoeia, 2007) (Fig. 1a) and chromatogram of picroliv isolated sample showed identical peaks (Fig. 1b). Under the chromatographic conditions, the retention time of the isolated picroliv was about 13.80 min identical to that of picroliv reference standard.

Body weights of all the animals were measured on 1st day before commencing the experiment then on 7th day after continuous drug treatment and again on 14th day. Vehicle control group showed 5.9 and 8.5% increase in body weight respectively on 7th and 14th day. Negative control group (CCl_4 treated) showed 24.6% decrease in

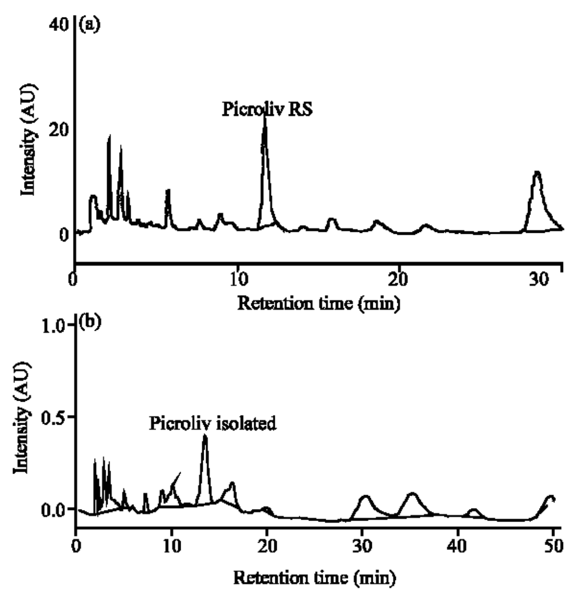

Fig. 1: (a) HPLC chromatogram (i) picroliv reference standard (Indian Pharmacopoeia, 2007), (b) HPLC chromatogram (ii) picroliv isolated

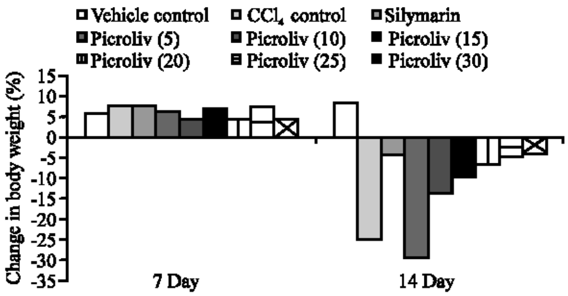

Fig. 2: Effects of picroliv treatment on body weight change after 7th and 14th of treatment on CCl_4 intoxicated rats. Values in parentheses dose in mg kg^{-1}

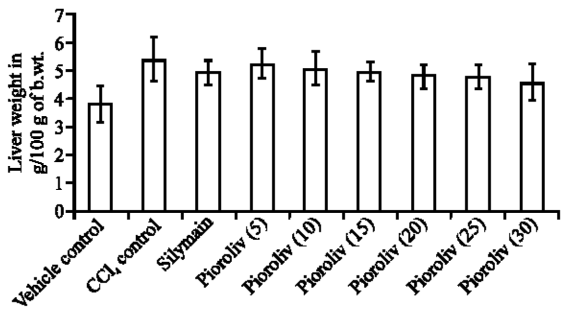

Fig. 3: Effects of picroliv treatment on liver weight comparative to body weight in CCl_4 intoxicated rats. Results are expressed as Mean±SEM, n = 6 in each group. Values in parentheses signify doses in mg kg^{-1}

body weight with reduced food consumption. Standard drug silymarin treatment showed only 4.3% decrease in body weight where as picroliv treatment showed dose dependent protection against body weight loss. Picroliv 30 mg kg^{-1} b.wt. dose treatment showed only 3.8% loss in body weight which is less than silymarin (Fig. 2). Picroliv showed extremely significant protection by reducing the rate of body weight loss at 15, 20, 25 and 30 mg kg^{-1} b.wt. doses.

Silymarin and picroliv treatment signifies hepatoprotection by reducing the liver weight of CCl_4 intoxicated animals. Liver weight in g/100 g b.wt. for vehicle control, CCl_4 control, silymarin and picroliv at 15, 20, 25 and 30 mg kg^{-1} b.wt. doses were 3.80±0.65, 5.39±0.79, 4.95±0.42, 4.98±0.34, 4.87±0.37, 4.76±0.44 and 4.58±0.68, respectively (Fig. 3).

The effect of picroliv treatment on the thiopental induced sleeping time in CCl_4 intoxicated rats indicated significant increase in thiopental induced sleeping time in CCl_4 treated groups compared to that of vehicle control group (Table 1). The treatment with silymarin and different

Table 1: Effects of picroliv treatment on the thiopental induced sleeping time of CCl_4 intoxicated rats

Treatment (mg kg^{-1})	Sleeping duration (min)	Hepatoprotection (%)
Vehicle control	18.50±1.02	-
CCl_4 control	222.48±5.68	-
Silymarin (20)	56.20±2.05***	85.51
Picroliv (5)	224.72±5.10^{ns}	1.098
Picroliv (10)	204.06±4.97*	9.03
Picroliv (15)	188.79±4.67***	16.51
Picroliv (20)	109.44±3.68***	55.41
Picroliv (25)	75.86±2.60***	71.87
Picroliv (30)	24.53±2.31***	97.04

The values are expressed as Mean±SEM, n = 6 in each group. *$p<0.05$, ***$p<0.001$, ns: Non significant when compared with CCl_4 control. Hepatoprotection (%) = 1 - [T-V/C-V]×100. Where, T = mean value of group treated with test drug, C = Mean value of group treated with CCl_4, V = Mean value of group treated with vehicle

Table 2: Hepatoprotective effect of picroliv on in-vitro bromosulphalein (BSP) uptake of CCl_4 intoxicated rat liver slices

	Hepatoprotection (%)		
Treatment (mg kg^{-1})	After 10 min	After 20 min	After 30 min
Vehicle control	-	-	-
CCl_4 control	-	-	-
Silymarin (20)	35.80	50.53	81.40
Picroliv (5)	-6.82	-27.60	-51.10
Picroliv (10)	-3.97	17.06	30.21
Picroliv (15)	14.07	45.74	78.40
Picroliv (20)	43.23	59.65	88.75
Picroliv (25)	49.48	69.89	94.78
Picroliv (30)	61.94	92.25	115.10

n = 6 in each group. Hepatoprotection (%) = 1 - [T-V/ C-V]×100. Where, T = Mean value of group treated with test drug, C = Mean value of group treated with CCl_4, V = Mean value of group treated with vehicle

doses of picroliv (15, 20, 25 and 30 mg kg^{-1} b.wt.) resulted in extremely significant ($p<0.001$) decrease in thiopental induced sleeping time compared to the CCl_4 treated group. The percentage hepatoprotection in concern to thiopental induced sleeping time reveled maximum protection (97.04%) with picroliv (30 mg kg^{-1} b.wt.) treatment compared to silymarin (85.51%).

In vitro BSP uptake study of liver slices showed more than 50% hepatoprotection on picroliv 15, 20, 25 and 30 mg kg^{-1} b.wt. treatment after 30 min of incubation. Picroliv showed dose dependent hepatoprotection starting from 10 to 30 mg kg^{-1} b.wt. dose (Table 2). At 20, 25 and 30 mg kg^{-1} dose, percentage hepatoprotection were 88.75, 94.78 and 115.10%, respectively, greater than that showed by silymarin (81.40%).

Hepatotoxic CCl_4 gets converted in CCl_3O^- by liver enzymes, which attack the unsaturated fatty acids of cell membrane giving rise to lipid peroxides that alters the functional integrity of liver mitochondria. As a result the level of marker enzymes in plasma severely increases in CCl_4 intoxicated animals. Serum level of SGOT, SGPT and ALP were increased to 168.37, 130.26 and 186.17 IU L^{-1}, respectively in comparison to vehicle control values 25.50,

Table 3: Effects of picroliv treatment on serum enzyme activities of CCl_4 intoxicated rats

Treatment (mg kg^{-1})	Serum biochemical parameters		
	SGOT (mg dL^{-1})	SGPT (mg dL^{-1})	ALP (IU L^{-1})
Vehicle control	25.50±1.29	30.17±1.68	94.08±4.10
CCl_4 control	168.37±3.12	130.26±4.29	186.17±4.92
Silymarin (20)	43.56±1.72***	26.73±1.75***	102.87±3.22***
Picroliv (5)	120.45±3.57***	95.92±3.61***	162.30±4.91**
Picroliv (10)	70.82±2.63***	56.70±3.37***	118.28±4.55***
Picroliv (15)	55.03±2.94***	38.14±2.24***	106.75±4.63***
Picroliv (20)	42.60±1.38***	31.22±2.33***	102.10±4.11***
Picroliv (25)	35.12±1.27***	28.50±1.92***	96.88±3.90***
Picroliv (30)	26.34±1.56***	23.32±1.68***	71.56±3.25***

The values are expressed as Mean±SEM, n = 6 in each group. ***p<0.001 and **p<0.01 when compared with CCl_4 control. SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate transaminase and ALP = Alkaline phosphatase

Table 4: Estimation of picroliv ED_{50} for hepatic microsomal drug metabolizing enzyme functional integrity against CCl_4 induced hepatotoxicity on rats

Treatment (mg kg^{-1})	Log dose	Hepatoprotection on thiopental sleeping-time (%)	Hepatoprotection against BSP uptake 30 min after incubation of liver slices (%)
Picroliv (5)	0.6989	1.098	-
Picroliv (10)	1	9.03	30.21
Picroliv (15)	1.176	16.51	78.4
Picroliv (20)	1.301	55.41	88.75
Picroliv (25)	1.3979	71.87	94.78
Picroliv (30)	1.4771	97.04	115.1
Statistical analysis			
ED_{50}		17.49 (12.66-24.17) mg kg^{-1}	11.94 (10.49-13.58) mg kg^{-1}
p-value		<0.001	<0.001
r^2		0.8098	0.9339
F		17.036	42.376

30.17 and 94.08 IU L^{-1} in CCl_4 treated animals. In contrast, the groups treated with silymarin and different doses of picroliv showed extremely significant (p<0.001) decrease in SGOT, SGPT and ALP values toward normalization (Table 3).

The ED_{50} values of picroliv on thiopental induced hepatoprotection and *in vitro* BSP uptake were 17.49 (95% confidence limit: 12.66-24.17) and 11.94 (95% confidence limit: 10.49-13.58) mg kg^{-1}, respectively (Table 4).

DISCUSSION

The liver is the largest organ in the body and serves many vital functions in human body such as remove damaged red blood cells from the blood in co-ordination with spleen, produces bile, clotting factors, stores vitamins, minerals, protein, fats and glucose from diet (Dyce *et al*., 1987). The most important task of the liver is detoxification substances like alcohol and, different medications such as chemotherapeutic drugs, antibiotics and toxicants. Chemical agents and toxins impose excess stress on the liver filtering function. Liver removes harmful chemical agents and toxins through the bile or urine. If accumulation of toxins is faster than the liver metabolizing ability, hepatic damage may occur (Bigoniya *et al*., 2009).

Herbal drugs play a crucial role in the management of various liver disorders in addition to promotion of natural healing processes (Subramoniam *et al*., 1998). Because of today's increasing demands for the herbal agents that have been regarded as relatively safe in use, numerous type of herbal extracts were tested in various *in vivo* or *in vitro* systems (Lee *et al*., 2006). Many plant derived pharmaceuticals are available for the treatment of ailments including acute or chronic liver diseases.

Picrorrhiza kurroa has been shown to protect the liver from a wide variety of insults including galactosamine, ethanol and aflatoxin B1 (Rastogi *et al*., 1996; Dwivedi *et al*., 1993a, b). Picroliv is a standardized iridoid glycoside fraction isolated from roots and rhizomes of *P. kurroa*. It contains 60% picroside I and kutkoside in the ratio of 1:1.5 and is mainly responsible for its hepatoprotective activity (Singh *et al*., 2005). Picroliv was selected for the present study as clinical and pharmacokinetic studies reveal that it has no side effect (Anonymous, 2004) and more efficacious than silymarin (Ansari *et al*., 1991).

The CCl_4 induced hepatotoxicity has chosen as the experimental model, since, the changes associated with the CCl_4 induced liver damage are similar to that of viral hepatitis. The liver toxicant CCl_4 causes lipid peroxidative degradation of biomembrane which is one of the principle causes of hepatotoxicity (Cotran *et al*., 1994). In liver CCl_4 is biotransformed by cytochrome P_{450} to produce its active metabolite trichloromethyl free radical (Kaplowitz *et al*., 1986), which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide which in turn gives toxic aldehyde that causes damage to liver. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. Necrosis or membrane damage releases the enzyme SGOT, SGPT and ALP into circulation, therefore, it can be measured in serum. Estimation of these enzymes in the serum is useful quantitative markers of the extent and type of hepatocellular damage. High levels of SGOT indicate the loss of functional integrity of liver, such as that of viral hepatitis, as well as cardiac infraction and muscle injury. The SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, SGPT is more specific to liver and thus a better parameter for detecting liver injury (Willianson *et al*., 1996).

Present results on CCl_4 induced hepatotoxicity of rats demonstrated that the picroliv at the different doses cause significant reduction in the levels of SGPT and SGOT elevated by CCl_4. Serum ALP is related to the function of hepatic cells. Increase in serum level of ALP is due to increased synthesis in presence of increasing billiary pressure (Moss and Butterworth, 1974). The results of the experiment revealed that different doses of picroliv caused significant inhibition of serum ALP in comparison to negative control group. Pilot experiments, using different liver toxicant and hepatic models are evaluated earlier, where, the picroliv dose ranges from 1.5 to 200 mg kg^{-1} b.wt. depending on treatment schedule (1 to 45 days) as reported by Rajeshkumar and Kuttan (2000); Saraswat *et al.* (1999) and Shukla *et al.* (1991).

Drugs used for treatment of liver diseases usually have to be given for several days before the therapeutic effects become evident. It is therefore, always not possible to predict and follow such a schedule with drugs like paracetamol and galactosamine where the hepatic damage is of acute onset (Ansari *et al*., 1991). Two weeks schedule was followed here to stimulate a clinically effective course of treatment. The present study establishes the median effective dose of picroliv on moderate treatment schedule in order to maintain functional integrity of liver. The findings indicated that liver damaged by CCl_4 metabolizes the thiopental with slower rate of metabolism as evident by increased sleeping time in comparison to picroliv treated group and normal group. Picroliv treatment showed more profound hepatoprotection comparative to standard silymarin on *in vitro* BSP uptake study. The median effective dose (ED_{50}) of picroliv was calculated on the basis of percentage hepatoprotection on thiopental induced sleeping time and protection against BSP uptake by liver slices. Picroliv restores the functional integrity of liver by preventing penetration of the liver toxin into the interior of the cell and stimulates the action of nucleolar polymerase A, resulting in ribosomal protein, nucleic acid synthesis and thus stimulates the regenerative ability of the liver and formation of new hepatocytes (Singh *et al*., 1992; Chander *et al*., 1990). The pretreatment with picroliv has prevented oxygen free radicals and thereby prevented the formation of peroxy radicals (Kapoor *et al*., 1992).

Picroliv primarily effects hepatocyte ionic transport, mitochondrial electron transport chain reaction and finally disposal of free radicals. Picroliv is reported to have preventive as well as prophylactic activity against various hepatotoxic models. Picroliv can prevent and preserve liver histology given before, simultaneously or after liver intoxication (Rastogi *et al*., 1995). This study for the first time ever report effectiveness of picroliv pretreatment on liver metabolizing enzyme integrity. The ED_{50} values for effectiveness of liver enzyme metabolizing activity on BSP uptake test and sleeping time were 11.94 and 17.49 mg kg^{-1} b.wt. respectively. Picroliv treatment for 45 days effectively reverses ethanol induced liver damage in rats (Saraswat *et al*., 1999). Picroliv oral pretreatment for 7 days at 12 mg kg^{-1} b.wt. dose prior to induction of ischaemia demonstrated improved hepatocyte glycogen preservation and reduced apoptosis (Singh *et al*., 2000). Although, picroliv was administered by different researchers in dose ranging from 1.5 to 200 mg kg^{-1} b.wt. the effective dose range is above 18 mg kg^{-1} b.wt. as established by this study. Picroliv in dose of 20 to 30 mg kg^{-1} b.wt. showed excellent hepatoprotection.

As herbs are believe to be safe for treating ailments but there are few examples which showed that herbs are also having side effects on long term use. Many natural drugs like senna, ephedra and sarsparilla causes liver damage. In the present scenario, changes in life style make the liver highly exposed to the drugs and toxicants which are credited for liver diseases. Picroliv should not be administered unnecessarily in higher doses that would also increases the chances of undesirable effects as crude extract of *P. kurroa* rhizomes have side effects like loose stool and cholic (Chaturvedi and Singh, 1966).

CONCLUSION

Picroliv seems to be a better hepatoprotective agent compared to silymarin as picoliv in dose of 20 to 30 mg kg^{-1} showed excellent hepatoprotection. The quantitative differences on reversal could be due to differences in mode of action of the two hepatoprotective agents. The hepatoprotective activity of picroliv may depend on preventing the formation of free radicals at the level of O_2^- anions, possibly acting like superoxide dimutase, xanthine oxidase inhibitors and metal ion chelators. Recovery induced by picroliv might be due to restoration of plasma membrane permeability including repair of injured hepatic cells and increasing protein and nucleic acid synthesis.

REFERENCES

Anonymous, 2004. Better health through research: Traditional medicine. ICMR Annual Report 2003-2004, pp: 115-117. http://icmr.nic.in/annual/hqds2004/bms.pdf.

Ansari, R.A., S.C. Tripathi, G.K. Patnaik and B.N. Dhawan, 1991. Antihepatotoxic properties of picroliv: An active fraction from rhizomes of *Picrorhiza kurroa*. J. Ethnopharmacol., 34: 61-68.

Bigoniya, P., C.S. Singh and A. Shukla, 2009. A comprehensive review of different liver toxicants used in experimental pharmacology. Int. J. Pharm. Sci. Drug Res., 1: 124-135.

Chander, R., Y. Dwivedi, R. Rastogi, S.K. Sharma, N.K. Garg, N.K. Kapoor and B.N. Dhawan, 1990. Evaluation of hepatoprotective activity of picroliv (from *Picrorrhiza kurroa*) in *Mastomys natalensis* infected with *Plasmodium bergheri*. Indian J. Med. Res., 92: 34-37.

Chaturvedi, G.N. and R.H. Singh, 1966. Jaundice of infectious hepatitis and its treatment with an indigenous drug, *Picorrhiza kurroa* [sic]. J. Res. Indian Med., 1: 1-13.

Cotran, R.S., V. Kumar and S.L. Robbins, 1994. Pathologic Basis of Diseases. 5th Edn., W B Saunder Company, Philadelphia, USA., pp: 178-189.

Dorsch, W., H. Stuppner, H. Wagner, M. Gropp, S. Demoulin and J. Ring, 1991. Antiashthmatic effect of *Picrorrhiza kurroa*: Androsin prevents allergen and PAF-induced bronchial obstruction in ginea pigs. Int. Arch. Allergy Applied Immunol., 95: 128-133.

Dwivedi, Y., R. Rastogi, N.K. Garg and B.N. Dhawan, 1992. Picroliv and its components kutkoside and picroside I protect liver against galactosamine-induced damage in rats. Pharmacol. Toxicol., 71: 383-387.

Dwivedi, Y., R. Rastogi, N.K. Garg and B.N. Dhawan, 1993a. Perfusion with picroliv reverses biochemical changes induced in livers of rats toxicated with galactosamine or thioacetamide. Planta Med., 59: 418-420.

Dwivedi, Y., R. Rastogi, R. Mehrotra, N.K. Garg and B.N. Dhawan, 1993b. Picroliv protects against aflatoxin B1 acute hepatotoxicity in rats. Pharmacol. Res., 27: 189-199.

Dyce, K.M., W.O. Sack and C.J. Wensing, 1987. Text Book of Veterinary Anatomy. W B Saunders Company, Philadelphia.

Indian Pharmacopoeia, 2007. Government of India. Ministry of health and Family Welfare.

Jagetia, G.C. and M.S. Baliga, 2004. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: A preliminary study. J. Med. Food, 7: 343-348.

Jeena, K.J., K.L. Joy and R. Kuttan, 1999. Effect of *Emblica officinalis*, *Phylanthus amarus* and *Picrorrhiza kurroa* on N- nitrosodiethylamine induced hepatocarcinogenesis. Cancer Lett., 136: 11-16.

Joy, K.L. and R. Kuttan, 1999. Anti-diabetic activity of *Picrorrhiza kurroa* extract. J. Ethropharmacol., 67: 143-148.

Joy, K.L., N.V. Rajeshkumar, G. Kuttan and R. Kuttan, 2000. Effect of *Picrorrhiza kurroa* extract on transplanted tumours and chemical carcinogenesis in mice. J. Ethanopharmacol., 71: 261-266.

Kaplowitz, N., T.Y. Aw, F.R. Simon and A. Stolz, 1986. Drug induced hepatotoxicity. Ann. Int. Med., 104: 826-839.

Kapoor, N.K., R. Chander and B.N. Dhawan, 1992. Picroliv, picroside-I and kutkoside from *Picrorrhiza kurroa* are scavengers of superoxide anions. Biochem. Pharmacol., 44: 180-183.

Kind, P.R.N. and E.J. King, 1954. Estimation of plasma phosphatases by determination of hydrolyzed phenol with amino-antipyrine. J. Clin. Pathol., 7: 322-330.

Lee, H.S., H.C. Ahn and S.K. Ku, 2006. Hypolipidemic effect of water extracts of *Picrorrhiza kurroa* in PX-407 induced hyperlipemic ICR mouse model with hepatoprotective effects: A prevention study. J. Ethanopharmacol., 105: 380-386.

Lemke, T.L., D.A. William, V.F. Roche and S.W. Zito, 2007. Foye's Principles of Medicinal Chemistry. 6th Edn., Lippincott Williams and Wilkins, USA.

MacLennan, A.P., H.M. Randall and D.W. Smith, 1959. Detection and identification of deoxysugars on paper chromatograms. Anal. Chem., 31: 2020-2022.

Mehrotra, R., 1990. *In vitro* studies on the effect of certain natural products against hepatitis B virus. Indian J. Med. Res., 92: 133-138.

Mitra, V. and J. Metcalf, 2009. Metabolic functions of liver. Anaesth. Intensive Care Med., 10: 334-335.

Moss, D.W. and P.J. Butterworth, 1974. Enzymology and Medicine. Pitman Medical, London, pp:139.

Pandey, B.L. and P.K. Das, 1989. Immunopharmacological studies on *Picrorrhiza kurroa* Royle-ex-Benth. Part IV: Cellular mechanism of anti-inflammatory action. Indian J. Physiol. Pharmacol., 33: 28-30.

Puri, A., R.P. Saxena, Sumati, P.Y. Guru, D.K. kulshreshtha, K.C. Saxena and B.N. Dhawan, 1992. Immunostimulant activity of picroliv, the iridoid glycoside fraction of *Picrorrhiza kurroa* and its protective action against *Leishmania donovani* infection in hamsters. Planta Med., 58: 528-532.

Rajeshkumar, N.V. and R. Kuttan, 2000. Inhibition of N-nitrosodiethylamine-induced hepatocarcinogenesis by Picroliv. J. Exp. Clin. Cancer Res., 19: 459-465.

Rajpal, V., 2002. Standardization of Botanicals Testing and Extraction Methods of Medicinal Herbs. Eastern Publishers, New Delhi, pp: 95.

Ranjan, R. and K. Subramanyam, 1965. Uptake of Sodium phenol tetrabromosulphalein (bromosulphalein) by rat liver slices under different conditions. Indian J. Exp. Biol., 24: 100-103.

Rastogi, R., S. Saksena, N.K. Garg and B.N. Dhawan, 1995. Effect of picroliv on antioxidant system in the liver of rats after partial hepactomy. Phytother. Res., 9: 364-367.

Rastogi, R., S. Saksena, N.K. Garg, N.K. Kapoor, D.P. Agarwal and B.N. Dhawan, 1996. Picroliv protects against alcohol-induced chronic hepatotoxicity in rats. Planta Med., 62: 283-285.

Reitman, S. and S. Frankel, 1957. A calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-63.

Saraswat, B., P.K. Visen, G.K. Patnaik and B.N. Dhawan, 1999. *Ex vivo* and *in vivo* investigations of picroliv from *Picrorrhiza kurroa* in alcohol intoxication level in rats. J. Ethnopharmacol., 66: 263-269.

Shukla, B., P.K. Visen, G.K. Patnaik and B.N. Dhawan, 1991. Choleretic effect of picroliv, the hepatoprotective principle of *Picrorrhiza kurroa*. Planta Med., 57: 29-33.

Singh, V., N.K. Kapoor and B.N. Dhawan, 1992. Effect of picroliv on protein and nucleic acid synthesis. Indian J. Exp. Biol., 30: 68-69.

Singh, G.B., S. Bani, S. Singh, A. Khajuria, M.L. Sharma, B.D. Gupta and S.K. Banerjee, 1993. Antiinflamatory activity of the iridoids kutkin, picroside-I and kutkoside from *Picrorrhiza kurroa*. Phytother. Res., 7: 402-407.

Singh, A.K., H. Mani, P. Seta, J.P. Gaddipati and R. Kumari *et al*., 2000. Picroliv preconditioning protects the rats liver against ischaemia-reperfusion injury. Eur. J. Pharmacol., 395: 229-239.

Singh, M., V. Tiwari, A. Jain and S. Ghosal, 2005. Protective activity of picroliv on hepatic amoebiasis associated with carbon tetrachloride toxicity. Indian J. Med. Res., 121: 676-682.

Smith, G., M.J. Stubbins, L.W. Harries and C.R. Wolf, 1998. Molecular genetics of the human cytochrome P_{450} monooxygenase superfamily. Xenobiotica, 28: 1129-1165.

Subramaniam, H.S., R. Anandan, T. Devaki and S. Kumar, 2001. Cardioprotective effects of *Picrorrhiza kurroa* against isoproterenol-induced myocardial stress in rats. Fitoterapia, 72: 402-405.

Subramoniam, A., D.A. Evans, S. Rajasekharan and P. Pushpangadan, 1998. Hepatoprotective activity of *Trichopus zeylanicus* extract against paracetamol induced damage in rats. Indian J. Exp. Biol., 36: 385-389.

Visen, P.K., B. Shukla, G.K. Patnaik, R. Chander, V. Singh, N.K. Kapoor and B.N. Dhawan, 1991. Hepatoprotective activity of picroliv isolated from *Picrorrhiza kurroa* against thioacetamide toxicity on rat hepatocytes. Phytother. Res., 5: 224-227.

Werck-Reichhart, D. and R. Feyereisen, 2000. Cytochromes P_{450}: A success story. Genome Biol., 1: 3003-3003.

Willianson, E.M., D.T. Okpako and F.J. Evans, 1996. Selection, Preparation and Pharmacological Evaluation of Plant Material. John Wiley, England.

Zafar, R. and S.M. Ali, 1998. Anti-hepatotoxic effect of root and root callus extract of *Cichorium intybus* L. J. Ethnopharmacol., 63: 227-231.