Evaluation of Hepatic Microsomal Enzyme Functional Integrity on Picroliv Pretreatment Against CCl₄-Induced Hepatotoxicity

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Abstract: The effect of picroliv isolated from Picrorrhiza kurroa was evaluated at different dose ranges to establish its ED₅₀ against carbon tetrachloride (CCl₄) induced hepatotoxicity. The CCl₄ 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 assessed 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 ED₅₀ 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 P₄₅₀, 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 P₄₅₀ group (Smith et al., 1998; Werck-Reichhart and Feyereisen, 2000).

Unique gene encodes in each P₄₅₀ 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, chickory 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 (Jagasia and Baliga, 2004), cardiac 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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200
(Mehrotra, 1990) of *P. curroa* 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 CCl₄ induced hepatic damage. The present study was designed to standardize medium effective (ED₅₀) dose level of picroliv capable of maintaining metabolizing hepatic enzyme integrity on CCl₄ 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 CCl₄ induced hepatotoxic animal model by *in vitro* BSP uptake and barbiturate sleeping time assessment 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 isotropic 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 Radhramanan 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 CCl₄ control) were treated with CCl₄ (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 assess 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 silimarin was prepared freshly in 1% gum-acacia 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**: CCl₄ control group (1 mL kg⁻¹ b.wt.) on seventh day afterwards alternate days for one week
- **Group III**: Silimarin (20 mg kg⁻¹ b.wt.) + CCl₄ (1 mL kg⁻¹ b.wt.)
- **Group IV**: Picroliv (5 mg kg⁻¹ b.wt.) + CCl₄ (1 mL kg⁻¹ b.wt.)
- **Group V**: Picroliv (10 mg kg⁻¹ b.wt.) + CCl₄ (1 mL kg⁻¹ b.wt.)
- **Group VI**: Picroliv (15 mg kg⁻¹ b.wt.) + CCl₄ (1 mL kg⁻¹ b.wt.)
- **Group VII**: Picroliv (20 mg kg⁻¹ b.wt.) + CCl₄ (1 mL kg⁻¹ b.wt.)
- **Group VIII**: Picroliv (25 mg kg⁻¹ b.wt.) + CCl₄ (1 mL kg⁻¹ b.wt.)
- **Group IX**: Picroliv (30 mg kg⁻¹ b.wt.) + CCl₄ (1 mL kg⁻¹ b.wt.)

**Assessment 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 CCl₄ 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 kuttakside and picroside I, as 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 (CCl₄ treated) showed 24.6% decrease in

![Fig. 1](image-url): (a) HPLC chromatogram (i) picroliv reference standard (Indian Pharmacopoeia, 2007), (b) HPLC chromatogram (ii) picroliv isolated
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 CCl₄ intoxicated animals. Liver weight in g/100 g b.wt. for vehicle control, CCl₄ 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 thiopeental induced sleeping time in CCl₄ intoxicated rats indicated significant increase in thiopeental induced sleeping time in CCl₄ treated groups compared to that of vehicle control group (Table 1). The treatment with silymarin and different doses of picroliv (15, 20, 25 and 30 mg kg⁻¹ b.wt.) resulted in extremely significant (p<0.001) decrease in thiopeental induced sleeping time compared to the CCl₄ treated group. The percentage hepatoprotection in concern to thiopeental induced sleeping time revealed 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 CCl₄ gets converted in CCl₃O⁻ 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 CCl₄ 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 picrolov treatment on serum enzyme activities of CCl4-intoxicated rats

<table>
<thead>
<tr>
<th>Treatment (mg kg⁻¹)</th>
<th>Serum biochemical parameters</th>
<th>Hepatoprotective effect on thiopeptal sleeping time (%)</th>
<th>Hepatoprotective effect of BSP uptake 30 min after induction of liver slices (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGOT (μg dl⁻¹)</td>
<td>SCPT (μg dl⁻¹)</td>
<td>ALP (IU L⁻¹)</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>25.5±0.1 29</td>
<td>30.17±1.68</td>
<td>94.0±8.10</td>
</tr>
<tr>
<td>CCl4 control</td>
<td>168.3±7.12</td>
<td>130.2±4.29</td>
<td>186.1±7.92</td>
</tr>
<tr>
<td>Silymarin (20)</td>
<td>43.5±6.1 72***</td>
<td>26.7±4.1 75***</td>
<td>102.8±3.22***</td>
</tr>
<tr>
<td>Picrolov (5)</td>
<td>120.4±5.3 57***</td>
<td>95.2±4.3 61***</td>
<td>162.3±4.91***</td>
</tr>
<tr>
<td>Picrolov (10)</td>
<td>70.8±2.6 63***</td>
<td>56.7±2.3 77***</td>
<td>118.2±4.55***</td>
</tr>
<tr>
<td>Picrolov (15)</td>
<td>55.5±2.9 64***</td>
<td>38.1±2.2 71***</td>
<td>106.7±2.53***</td>
</tr>
<tr>
<td>Picrolov (20)</td>
<td>42.6±1.3 39***</td>
<td>31.2±1.2 31***</td>
<td>102.1±4.1 11***</td>
</tr>
<tr>
<td>Picrolov (25)</td>
<td>35.1±1.2 77***</td>
<td>28.5±1.9 62***</td>
<td>98.8±3.5 90***</td>
</tr>
<tr>
<td>Picrolov (30)</td>
<td>26.3±1.8 56***</td>
<td>23.2±1.6 58***</td>
<td>71.5±3.2 25***</td>
</tr>
</tbody>
</table>

The values are expressed as Mean±SEM, n = 6 in each group. ***p<0.001 and **p<0.01 when compared with CCl4 control. SGOT = Serum glutamate oxaloacetate transaminase, SCPT = Serum glutamate pyruvate transaminase and ALP = Alkaline phosphatase.

Table 4: Estimation of picrolov ED30 for hepatic microosomal drug metabolizing enzyme functional integrity against CCl4 induced hepatotoxicity on rats

<table>
<thead>
<tr>
<th>Treatment (mg kg⁻¹)</th>
<th>Log dose</th>
<th>Hepatoprotection on thiopeptal sleeping-time (%)</th>
<th>Hepatoprotection against BSP uptake 30 min after induction of liver slices (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picrolov (5)</td>
<td>0.6989</td>
<td>1.98</td>
<td>-</td>
</tr>
<tr>
<td>Picrolov (10)</td>
<td>1</td>
<td>9.03</td>
<td>30.21</td>
</tr>
<tr>
<td>Picrolov (15)</td>
<td>1.176</td>
<td>16.51</td>
<td>78.4</td>
</tr>
<tr>
<td>Picrolov (20)</td>
<td>1.301</td>
<td>55.44</td>
<td>98.75</td>
</tr>
<tr>
<td>Picrolov (25)</td>
<td>1.397</td>
<td>71.87</td>
<td>94.78</td>
</tr>
<tr>
<td>Picrolov (30)</td>
<td>1.477</td>
<td>97.04</td>
<td>115.1</td>
</tr>
</tbody>
</table>

Statistical analysis

ED30 = 17.49 (12.66-24.17) mg kg⁻¹

p-value <0.001

r² 0.8098

F 17.036

30.17 and 94.08 IU L⁻¹ in CCl4 treated animals. In contrast, the groups treated with silymarin and different doses of picrolov showed extremely significant (p<0.001) decrease in SGOT, SCPT and ALP values toward normalization (Table 3).

The ED30 values of picrolov on thiopeptal 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 and 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 kurrooa_ 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). Picrolov is a standardized iridoid glycoside fraction isolated from roots and rhizomes of _P. kurrooa_. 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). Picrolov was selected for the present study as clinical and pharmacokinetics studies reveal that it has no side effect (Anonymous, 2004) and more efficacious than silymarin (Ansari et al., 1991).

The CCl4 induced hepatotoxicity has chosen as the experimental model, since, the changes associated with the CCl4 induced liver damage are similar to that of viral hepatitis. The liver toxicant CCl4 causes lipid peroxidative degradation of biomembrane which is one of the principle causes of hepatotoxicity (Cotran et al., 1994). In liver CCl4, is biotransformed by cytochrome P450 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 inflation 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 (Williamson et al., 1996).
Present results on CCl₄ induced hepatotoxicity of rats demonstrated that the picroliv at the different doses cause significant reduction in the levels of SGPT and SGOT elevated by CCl₄. 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 biliary 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 CCl₄ metabolizes the thiopeptin 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 (ED₅₀) of picroliv was calculated on the basis of percentage hepatoprotection on thiopeptin 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 peroxo radicals (K 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 ED₅₀ 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 sarsaparilla 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 picroliv in dose of 20 to 30 mg kg⁻¹ showed excellent hepatoprotection. The quantative 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 O₂⁻ 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


