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Hepatoprotective and Anti-cancer activities of *Picrorhiza kurroa* associated with Paracetamol induced Hepatotoxicity

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ABSTRACT: Background: The dried root decoctions of *Picrorhiza kurroa* are traditionally used orally for abdominal pain, liver complaints, jaundice, malarial fever, and anemia. **Aim:** The present study was aimed to explore the hepatoprotective and anti-cancer activities of *P. kurroa* root. **Method:** The hepatopretective (*in-vitro* anti-oxidant) activity of chloroform fraction of *P. kurroa* were studied by 2,2-diphenylpicryylhydrazide (DPPH) free radical scavenging and COX-2 inhibitory methods, whereas anti-cancer activity of extract was done on HepG2 cell lines by the MTT [3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide] assay. **Results:** The i*n-vitro* antioxidant study revealed that the IC_{50} value was found to be 305 and 585 µg/ml for chloroform fraction of the roots of *P. kurroa* and Ascorbic acid respectively. In COX-2 inhibitory study, the chloroform fraction exhibited an inhibition of cyclooxygenase-2 respectively at highest concentration. The 50 % cell viability value was found to be 62.5 µg/ml. From the above studies it revealed the root of *P. kurroa* has significant hepatoprotective activity. **Conclusion:** It could be concluded that the root of *P. Kurroa* can be used effectively for its hepatoprotective and cytotoxic activities. Further studies to be carried out on isolation of the active moiety, which is responsible for such activities and exploration of route of administration for delivery of the phyto-constituents effectively.

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INTRODUCTION:

Many toxins damage the liver and affect its functions resulting in poor health and production. For prevention of hepatocytes, some drugs or chemicals are used which also antagonize the toxins and help to regain its power of metabolism, during early days, liver extract derived from liver of other mammals or fishes was the drug of choice. But such drugs posed serious risk of transmitting infections from animals to animals or to human [1]. Moreover, the cost of liver extract is high especially if economy of the farm and farm products become a matter

of concern. Now-a-day herbal liver formulations become more important in treating hepatic diseases. In that *Picrorhiza kurroa* (P. kurroa; Family Scrophulariaceae), a well-known herb in the Indian traditional Ayurveda system of medicine, has been used to treat disorders of the liver and is an important ingredient of many herbal preparations used for treatment of liver ailments [2]. In India, hepatoprotective medicinal plants and their formulations have been traditionally used in Ayurveda for the prevention and treatment of diverse liver diseases [3]. *Picrorhiza kurroa* has been commonly used and well investigated for the treatment of jaundice. The plant has also been shown to be hepatoprotective in various animal models of hepatotoxicity like carbon tetrachloride, d-galactosamine, paracetamol, and thioacetamide [4]. In the present study, we evaluated the antioxidant activity, COX 2 inhibitory activity and hepatoprotective activity of P. *kurroa* against paracetamol-induced hepatotoxicity and Celecoxib were used as standard against COX 2 inhibitory activity.

(A) **(B)**

Fig 1. The flowering plant (A) and dried roots (B) of *Picrorhiza kurroa*.

MATERIALS AND METHODS:

MEM was purchased from Hi Media Lab, Mumbai. The Fetal bovine serum (FBS) was purchased from Cistron Lab, India. Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from Sisco Research Laboratory Chemicals, Mumbai. All of other chemicals and reagents were obtained from Sigma Aldrich, Mumbai. All chemicals and reagents used in this study were of analytical grades and procured from authorized dealers.

In-vitro Antioxidant (Hepatoprotective assay):

DPPH radical scavenging activity:

The effect of chloroform fraction of *P. kurrora* on DPPH radical was estimated by with minor modification. In brief, 2 ml of DPPH in methanol (3.6 × 10 to 5 M) were added to 50 μL of various concentrations test substance (25 μl to 1 ml). The mixture was vortexes for 15 s and left to stand at 37 °C for 30 min. The decrease in the absorbance at 517 nm was continuously recorded in a UV-Visible spectrophotometer (UV-1900, Shimadzu, Japan) for 15 min at room temperature [5-8].

COX-2 Inhibitory Activity:

The *in vitro* COX-2 inhibiting activities of the compounds have been evaluated using 'COX (ovine) inhibitor screening assay' kit with 96-well plates. Both ovine COX-1 and COX-2 enzymes were included. This screening assay directly measures PGF_2 produced by $SnCl_2$ reduction of COX-derived PGH_2.

The COX-1, COX-2, initial activity tubes were prepared taking 950 µL of reaction buffer, 10 µL of heme and 10 µL of COX-1 and COX-2 enzymes in respective tubes. Similarly, COX-1, COX-2 inhibitor tubes were prepared by adding 20 µL of inhibitor [9-11].

Chloroform fraction of the *P. kurroa* is added in each tube in addition to the above ingredients. The inhibitory assays were performed in the presence of extracts at different concentrations (10 to 80 µg/ml).The background tubes correspond to inactivated COX-1 and COX-2 enzymes obtained after keeping the tubes containing enzymes in boiling water for 3 min along with vehicle control. Reactions were initiated by adding 10 µL of arachidonic acid in each tube and quenched with 50 µL of 1M HCl. PGH_2 thus formed was reduced to PGF2α by adding 100 µL $SnCl_2$. The prostaglandin produced in each well was quantified using broadly specific prostaglandin antiserum that binds with major prostaglandins and reading the 96-well plate at 405 nm. The wells of the 96-well plate showing low absorption at 405 nm indicated the low level of prostaglandins in these wells and hence the less activity of the enzyme. Therefore, the COX inhibitory activities of the compounds could be quantified from the absorption values of different wells of the 96-well plate [12,13].

The celecoxib (selective COX-2 inhibitor) was used as positive controls in the study. The test extract

concentration causing 50 % inhibition of PGE2 release (IC_{50}) was calculated from the concentration-inhibition response curve by regression analysis.

Anti-cancer activity:

***Cell line and culture*:**

HepG2 cell line was obtained from Tamil Nadu Veterinary College, Chennai. The cells were maintained in Minimal Essential Media supplemented with 10 % FBS, penicillin (100 U/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

In vitro assay for Cytotoxicity activity (MTT assay):

The Cytotoxicity of samples (The chloroform fraction of roots of *P. kurroa*) on HepG2 was determined by the MTT assay. Cells (1 × 105/well) were plated in 1 ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 48 h of incubation, the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1 % DMSO for 48 h at 37°C.

After removal of the sample solution and it was washed with phosphate-buffered saline (pH 7.4), 200 µL/well (5 mg/ml) of 0.5 % 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl tetrazolium bromide cells (MTT) solution was added. After 4 h of incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570 nm [12-15].

Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HepG2 was expressed as the % cell viability, using the following formula:

$CV\ (\%) = [A_{570}\ TC / A_{570}\ CC] \times 100$ ……..(1)

Where, CV is cell viability, A_{570} TC and A_{570} CC are absorbance of treated cell and control cell at λ_{max} of 570 nm.

RESULTS AND DISCUSSION:

Hepatoprotective activity study by DPPH method:

In the DPPH radical scavenging assay, the DPPH radical was used as a substrate to evaluate free radical scavenging activity of Chloroform fraction of *P. kurroa*. This involves the reaction between the specific antioxidant (Which might be present as phytochemical in root extract of *P. kurroa* chloroform fraction) and the 2,2-diphenylpicryylhydrazide (DPPH). As a result there is a reduction of DPPH concentration by the antioxidant principle present in the extract which was confirmed by optical absorbance of DPPH and this was detected by spectrophotometer at 517 nm. Ascorbic acid was used as a standard. The IC_{50} value was found to be 305 and 585 µg/ml for chloroform fraction of the roots of *P. kurroa and* ascorbic acid respectively. The result was shown in Fig 1.

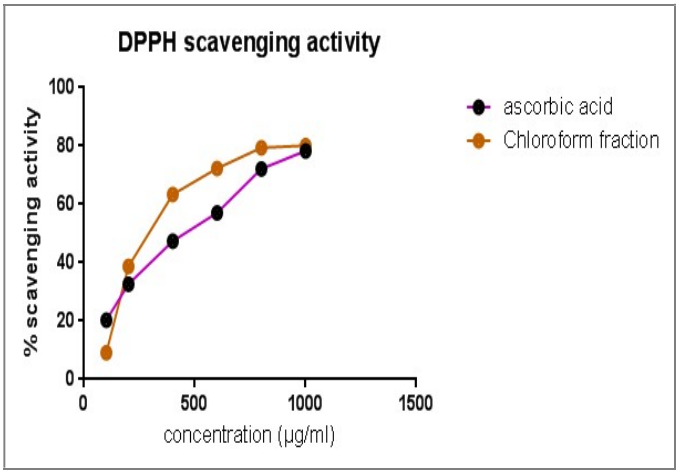

Fig 1. The DPPH scavenging activity of *P. kurroa* root chloroform fraction.

Hepatoprotective activity study by COX-2 inhibition method:

Inhibition of enzymes of the Arachidonic acid pathway may also contribute to the anti-inflammatory effects. The COX-2 is responsible for the biosynthesis of prostaglandins (PGs) under acute inflammatory conditions. So, the COX-2 plays an important role to be target enzyme on anti-inflammatory activity.

Table 1. The COX-2 percentage inhibition of *P. kurroa* root extract.

Sl. No.	Drug	Conc. (µg/ml)	Inhibition (%)
1	Chloroform Fraction	10	6.80±0.65
		20	15.82±0.88
		40	48.46±1.32
		80	65.85±0.72
2	Celecoxib (Standard)	30	85.24±0.90

The inhibitory effects of chloroform fraction of *P. kurroa* on *in vitro* enzymatic activities were measured against COX-2. Therefore, the chloroform fraction was studied at a concentration of 10 to 80 µg/ml, whether they inhibit cyclooxygenase-2. Interestingly, the chloroform fraction exhibited an inhibition of

cyclooxygenase-2 respectively at highest concentration. The result was shown in Table 1.

Cytotoxicity (Anti-cancer) study:

The cytotoxicity of sample on HepG2 was determined by the MTT assay. Cells (1 × 105/well) were plated in 1 ml of medium/well in 24-well plates. This initial study using the HepG2 cell line is a useful guide for primary hepatocyte growth and toxicological studies on 24-well plates. The 50 % cell viability value was found to be 59 µg/ml. The result was showed in Table 2, Fig 2 and 3.

Table 2. Anti-cancer effect of sample (*P. kurroa* root extract) on HepG2 cell line.

Sl. No.	Conc. (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.07	14.28
2	500	1:1	0.13	26.53
3	250	1:2	0.19	38.77
4	125	1:4	0.23	46.93
5	62.5	1:8	0.26	53.06
6	31.2	1:16	0.32	65.30
7	15.6	1:32	0.38	77.55
8	7.8	1:64	0.44	89.79
9	Cell control	-	0.49	100

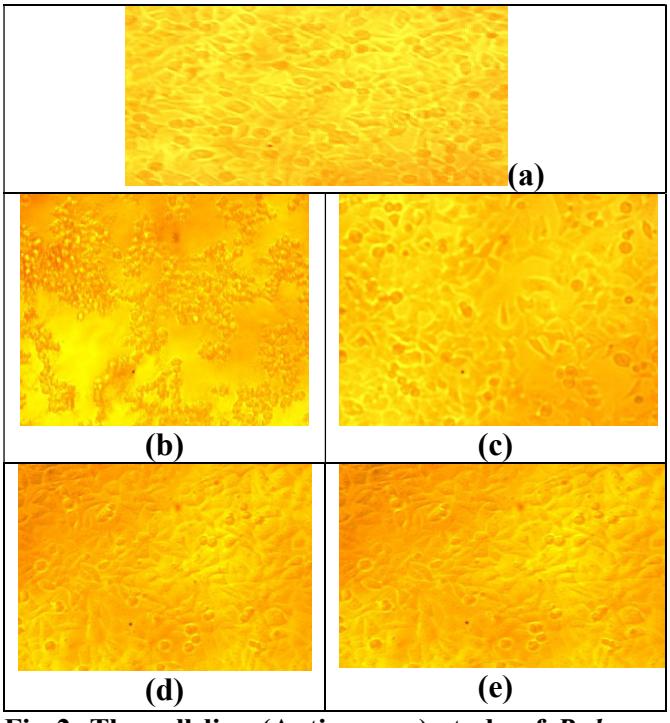

Fig 2. The cell line (Anti-cancer) study of *P. kurroa* root extract on HepG2 Cell line.

a - Normal HepG2 cell line, b, c, d and e are cell toxicities of *P. kurroa* root extract at concentration of 1000, 125, 62.5 and 31.25 µg/ml respectively.

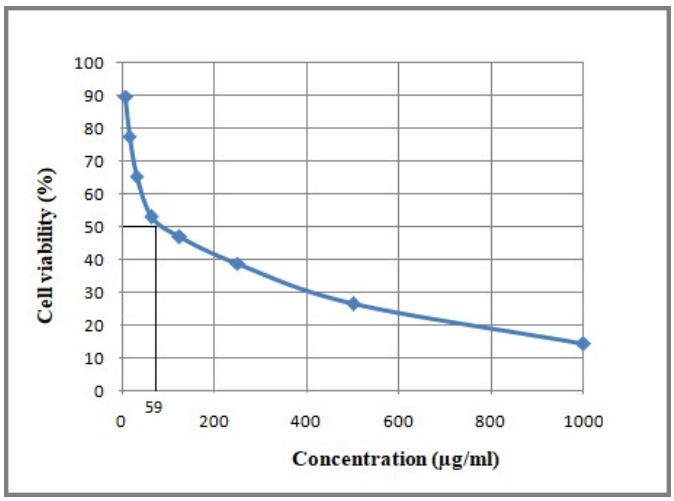

Fig 3. The MTT assay of *P. kurroa* roots.

1 - Corresponds to Cell control.

CONCLUSION:

Thus the present data provide a rationale for use of *P. kurroa* root as a suitable herbal treatment from Indian medicinal plants for the management of Hepatotoxicity and cancer diseases.

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