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Antioxidant Effects of *Picrorhiza kurrooa* Rhizome Extracts in Alcoholic Cirrhosis of Liver

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Abstract *Picrorhiza kurrooa* is mentioned in Ayurveda for the treatment of many disorders, but it has not been subjected to systematic scientific investigations to assess its antioxidant activity and hepatoprotective properties in alcoholic cirrhosis of liver. The methanol extracts of *P. kurrooa* Rhizomes significantly reduced oxidative stress and elevated antioxidants which were measured spectrophotometrically. The results of the study revealed that there was significant reduction in the activities of liver enzymes among the liver cirrhosis patients after the treatment with the *P Kurrooa* plant extract. The post-treatment results also showed significant correlation in the activities of malondialdehyde, glutathione peroxidase and superoxide dismutase in the liver of alcoholic patients.

Keywords: *Picrorhiza kurrooa, antioxidant activity, alcoholic cirrhosis of liver*

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1. Introduction

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of human kind [1,2,3,4]. Use of herbal drugs in the treatment of liver diseases has been a long tradition in India [5]. Now-a-day's emphasis has been laid on plants which yield effective antioxidative effect thereby adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. However standardization of herbal extracts has been a problem. Some herbal extracts recommended for gastrointestinal or bilary disorders have been found to posses potent hepatotoxic alkaloids which are harmful. Some of these extracts have yielded molecules, often related to flavonoids, which proven antioxidative, antifibrotic, antiviral or anticarcinogenic properties, including glycyrrhizin, phyllanthin, silibinin, picroside, and baicalein, which derive from licorice root, phyllanthus amarus, milk thistle, picrorhiza kurrooa, and sho-saiko-to, respectively, that can serve as primary compounds, for the development of specific hepato-toxic drugs [6]. *Picrorhiza kurroa*, (*Royleex Benth*) belonging to the family *Scrophuleriaceae* and grown in North-Western Himalayas from Kashmir to Sikkim has been mentioned in the Ayurveda for the treatment of asthma, liver and infectious diseases [7]. In view of these facts and lack of systematic studies on the liver and alcohol abused patients, we carried out this study to evaluate antioxidant activity, which we now present in this communication.

2. Materials and Methods

2.1. Blood sample procurement

Liver autopsy sample were obtained from forensic department of the government medical college. The study included 50 patients with alcoholic liver cirrhosis.

Primary cell culture establishment: Liver tissue was chopped with bend scissor in the sterile condition. Ham's F10 medium containing penicillin and gentamycin as antibiotic was obtained from sigma chemicals (USA). Small pieces of liver tissues were washed twice with complete medium and then treated with colligenase 5 at 37°C for 30 minutes. After incubation single cell of hepatocytes were incubated in Ham's F10 medium in Co_2 incubator at 37°C for 3 hours.

2.2. Plant Material and Extraction

The fresh rhizome of *Picrorhiza kurrooa* was collected and the plant was identified and authenticated by a Botanist from Department of Botany, Nagpur University, Nagpur. The voucher specimen has been kept in the department of Botany, Nagpur University, Nagpur, India (Acc. No. 5147/C). The rhizomes of *P. kurrooa* were washed, chopped, shade dried and powdered in grinding mill using Soxhlet extractor and then extracts were dried under reduced pressure using a rotary flash evaporator and kept in refrigerator. The yield of methanoic (MEP) extracts was 16.22%.

2.3. Experimental Design

In the culture flask 50 * 10^6 number of hepatic cells were incubated with or without plant extract for 1 hour at 37°C. After incubation cells were washed twice with complete Ham's F10 medium and proceed for different biological parameters.

2.4. Biochemical Determinations

In blood, serum alanine transaminase (ALT) [8], aspartase transaminase (AST) [9], total Bilurubin [10], albumin [11] and gamma glutamyl transferase(8) were measured. Levels of malondialdehyde (MDA) [12] as a marker of lipid peroxidation and Superoxide dismutase (SOD) [13], glutathione peroxidase (GPx) [14]as enzymatic markers of antioxidant defence systems were estimated in liver homogenate of the cultured cells.

3. Statistical Analysis

The results are presented as mean ± SD. The exact measures of associations in results between patients and control were compared using chi square test and fisher statistics. The Mann-Whitney 'U' test was used for intragroup comparisons. The significance was taken at P < 0.001.

4. Results

Significant decrease in the levels of alanine and aspartate transaminase, and gamma glutamyl transferase (Table 1) on administration of *P. Kurrooa* extracts to cirrhotic patients with decompensation was observed while the levels of albumin returned to near normal values. Significantly elevated antioxidants levels of superoxide dismutase and glutathione peroxidase was observed with concurrent decrease in oxidative stress which reflected with decreased MDA values near to normal control. Culture without *P.kurrooa* extract (with methanol supplement as control)showed elevated levels of MDA levels and decreased levels superoxide dismutase and glutathione levels as in case of untreated decompensated patients were observed in contrast to culture with *P. kurrooa* extract denoted elevated enzymatic levels and decreased oxidative stress (Table 2).

Table 1. Liver functions and GGT levels in cirrhotic patients with and without *P. kurrooa* treatment

LIVER FUNCTION TESTS	CULTURE WITHOUT EXTRACT n =50	CULTURE WITH EXTRACT n =50	p VALUE
ALT U/ml	80.04 ± 14.76	32.90 ±7.64	p < 0.0001
AST U/ml	133.56 ± 41.36	40.12 ± 4.96	p <0.0001
T.BILURUBIN mg/dl	6.8 ± 2.97	0.90 ± 0.27	p <0.0001
ALBUMIN g/dl	2.68 ± 0.29	3.11 ± 0.29	p <0.0001
GGT U/ml	35.38 ± 19.38	31.58 ± 16.06	p <0.0001

serum alanine transaminase (ALT), aspartase transaminase (AST), total Bilurubin, albumin and gamma glutamyl transferase (GGT).

Table 2. Comparison of variations in plasma activities of malondialdehyde, Superoxide dismutase and glutathione peroxidase in cultures with and without *P. kurrooa* treatment

	CULTURE WITHOUT EXTRACT n =50	CULTURE WITH EXTRACT n =50	p VALUE
MDA nmol/ml	10.16 ± 1.21	3.40 ± 0.88	p < 0.001
SOD	3.51 ± 0.79	5.65 ± 1.27	p < 0.001
GPx	8.51 ± 1.32	12.79 ± 1.67	p < 0.001

malondialdehyde (MDA), Superoxide dismutase (SOD), glutathione peroxidase (GPx).

5. Discussion

The view shared by many western supports of alternate medicine is that eastern traditional medicine relies on empiricism and holistic philosophy and controlled studies are considered unnecessary [6]. However various scientists have shown that herbal extracts possess free radical scavenging activity. The antioxidant reactions have been shown to involve multiple steps including the initiation, propagation, branching and termination of free radicals. The antioxidants which inhibit the formation of free radicals are called preventive antioxidants and those which interrupt the radical chain reaction (propagation and branching) are chain breaking antioxidants [15]. In present study Methanol Extract of *P. kurrooa* in the primary culture of hepatic cells showed reduced levels of liver function biomarkers and gamma glutamyl transferase levels. The culture contains decompensated hepatic cells and also showed to possess experimental model which can be used to justify our studies showing reduction in oxidative stress and resemblance with earlier studies. The increase of lipid peroxidation (MDA levels) and decrease levels of superoxide dismutase and glutathione peroxidase (Table 2) is in conformity with the earlier report that cirrhotic patients develop compromise of antioxidants thereby disturbance in the balance of pro-oxidants and antioxidants [16]. The results indicate that methanol extract of *P. kurrooa* acts as antioxidant or free radical scavenger. It is known that the enzymes of the antioxidant systems namely glutathione peroxidase and superoxide dismutase and non enzymatic antioxidants like vitamins have a prominent role in detoxifying H_2O_2 and the lipid peroxides generated during the metabolic processes of the body [17]. In the present study, there was a decrease of all the antioxidant enzyme s mentioned above (Table 2) in alcoholic cirrhosis of liver. The active substance called picrohizin in the rhizomes of *P. kurrooa* may be involved in the restoration of blood enzymes to near normal levels [18]. This might help in restoring the normal functioning of the hepatocyte compromised due to alcoholic bringe.

6. Conclusion

Further studies are necessary to find out whether picrorhizin and some other compounds present in the methanol extract of *P kurrooa* either individually or collectively are possibly responsible for reducing oxidative stress and improving liver function. It can be concluded from our results that the methanol extract of *P. kurrooa* rhizomes have antioxidant activities in cirrhotic hepatocytes. This finding may help in treatment of patients with alcohol abuse and cirrhosis of liver.

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