International Scholars Journals

International Journal of Medicinal Plants Research ISSN 2169-303X Vol. 8 (5), pp. 001-003, May, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Short Communication

Antihepatotoxic effect of *Picrorhiza kurroa* on mitochondrial defense system in antitubercular drugs (isoniazid and rifampicin)-induced hepatitis in rats

R. Jeyakumar[1], R. Rajesh[1], B. Meena[1], D. Rajaprabhu[1], B. Ganesan[1,2], S. Buddhan[1] and R. Anandan[2,*]

[1]Vinayaka Missions University, Ariyanoor, Salem-636308, India.

[2]Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin-682029, India.

Accepted 03 January, 2019

Oxidative stress is one of the mechanisms with a central role involved in the pathogenesis of antitubercular drugs (isoniazid and rifampicin)-induced hepatitis. In the present study the antihepatotoxic effect of the ethanol extract of *Picrorhiza kurroa* rhizomes and roots (PK) on liver mitochondrial antioxidant defense system in antitubercular drugs (isoniazid and rifampicin)-induced hepatitis in rats has been investigated. In liver mitochondria of antitubercular drugs administered rats, a significant elevation in the level of lipid peroxidation with concomitant decline in the level of reduced glutathione and the activities of antioxidant enzymes was observed. Co-administration of PK (50 mg/kg/day for 45 days) significantly prevented these antitubercular drugs-induced alterations and maintained the rats at near normal status. The results of the present investigation indicated that the hepatoprotective effect of the ethanol extract of *P. kurroa* rhizomes and roots (PK) might be ascribable to its membrane-stabilizing action and/or antioxidant property.

Key words: *Picrorhiza kurroa,* antihepatotoxic activity, antitubercular drugs, mitochondria, lipid peroxidation, antioxidant system.

INTRODUCTION

Plant

The rhizomes and roots of *Picrorhiza kurroa* Royle ex Benth. (Scrophulariacae), Indian name, 'Kutki', were col-lected from the hilly areas of Sikkim and authenticated by Captain Srinivasamurthi Drug Research Institute for Ayurveda, Arumbakkam, Chennai, India.

Uses in traditional medicine

In Indian ayurvedic medicine, the oral administration of extract of dried rhizomes and roots is claimed as a cure for human viral hepatitis (Kapahi et al., 1993; Anandan and Devaki, 1998). In traditional medicine, the plant has also been used to cure heart ailments, abdominal pain, stomach disorders, anaemia, jaundice, and for promoting bile secretion (Anand, 1990; Anandan and Devaki, 1999).

Previously isolated constituents

Kutkin, kurrin, kutkiol, picroside I, picroside II, kutkoside, apocyanin and androsin (Anand, 1990; Dorch et al., 1995; Anandan et al., 1998).

Tested material

Crude ethanol extract of dried rhizomes and roots of *P. kurroa* [PK] (yield: 8.2%).

Studied activity

Hepatoprotective activity of PK on hepatic mitochondrial defense system against antitubercular drugs -induced hepatotoxicity was investigated in male wistar albino rats.

Animals

Male Wistar strain albino rats, weighing 120 – 150 g were selected for the study. The animals were housed individually in polypropy-

*Corresponding author. E-mail: kranandan@inbox.com

Table 1. Hepatoprotective activity of the ethanol extract of *Picrorhiza kurroa* [PK] on liver mitochondrial lipid peroxidation [basal and in the presence of promoters like ascorbate, FeSO4 and *t*-butyl hydroperoxide] in antitubercular drugs-induced hepatitis in rats.

Group Parameters	Group I Normal control	Group II PK-administered (A)	Group III Antitubercular drugs-administered (B)	Group IV (A+B)
Basal	0.98 ± 0.06	0.83 ± 0.04	2.34 ± 0.11*	1.18 ± 0.05*
Ascorbate	2.78 ± 0.14	2.47 ± 0.10	4.63 ± 0.29*	2.91 ± 0.18*
FeSO4	4.15 ± 0.31	3.88 ± 0.25	6.89 ± 0.47*	4.36 ± 0.28*
t-butyl hydroperoxide	5.61 ± 0.42	5.49 ± 0.47	8.94 ± 0.67*	5.98 ± 0.54*

Values are expressed as mean ± S.D.; n = 6; * $P < 0.001$; Group III vs. Group I; Group IV vs. Group III; Student's *t*-test.
(A): PK, 50 mg/ kg body weight / day for 45 days. (B): Antitubercular drugs (isoniazid and rifampicin), [isoniazid and rifampicin, 200 mg each/ kg body weight/ day for 45 days]. Lipid peroxidation = nmol malondialdehyde / mg protein.

Table 2. Hepatoprotective activity of the ethanol extract of *Picrorhiza kurroa* [PK] on liver mitochondrial defense system [reduced glutathione (GSH), glutathione peroxidase (GPX), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD)] in antitubercular drugs-induced hepatitis in rats.

Group Parameters	Group I Normal control	Group II PK-administered (A)	Group III Antitubercular drugs-administered (B)	Group IV (A+B)
GSH	4.08 ± 0.34	4.36 ± 0.39	2.18 ± 0.12*	3.94±0.27*
CAT	7.11 ± 0.55	6.94 ± 0.46	3.49 ± 0.24*	6.43 ± 0.41*
SOD	3.73 ± 0.19	3.85 ± 0.21	1.68 ± 0.09*	3.38 ± 0.18*
GPx	3.05 ± 0.17	3.27 ± 0.21	1.86 ± 0.11*	2.77 ± 0.15*
GST	1412 ± 118	1374 ± 105	612 ± 58.4*	1211 ± 98.3*

Values are expressed as mean ± S.D.; n = 6; * $P < 0.001$; Group III vs. Group I; Group IV vs. Group III; Student's *t*-test.
(A): **PK**, 50 mg/kg/day for 45 days. (B): Antitubercular drugs (isoniazid and rifampicin), [isoniazid and rifampicin, 200 mg each/ kg body weight/ day for 45 days].
GSH, nmol g^{-1} wet tissue; GPx, nmol GSH oxidized min^{-1} mg^{-1} protein; GST, mol 1-chloro-2,4- dinitrobenzene conjugate formed min^{-1} mg^{-1} protein; CAT, nmol H_2O_2 decomposed min^{-1} mg^{-1} protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation.

pylene cages under hygienic and standard environmental conditions (28 ± 2ºC, humidity 60 - 70%, 12 h light/dark cycle). The animals were allowed a standard diet [M/s Sai Feeds, Bangalore, India] and water *ad libitum.* The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental protocol

Seven days after acclimatization, the experimental animals were divided into four groups, comprising six rats each. Rats in Group I (normal control) received standard diet for a period of 45 days. Group II animals were orally administered with PK, 50 mg/kg body weight/day for a period of 45 days. In Group III, rats were administered with antitubercular drugs (isoniazid and rifampicin, 200 mg each/ kg body weight/ day) for 45 days. In Group IV, the animals were co-administered with PK and antitubercular drugs for a period of 45 days at the above mentioned dosage regimen.

At the end of the experimental period, the animals were killed and the liver tissue was dissected out immediately, and washed with chilled physiological saline. Mitochondria isolated from the liver tissue by the method of Johnson and Lardy (1967) were used for the determination of lipid peroxides (LPO) (Ohkawa et al., 1979), reduced glutathione (GSH) (Ellman, 1959), glutathione peroxidase (GPX) [EC 1.11.1.9] (Paglia and Valentaine, 1967), glutathione-S-transferase (GST) [EC 2.5.1.18] (Habig et al., 1974) superoxide dismutase (SOD) [EC 1.15.1.1] (Misra and Fridovich, 1972) and catalase (CAT) [EC 1.11.1.6] (Takahara et al., 1960) .

Statistical analysis

Results are expressed as mean ± SD, and Student's *t*-test was used to assess the statistical significance. A *P*-value <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Rifampicin and isoniazid, alone or in association, are still widely used in most antitubercular chemotherapeutic regimens. However, these drugs are also well known as hepatotoxic agents (Prabakan et al., 2000; Saraswathy and Devi, 2001). Oxidative stress is one of the mechanisms with a central role involved in the pathogenesis of antitubercular drugs (isoniazid and rifampicin)-induced hepatitis (Attri et al., 2000). It is the result of excessive production of oxidant species and/or depletion of intracellular antioxidant defenses, leading to an imbalance in

the redox status of the hepatic cells (Sodhi et al., 1998). The focus of the current study was to evaluate the effects of ethanol extract of *P. kurroa* rhizomes and roots (PK) for its antioxidant and membrane stabilizing properties during antitubercular drugs-induced hepatotoxicity injury in rats.

The effects of oxidative stress can be evidenced by cellular accumulation of lipid peroxides. In the present study, the oral administration of antitubercular drugs [isoniazid and rifampicin, 200 mg each/ kg body weight/ day for 45 days], caused a significant elevation in the level of lipid peroxidation with concomitant decline in the level of reduced glutathione [GSH] and the activities of glutathione-dependent antioxidant enzymes (glutathione peroxidase [GPX] and glutathione-S-transferase [GST]) and antiperoxidative enzymes (catalase [CAT] and superoxide dismutase [SOD]) in liver mitochondria of experimental groups of rats as compared to that of normal control animals. Our findings are in accordance with earlier reported investigations (Dhuley, 2002; Sodhi et al., 1998); lack of antioxidant defense might have resulted in increased lipid peroxidation and subsequent deleterious effects on the hepatocellular membranes in antitubercular drugs- induced hepatitis condition. Oral co-administration of PK [50 mg/kg/day for 45 days] significantly (P<0.001) these antitubercular drugs-induced adverse effects and maintained the rats at near normal status. Previously, Anandan et al. (1999) reported that administration of ethanol extract of *P. kurroa* protected liver mitochondrial membranes against D-galactosamine-induced hepatotoxicity by its membrane-stabilizing and antioxidant properties. The unpaired electron present in the hydroxyl free radical generated during antitubercular drugs-induced hepatitis might have been trapped and dismuted by the electrophilic substances such as Picroside I, Picroside II and Kutkoside, which are present in rich quantities in the roots and rhizomes of *P. kurroa* (Anandan and Devaki, 1999).

In conclusion, the hepatoprotective effect of PK is probably due to the increase of the activities of the antioxidant enzymes, or to a counteraction of the free radicals by the presence of the electrophilic constituents picroside I, picroside II and kutkoside (Kumar et al., 2001; Ramesh et al., 1992), or to an activated conjugation of antitubercular drugs with GSH in liver.

REFERENCES

Anand N (1990). In: Hansch C, Sammes PG, Taylor JB, Kennewell PD, editors. Comprehensive medicinal chemistry, vol. 1.Oxford; Pergamon Press. pp. 113-131.

Anandan R, Devaki T (1998). Biochemical studies on the antihepatotoxic, potential of *Picrorhiza kurroa* on mitochondrial damage in D-galactosamine–induced liver intoxication in rats. Med. Sci. Res. 26: 349-352.

Anandan R, Devaki T (1999). Hepatoprotective effect of *Picrorhiza kurroa* on tissue defence system in D-galactosamine–induced hepatitis in rats. Fitoterapia. 70: 54-57.

Anandan R, Devi KP, Devaki T, Govindaraju P (1998). Preventive effects of *Picrorhiza kurroa* on D-galactosamine–induced hepatitis in rats. J Clin Biochem Nutr. 25: 87-95.

Anandan R, Rekha RD, Devaki T (1999). Protective effect of *Picrorhiza kurroa* on mitochondrial glutathione antioxidant system in D-galactosamine–induced hepatitis in rats. Curr. Sci. 76: 1543-1545.

Attri S, Rana SV, Vaiphei K, Sodhi CP, Katyal R, Goel RC, Nain CK, Singh K (2000). Isoniazid- and rifampicin-induced oxidative hepatic injury: Protection by N-acetylcysteine. Hum. Exp. Toxicol. 19: 517-522.

Dhuley JN (2002). Hepatoprotective effect of rhinax on antitubercular drug-induced hepato-toxicity in rats. Hindustan Antibiot Bull. 44: 53-59.

Dorch W, Stuppner H, Wagner H, Gropp M, Demoulin S, King J (1995). Antiasthmatic effects of *Picrochiza kurroa.* Androsin prevents allergen and PAF-induced bronchial obstruction in guinea pigs. Int. Arch. Allergy Appl. Immun. 95: 128-133.

Ellman GL (1959). Tissue sulfhydril groups. Arch. Biochem. Biophys. 82: 70–77.

Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130–7139.

Johnson D, Lardy H (1967). Isolation of liver or kidney mitochondria. In: Methods in Enzymology. Vol. 10. London; Academic Press. pp 94-96.

Kapahi BK, Srivastava TN, Sarin YK (1993). Description of *Picrorhiza kurroa*, a source of the ayurvedic drug kutuki. Int. J. Pharmacogn. 31: 217.

Kumar SHS, Anandan R, Devaki T, Kumar MS (2001). Cardioprotective effects of *Picrorhiza kurroa* against isoproterenol-induced myocardial stress in rats. Fitoterapia. 72: 402-405.

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170–3175.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95: 351–358.

Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70: 158–169.

Prabakan M, Anandan R, Devaki T (2000). Protective effect of *Hemidesmus indicus* against rifampicin and isoniazid-induced hepatotoxicity in rats. Fitoterapia 71: 55-59.

Ramesh C, Kapoor NK, Dhawan BN (1992). Picroliv, Picroside I and Kutkoside from *Picrorhiza kurroa* are scavengers of superoxide anions. Biochem. Pharmacol. 44:180-183.

Saraswathy SD, Shyamala Devi CS (2001). Modulating effect of Liv.100, an ayurvedic formulation on antituberculosis drug-induced alterations in rat liver microsomes. Phytother. Res. 15: 501–505.

Sodhi CP, Rana SF, Attri S, Mehta S, Yaiphei K, Mehta SK (1998). Oxidative-hepatic injury of isoniazid-rifampicin in young rats subjected to protein and energy malnutrition. Drug. Chem. Toxicol. 21: 305-317.

Takahara S, Hamilton BH, Nell JV, Ogubra TY, Nishimura ET (1960). Hypocatalasemia: a new genetic carrier state. J. Clin. Invest. 39: 610–619.