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Hepatoprotective Effect of *Rheum emodi* Roots (*Revand chini*) and *Akseer-e-Jigar* Against Paracetamol-induced Hepatotoxicity in Rats

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Abstract

Hepatoprotective effects of *Rheum emodi* roots and their aqueous and methanolic extracts were studied against liver damage induced by paracetamol in albino rats. In addition, the effects of herbal preparation, *Akseer-e-Jigar* and a control drug, silymarin were also studied. Pretreatment and post-treatment hepatoprotective effects of all these drugs were determined. The prevention of liver damage and curative effects of the drugs were judged by changes in serum ALT, AST, ALP, albumin and bilirubin (total and direct) levels. Powdered *Rheum emodi* roots (1 and 1.5 g/kg) and their aqueous extract did not significantly affect serum enzymes, albumin and bilirubin levels. However, treatment with powder (2 g/kg), methanolic extract (0.6 g/kg), *Akseer-e-Jigar* (1 g/kg) and silymarin (50 mg/kg) in both pre and post-treatment studies significantly prevented the paracetamol-induced rise of serum enzymes and bilirubin levels whereas serum albumin was raised after treatment with these drugs. It is conceivable, therefore, that *Rheum emodi* roots and Akseer-e-Jigar possess hepatoprotective principles that can prevent and/or treat liver damage due to paracetamol. The study has supported empirical use of the plant and its compound preparation used in traditional medicine.

Introduction

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factor [Nadeem, 1997]. The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or the other for this purpose. Scientific evaluation of plants has often shown that active principles in these are responsible for therapeutic success. A large number of medicinal plants have been tested and found to contain active principles with curative properties against a variety of diseases [Lewis, 1977]. Recent experience has shown that plant drugs are relatively non-toxic, safe and even free from serious side effects [Momin, 1987].

Rheum emodi root has been claimed to possess purgative, anthelmintic and hepatoprotective actions [Saeed,1970] and its tuber as bitter, laxative, appetite stimulant, diuretic, anti-bilious, and curative for sore eyes and bruises [Alam, 2005]. It has also been reported to have antifungal [Agarwal, 200] and hypoglycaemic properties [Li J, 1997]. Although *Rheum emodi* root is being commonly used in traditional medicine as hepatoprotective yet its efficacy has

not been well documented. Therefore, a study was conducted to investigate hepatoprotective effect of crude drug as well as aqueous and methanolic extracts of *Rheum emodi* roots and a popular compound preparation containing the roots against paracetamol-induced liver damage in albino rats.

Materials and Methods

Plant Material

Dried *Rheum emodi* roots locally called "*Revand chini*" were purchased from local herbal market. *R. emodi* roots were identified and authenticated by Department of Botany, University of Agriculture, Faisalabad. The dried roots were powdered finely with herbal grinder and stored in well-closed cellophane bags at 4 °C temperature.

Chemicals and Drugs

Paracetamol and methanol were obtained from GSK Pakistan Ltd., Karachi and Merck, Germany, respectively. Silymarin was that of Abbott Laboratories, Karachi. The diagnostic kits for the estimation of ALT, AST, ALP, albumin and bilirubin were that of Breuer & Breuer, Germany; Merck, Germany; Medi, Italy; Giesse, Italy; Human, Germany, respectively. *Akseer-e-Jigar* was supplied by Al-Mansoor Clinics, Medina Town, Faisalabad which consists of *R. emodi* roots, ammonium chloride and potassium nitrate.

Experimental Animals

Healthy adult male albino Wister rats weighing 200-300 g were procured from National Institute of Health, Islamabad. Three animals per cage were housed in metal cages and free access to standard feed and tap water was provided *ad libitum*. The animals were kept under observation for one week before experimentation under usual managemental conditions in the animal room. The animals were kept under stander laboratory conditions and the study protocol was approved by local ethical committee.

Induction of hepatic toxicity

Hepatic toxicity in rats was induced by oral administration of a single dose of paracetamol (640 mg/kg) using 1% methylcellulose (13 ml/kg) solution as vehicle. The test drugs were administered to the treatment groups orally, using normal saline (10 ml/kg) as vehicle. The control animals received equal volumes of the vehicle.

Pretreatment Hepatoprotective Effects

Animals were randomly divided into 9 groups of six animals each. Group I, serving as control, received 4 doses of normal saline (10 ml/kg) at 12 hrs intervals; 1 hr after the last saline dose, 1% methylcellulose solution (13 ml/kg) was administered. Group II received 4 doses of normal saline (10 ml/kg) at 12 hrs intervals; 1 hr after the last saline dose, paracetamol (640 mg/kg) was administered. Group III received 4 doses of powdered *Rheum emodi* roots (1 g/kg) at 12 hrs intervals; 1 hr after the last dose, paracetamol (640 mg/kg) was administered. Group IV received 4 doses of powdered *Rheum emodi* roots (1.5 g/kg) at 12 hrs intervals; 1 hr after the last dose, paracetamol (640 mg/kg) was administered. Group V received 4 doses of powdered *Rheum emodi* roots (2 g/kg) at 12 hrs intervals; 1 hr after the last dose, paracetamol (640 mg/kg) was administered. Groups VI, VII, VIII & IX were treated similar to the group V except that they received methanolic extract of *Rheum emodi* root (0.6 g/kg), aqueous extract of *Rheum emodi* roots (eq. to 2 g/kg), Akseer-e-Jigar (1 g/kg) and Silymarin (50 mg/kg), respectively.

Post-treatment Hepatoprotective Effects of Test Drugs

Animals were randomly divided into 9 groups of six animals each. Group I, serving as control, was administered the vehicle (1% methylcellulose orally; 13 ml/kg) at 0 hr and 4 doses of normal saline (10 ml/kg) at 6 hours intervals. Group II received paracetamol (640 mg/kg) at 0 hr and 4 doses of normal saline (10 ml/kg) at 6 hrs intervals, Group III received paracetamol (640 mg/kg) at 0 hr and 4 doses of powdered *Rheum emodi* roots (1 g/kg) at 6 hrs intervals, Group IV was given paracetamol (640 mg/kg) at 0 hr and 4 doses of powdered *Rheum emodi* roots (1.5 mg/kg) at 6 hrs intervals, Group V received paracetamol (640 mg/kg) at 0 hr and 4 doses of powdered *Rheum emodi* roots (2 g/kg) at 6 hrs intervals, Groups VI, VII, VIII & IX were treated similar to the group V except that they received methanolic extract of *Rheum emodi* roots (0.6 g/kg), aqueous extract of *Rheum emodi* roots (eq. to 2 g/kg), Akseer-e-Jigar (1 g/kg) and Silymarin (50 mg/kg), respectively.

Preparation of Methanolic and Aqueous Extracts of Roots:

Methanolic Extract

Methanolic extract was prepared by continuous extraction technique using Soxhlet's apparatus. Slow heating at 40 ° C and continuous stirring evaporated the methanolic extract. The process of evaporation was continued till complete evaporation of methanol was ensured. The dried extract was dissolved in 1% methylcellulose sol. just before administration to rats. The methanolic extract eq. to 2 g/kg b.w. of powdered *R. emodi* roots i.e. 0.6 g/kg was administered orally.

Aqueous Extract

A weighed amount of powdered roots was macerated in distilled water at room temperature and agitated occasionally for one day [Osol,1975]. Filtered through a fine filter paper and marc was pressed to avoid loss. The extract obtained was in liquid form at room temperature. The extract was stored in a sterilized PVC bottle at 4 °C. The extract was used in liquid form by calculating the volume eq. to the extract from 2 g of powdered *Rheum emodi* roots.

Collection of Blood Samples

Blood samples were collected 24 hrs after the last treatment. Animals were anaesthetized with ether, and put in a desiccator. Up to 5-8 ml of blood from each rat was collected by cutting the carotid artery in centrifuge tubes. These blood samples were kept at 4°C for a period of 12 hrs. Serum was separated by centrifugation (3000 r.p.m., for 15 min.) The serum thus obtained was used for determination of levels of ALT, AST, ALP, albumin and bilirubin.

Determination of Serum Enzymes, Albumin and Bilirubin

Serum samples were analyzed by following kinetic methods using B.T.S. 330 (Biosystems, Spain). Diagnostic kits have been used in the above mentioned semi-automated analyzer for the quantitative estimation of ALT (SGPT), AST (SGOT) ALP, albumin and bilirubin.

Statistical Analysis

The data obtained were analyzed by using one way analysis of variance technique. The means were compared for significance by using Least Significance Difference (LSD) test (Steel and Torrie, 1980).

Results

Pretreatment Hepatoprotective Effects

Paracetamol-treated group had ALT level which was significantly higher than control (Table 1). The

powdered *R. emodi* roots (2g/kg), methanolic extract, *Akseer-e-Jigar* and silymarin pretreatment inhibited the rise of serum ALT, AST, ALP and bilirubin levels effectively as their values were non-significantly different from control whereas level of albumin was decreased. The lower doses of *R. emodi* roots and their aqueous extract did not significantly affect the paracetamol-induced rise of serum enzymes and bilirubin whereas the decline in albumin level was also not prevented (Table 1).

Table 1. Pretreatment hepatoprotective effects of powdered *Rheum emodi* roots, methanolic extract, aqueous extract, *Akseer-e-Jigar* and Silymarin on paracetamol-induced rise of ALT, AST, ALP, albumin and bilirubin (total and direct).

Group	Treatments	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dl)	Bilirubin Tot. (g/dl)	Bilirubin Dir. (g/dl)
1	Saline + Vehicle	36.33 ± 2.03 D	49.83 ± 2.73 F	129.2 ± 11.7 G	3.50 ± 0.10 A	0.43 ± 0.07 E	0.25 ± 0.04 E
2	Saline+ Paracetamol	693.7 ± 30.0 A	921.7 ± 23.6 A	369.5 ± 15.8 A	2.33 ± 0.13 E	4.70 ± 0.11 A	3.26 ± 0.10 A
3	*R. emodi* roots (1 g/ kg) + Paracetamol	527.8 ± 25.2 B	608.5 ± 44.3 B	312.2 ± 15.6 B	2.78 ± 0.06 D	3.60 ± 0.24 B	2.41 ± 0.18 B
4	*R. emodi* roots (1.5g/ kg) + Paracetamol	194.7 ± 23.9 C	277.0 ± 31.0 CD	223.5 ± 15.3 CD	2.85 ± 0.08 CD	1.71 ± 0.16 C	1.15 ± 0.09 C
5	*R. emodi* roots (2 g/ kg) + Paracetamol	52.67 ± 2.91 D	111.0 ± 15.1 FG	138.5 ± 15.2 FG	3.28 ± 0.14 AB	1.11 ± 0.11 D	0.61 ± 0.08 D
6	Methanolic Extract + Paracetamol	42.17 ± 2.10 D	62.50 ±4.15 EFG	165.8 ± 12.7 EFG	3.25 ± 0.08 AB	0.41 ± 0.05 E	0.28 ± 0.04 E
7	Aqueous Extract + Paracetamol	66.17 ± 4.61 D	172.7 ± 12.2 C	227.8 ± 18.7 C	3.15 ± 0.14 B	1.03 ± 0.07 D	0.60 ± 0.03 D
8	*Akseer-e-Jigar* + Paracetamol	54.67 ± 5.14 D	76.67 ± 8.57 DE	186.0 ± 5.21 DE	3.06 ± 0.05 BCD	0.51 ± 0.10 E	0.35 ± 0.07 DE
9	Silymarin + Paracetamol	48.50 ± 3.75 D	81.00 ± 6.94 EF	169.8 ± 9.65 EF	3.13 ± 0.06 BC	0.51 ± 0.08 E	0.31 ± 0.07 E

Means sharing similar letters are statistically non-significant ($P > 0.05$), n=6

Post-treatment Hepatoprotective Effects

Table 2 shows that treatment with test substances significantly lowered paracetamol-induced rise of ALT level except the lower doses of *R. emodi* roots. The effect was highest with methanolic extract followed by *Akseer-e-Jigar* and silymarin, respectively. The test drugs also lowered serum AST, ALP and bilirubin levels in paracetamol treated rats (Table 2). The maximum decrease in the levels of AST and bilirubin were resulted from methanolic extract while maximum depression in ALP level was caused by *Akseer-e-Jigar*. The methanolic extract proved to be most efficient in increasing the decreased level of albumin in paracetamol treated rats (Table 2).

Table 2. Post-treatment hepatoprotective effects of powdered *Rheum emodi* roots, methanolic extract, aqueous extract, *Akseer-e-Jigar* and Silymarin on paracetamol-induced rise of ALT, AST, ALP, albumin, bilirubin (total and direct).

Group	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dl)	Bilirubin Tot. (g/dl)	Bilirubin Dir. (g/dl)
1	Saline + Vehicle	43.67 ± 2.76 E	57.67 ± 2.54 F	141.0 ± 9.04 EF	3.43 ± 0.11 A	0.31 ± 0.07 G	0.21 ± 0.04 F
2	Saline+ Paracetamol	690.5 ± 30.2 A	1027.5 ± 30.2 A	385.5 ± 10.0 A	2.08 ± 0.08 F	5.11 ± 0.12 A	3.43 ± 0.11 A
3	*R. emodi* roots (1 g/ kg) + Paracetamol	567.0 ± 31.3 B	812.8 ± 29.6 B	312.5 ± 14.0 B	2.56 ± 0.12 E	4.36 ± 0.21 B	2.90 ± 0.22 B
4	*R. emodi* roots (1.5g/kg) + Paracetamol	220.5 ± 16.6 C	354.8 ± 26.4 C	236.0 ± 10.5 C	2.85 ± 0.08 D	2.81 ± 0.18 C	1.93 ± 0.12 C
5	*R. emodi* roots (2 g/ kg) + Paracetamol	66.83 ± 5.18 DE	136.0 ± 17.6 DE	173.2 ± 17.9 DE	3.10 ± 0.07 BC	0.98 ± 0.10 DE	0.60 ± 0.09 DE
6	Methanolic Extract + Paracetamol	51.67 ± 2.44 DE	74.50 ± 3.92 F	153.3 ±13.6 EF	3.26 ± 0.09 AB	0.58 ± 0.07 FG	0.33 ± 0.06 EF
7	Aqueous Extract + Paracetamol	89.00 ± 4.44 D	183.3 ± 14.1 D	204.2 ± 18.5 CD	2.98 ± 0.07 CD	1.20 ± 0.03 D	0.66 ± 0.03 D
8	Akseer-e-Jigar + Paracetamol	57.50 ± 4.99 DE	93.83 ± 5.76 EF	130.0 ± 6.89 F	3.10 ± 0.03 BC	0.78 ± 0.10 EF	0.51 ± 0.07 DEF
9	Silymarin + Paracetamol	58.83 ± 2.87 DE	101.0 ± 5.79 EF	140.83 ± 8.21 EF	3.08 ± 0.04 BCD	0.63 ± 0.06 FG	0.35 ± 0.05 EF

Means sharing similar letters are statistically non-significant ($P > 0.05$), n=6

Discussion

Paracetamol-induced hepatic damage has been commonly used as experimental model for screening of newer hepatoprotectives [Plas,1982] and the extent of hepatic damage is assessed by the circulating levels of released cytoplasmic enzymes such as ALT, AST and ALP [Singh,1998]. The data obtained in the present study have shown that pretreatment of animals with test drugs have resulted in prevention of rise of serum enzymes, bilirubin and decline of albumin levels (Table 1). It is, conceivable, therefore that powdered *R. emodi* root, its methanolic extract and *Akseer-e-Jigar* contain MDME inhibitory constituents that provide hepatoprotection [Agarwal, 2000; Li J, 1975]. All the test drugs have marked effect, excepting the low doses of *R. emodi* and its aqueous extract that have failed to yield significant change in the curative trials. These results show that the powdered *Rheum emodi* in high dose, its methanolic extract and *Akseer-e-Jigar* have hepatoprotective principles. Our data have also supported empirical use of the plant drug in traditional eastern medicine and the herbal preparation, *Akseer-e-Jigar being* used very commonly by Hakims in the treatment of some liver disorders,

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