Indian Journal of Clinical Biochemistry, 2008 / 23 (3) 238-242

ANTIDIABETIC AND ANTIOXIDANT EFFECTS OF *PICRORHIZA KURROOA* RHIZOME EXTRACTS IN DIABETIC RATS

Shivkumar Chauhan* , Nirmalendu Nath and Vinay Tule *

Department of Biochemistry, Nagpur University, L.I.T. Premises, Amravati Road, Nagpur- 440010, India
**Eugeniks Genetic Research Institute, Lokmat Bhavan, Ramdaspeth, Nagpur 440012, India*

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 antidiabetic effect. The oral administration of aqueous and methanol extracts of P. kurrooa rhizomes (250 and 500 mg / kg body weight / day) for 15 days significantly reduced blood glucose, glycosylated haemoglobin and increased total hemoglobin, plasma insulin in alloxan-induced diabetes in albino rats. The treatment also showed significant correction in the level of nitric oxide radicals, superoxide radicals, peroxynitrite radical, lipid peroxidation, glutathione, glutathione reductase, glutathione-S-transferase, glutathione peroxidase, superoxide dismutase and catalase in the pancreas of alloxan diabetic rats.

KEY WORDS

Picrorhiza kurrooa, Antidiabetic, Alloxan, Antioxidant.

INTRODUCTION

It is known that diabetes may be due to damaged pancreas, insulin resistance due to its receptor defect or altered peroxidation-oxidation system (1, 2). Many drugs like sulphonylureas, biguanides, thioglitazones, etc. have many side effects. So the emphasis now a day is on plants, which have been in use for diabetes in *Ayurvedic* system of India from ancient times. Some plants studied for their use in diabetes have been reviewed (3, 4). *Picrorhiza kurrooa*, (Royle ex Benth.) belonging to the family Scrophuleriaceae and grown in North-Western Himalayas from Kashmir to Sikkim has been mentioned in *Ayurveda* for the treatment of asthma, liver and infectious diseases (5). But there are no systematic studies on the antidiabetic and antioxidant activity, which we now present in this communication.

Address for Correspondence :

Dr. Shivkumar Chauhan,
Eugeniks, Genetic Research Institute,
106/A-wing, First floor,
Lokmat Bhavan, Ramdaspeth, Nagpur 440012
E-mail: shivkumar_chauhan@rediffmail.com

MATERIALS AND METHODS

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. The powder was successively extracted with methanol and distilled water using Soxhlet extractor and then extracts were dried under reduced pressure using a rotary flash evaporator and kept in refrigerator. The yield of aqueous (AEP) and methanolic (MEP) extracts was 8.47% and 16.22% respectively. Both the extracts were administered to the animals as a suspension in propyleneglycol (100 mg per ml).

Experimental animals : Adult albino rats of Wistar stain of either sex of 12-16 weeks of age weighing between 120-180 gm were used for the study. The animals were maintained in well-ventilated room temperature with natural day-night cycle in large polypropylene cages. They were fed balanced rodent pellet diet and water *ad libitum* throughout the experimental period. The animals were quarantined for one week, prior to the experiments to acclimatize to laboratory conditions. The study protocol was approved by the IAEC (Institutional Animal

Table 1: Antidiabetic effect of aqueous and methanol extracts of *Picrorhiza kurrooa* rhizomes in alloxan diabetic rats

Parameters	Healthy Control (Group I)	Untreated Diabetes (Group II)	Treatment			
			AEP		MEP	
			(Group III) 250 mg	(Group IV) 500 mg	(Group V) 250 mg	(Group VI) 500mg
Blood glucose (mg/dL)	72 ± 7.3	282 ± 9.8 *	99.4 ± 7.7 *	83 ± 8.3 *	92.7 ± 4.5 *	79.7 ± 9.1 *
Plasma Insulin (μ IU/ml)	43.2 ± 3.9	7.9 ± 2.8 *	31.8 ± 3.7 *	38.1 ± 3.8 *	35.0 ± 3.9 *	41.2 ± 2.7 *
Total haemoglobin (g/dl)	13.9 ± 2.3	6.2 ± 1.8 *	9.9 ± 2.1 *	12.1 ± 2.3 *	10.1 ± 2.2 *	12.1 ± 1.9 *
Glycosylated haemoglobin (Hb %)	5.5 ± 1.2	14.7 ± 1.5 *	10.8 ± 1.1 *	7.3 ± 1.3 *	9.8 ± 1.6 *	6.9 ± 1.2 *
Body weight (g)	168 ± 18	149 ± 14 *	159 ± 10 *	172 ± 13 *	151 ± 11 *	174 ± 15 *

Values are mean ± SD of 7 experiments

Group II is compared with Group I; Group III, IV, V and VI are compared with Group II; * $P < 0.001$.

Ethics Committee, Govt. of India) (Registration No. 414/01/ab/CPCSEA).

Induction of diabetes: The rats were starved overnight and diabetes was induced by a single subcutaneous injection of alloxan monohydrate (Sigma chemical company, St. Louie, USA) (80 mg/kg body weight), dissolved in freshly prepared 0.15 M sodium acetate buffer, pH 4.5 (6). After seven days, the animals were stabilized and considered as diabetic, if their blood glucose level was >200 mg/dL after single subcutaneous injection of alloxan. AEP and MEP treatment was started after confirmation of diabetes. Total duration of AEP and MEP treatment was 15 days.

Experimental design: Animals were divided into six groups of seven animals each. Group I healthy animals received only propyleneglycol (5 ml/kg per day p.o.) for fifteen days and served as control. All the other groups are diabetic animals. Group II animals served as untreated diabetic control. Group III and IV animals were treated with AEP at a dose level 250 and 500 mg/kg body weight/day p.o. for 15 days. Group V and VI animals were treated with MEP at a dose 250 and 500 mg/kg body weight/day p.o. for 15 days. All animals were sacrificed by decapitation under light ether anesthesia after completion of 15 days treatment. Immediately after sacrifice, blood was collected from all animals in heparinised vaccutube and pancreas was dissected out, washed in ice-cold saline, and the homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 1000 rpm for 5 minutes at 4°C and supernatant was collected for different biochemical assays.

Biochemical determinations: In blood, plasma glucose (7), total haemoglobin (8), glycosylated haemoglobin (9) and plasma insulin (10) were measured. Level of glutathione (11), glutathione reductase (12), glutathione-S-transferase (13),

Table 2: Effect of treatment with aqueous and methanol extracts of *Picrorhiza kurrooa* rhizomes on the lipid peroxidation and antioxidant status in the pancreas of diabetic rats

Parameters	(Group I)	(Group II)	(Group III)	(Group IV)	(Group V)	(Group VI)
Nitric oxide radicals (pM of nitric oxide released/mg protein)	5.3 ± 0.78	12.5 ± 1.05 *	8.7 ± 0.96 *	6.4 ± 0.73 *	7.5 ± 0.68 *	6.1 ± 0.78 *
Superoxide radicals (nM cytochome C reduced/mg protein)	4.85 ± 0.51	9.66 ± 0.83 *	7.18 ± 0.63 **	5.99 ± 0.48 *	7.0 ± 0.39 **	5.1 ± 0.43 *
Peroxynitrite radicals (μM of peroxynitrite generated/mg protein)	7.42 ± 0.61	11.54 ± 1.13 *	8.97 ± 0.92 **	7.78 ± 0.70 *	8.44 ± 0.59 **	7.5 ± 0.37 *
Lipid peroxidation (μM of malonaldehyde formed/mg protein)	5.31 ± 0.48	9.64 ± 0.96 *	7.74 ± 0.74 **	5.50 ± 0.55 *	7.77 ± 0.47 **	5.3 ± 0.81 *

The animals of Group I to IV represent the same treatment status mentioned in Table 1; Values are mean ± SD of 7 experiments.
Group II is compared with Group I; Group III, IV, V and VI are compared with Group II; * $P < 0.001$; ** $P < 0.01$

Table 3: Antioxidative activity of aqueous and methanol extracts of *Picrorhiza kurrooa* rhizomes in the pancreas of alloxan diabetic rats

Parameters	(Group I)	(Group II)	(Group III)	(Group IV)	(Group V)	(Group VI)
Glutathione (μg of GSH utilized/mg protein)	5.59 ± 0.09	1.74 ± 0.05 *	3.83 ± 0.03 *	5.9 ± 0.13 *	3.4 ± 0.13 *	5.6 ± 0.1 *
Glutathione peroxidase (nM of GSH oxidized/min/mg protein)	289 ± 21.7	147 ± 13.5 *	215 ± 16.3 *	279 ± 12.2 *	229 ± 13.7 *	281 ± 11 *
Glutathione-S-transferase (nM of CDNB conjugate formed/min/mg protein)	292 ± 26.5	127 ± 15.4 *	199 ± 14.8 *	271 ± 16.2 *	198 ± 13.7 *	287 ± 13 *
Glutathione reductase (nM of GSSG utilized/min/mg protein)	28.7 ± 1.68	12.9 ± 1.11 *	19.7 ± 1.15 *	27.8 ± 1.21 *	19.3 ± 1.21 *	27.9 ± 2 *
Superoxide dismutase (Units of activity/mg protein)	3.37 ± 0.28	0.76 ± 0.08 *	1.99 ± 0.3 *	3.13 ± 0.16 *	2.4 ± 0.16 *	3.2 ± 0.2 *
Catalase (nM of H_2O_2 decomposed/min/mg protein)	5.15 ± 0.26	1.86 ± 0.11 *	3.5 ± 0.15 *	5.0 ± 0.22 *	3.8 ± 0.21 *	5.1 ± 0.3 *

The animals of Group I to IV represent the same treatment status mentioned in Table 1. Values are mean ± SD of 7 experiments

Group II is compared with Group I; Group III, IV, V and VI are compared with Group II; * $P < 0.001$

glutathione peroxidase (14), catalase (15), superoxide dismutase (16), lipid peroxidation in terms of thiobarbituric acid reacting substances (TBARS) (17), nitric oxide radicals (18), superoxide radicals (19) and peroxynitrite radical (20) and protein (21) were estimated in pancreas homogenate of all experimental groups animals.

Histological study : For histopathological study, small pieces of pancreas were fixed in Bouin's fluid, dehydrated in graded alcohol and embedded in paraffin wax. Sections (4-5 μm thick) were cut and stained with acidified aldehyde fuchsin.

Statistical analysis : Data were analyzed using GraphPad Prism software (version 4). The results are expressed as mean ± SD and Student's 't' test was used to assess statistical significance. *P*-values < 0.05 were considered as significant.

RESULTS

Significant increase in blood glucose and glycosylated haemoglobin (HbA_{1C}) observed in untreated alloxan diabetic rats when compared with control (Table 1). Administration of *P. kurrooa* extracts to diabetic rats significantly decreased blood glucose level and increased body weight gain to near normal control. The diabetic rats (Group II) showed a significant decrease in total body weight, haemoglobin and plasma insulin level when compared with normal control (Group I). Oral administration of *P. kurrooa* rhizomes AEP (Group III & IV) and MEP (Group V & VI) extract to diabetic rats restored the blood glucose, total haemoglobin, HbA_{1C}, plasma insulin level and body weight to near normal values (Table 1).

The pancreas of untreated diabetic animals showed elevated levels of superoxide, nitric oxide and peroxynitrite radicals and abnormally high lipid peroxidation (Table 2). The oral treatment with AEP and MEP extract of *P. kurrooa* of diabetic rats restored the oxidant status (Group III, IV, V & VI). In the diabetic pancreas (Group II) the levels of the antioxidants namely glutathione, glutathione peroxidase, glutathione-S-transferase, glutathione reductase, superoxide dismutase and catalase were much less (Table 3) when compared with Group I ($P < 0.001$). The administration of AEP and MEP (Group III to IV) significantly restored in the antioxidant status of glutathione related enzymes, superoxide dismutase and catalase when compared with untreated diabetes animals.

Figure 1 shows the histology of normal pancreas. Histologically, pancreatic necrosis in untreated diabetic rats with obliteration of β-cells was noted (Figure 2). AEP and MEP (500 mg/kg/ day) treatment to such alloxan diabetic rats showed recovery of the β-cells from necrosis (Figure 3 and Figure 4, respectively).

DISCUSSION

Oral administration of aqueous and methanol extracts of *P. kurrooa* for 15 days to alloxan diabetic rats reduced the elevated blood glucose and glycosylated haemoglobin (HbA_{1C}) levels. Normally, HbA_{1C} reflects blood glucose status over the previous 2 to 3 months time. The interesting observation in the present study that HbA_{1C} level returned to normal level in 15 days could be due to the presence of lectin like compounds in *P. kurrooa* (22) which known that lectin like compounds which are reported to bring down glycosylated

haemoglobin (13). This along with normal glucose level might be the reason. The increase of lipid peroxidation (MDA level) and level of nitric oxide, superoxide and peroxynitrite radicals seen in the untreated diabetic animals (Table 2) is in conformity with the earlier report that in diabetes the balance between peroxidation products (H_2O_2 also) and antioxidant of the body is disturbed (1). The oral treatment of AEP and MEP to alloxan-induced diabetic rats showed suppressed free radicals level. This result indicates that AEP and MEP acts as antioxidant or free radical scavenger. It is known that the enzymes of the antioxidant system namely glutathione peroxidase, glutathione-S-transferase, glutathione reductase, superoxide dismutase and catalase and the non-enzymatic antioxidants like vitamin C, reduced glutathione and selenium have a prominent role in detoxifying H_2O_2 and other lipid peroxides generated during the metabolic processes of the body (1). In the present study, there was a decrease of all the antioxidant enzymes mentioned above (Table 3) in the untreated diabetic animals. Treatment with AEP and MEP reverse this change and restored the level of all these enzymes to near normal level. This helps in maintaining the antioxidant potential of the pancreatic β-cell in order to insure both its survival and insulin secretion. This view supported by the histological study of the animals. The architecture of alloxan diabetic pancreas showed considerable reduction in the islet size along with degenerative changes and necrosis in some loci. After the treatment with the aqueous and methanol extract of *P. kurrooa* rhizomes, diabetic pancreas showed considerable improvement in β-cell density. The number of insulin granules and β-cells population was significantly high in the treated groups of rats. The islet size was also larger. This may be an indication of the regeneration and rejuvenation of β-cells leading to increase in insulin production and secretion (Table 1) seen after treatment with AEP and MEP. Rhizomes of *P. kurrooa* contain large quantities

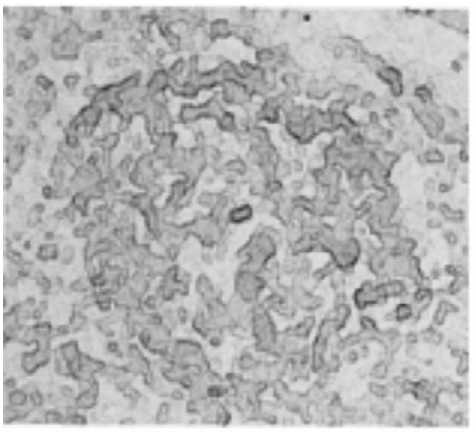

Fig 1

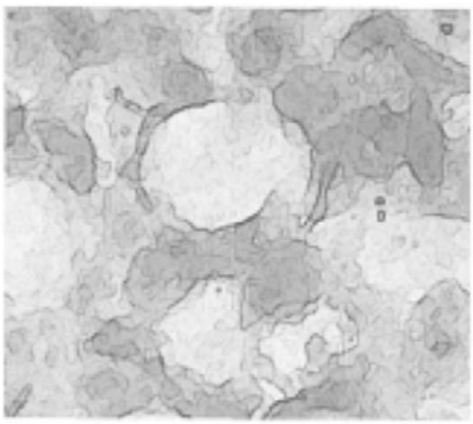

Fig 2

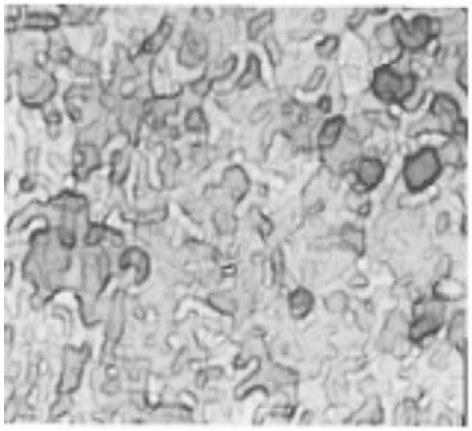

Fig 3

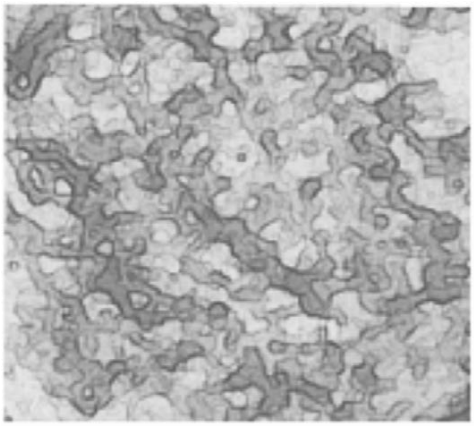

Fig 4

Figure 1: Histology of normal rat pancreas showing islet of Langerhans along with densely populated β-cells (1000x).
Figure 2: Histology of islet of pancreas of alloxan diabetic rat showing degenerative changes along with necrosis in some loci (1000x).
Figure 3: Histology of regenerated pancreatic islets of Langerhans from AEP treated diabetic rat showing densely populated and normal pancreatic β-cells (1000x).
Figure 4: Histology of the pancreas of diabetic rat treated with MEP showing recovery of β-cells (1000x).

of a substance called picrorhizin (5). Further studies are necessary to find out whether picrorhizin and/or lectin like compounds and some other compounds present in AEP and MEP are either individually or collectively responsible for the antidiabetic activity. It can be concluded from our results that the aqueous and methanol extract of *P. kurrooa* rhizomes have the antidiabetic and antioxidant activities in alloxan diabetic rats. These findings may help its use in the treatment of diabetes.

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