Antidiabetic activity of standardized extract of *Picrorhiza kurroa* in rat model of NIDDM

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**ABSTRACT:** The present study was undertaken to investigate the effect of standardized aqueous extract of *Picrorhiza kurroa* Royle ex Benth. on diabetes. Diabetes mellitus was induced with streptozotocin-nicotinamide and rats found diabetic were orally administered standardized aqueous extract of *Picrorhiza kurroa* (100 and 200 mg/kg, p.o.) or glibenclamide (10 mg/kg, p.o.) or vehicle (0.3% carboxy methyl cellulose suspension) for 14 days. Fasting blood glucose levels and lipid profiles were measured in control as well as diabetic rats after two week treatment. In addition, liver glycogen level of *Picrorhiza kurroa* extract (PKE) treated diabetic rats were compared to that of control and diabetic control rats. Oral glucose tolerance test was also performed on nondiabetic normal rats. Statistical analyses were performed by one way analysis of variance followed by Tukey-Kramer multiple comparisons test. PKE treatment induced significant reduction (*p* < 0.001) in elevated fasting blood glucose level in streptozotocin-nicotinamide induced type-2 diabetic rats. In oral glucose tolerance test, oral administration of PKE increased the glucose tolerance. PKE treatment also significantly (*p* < 0.001) reversed the weight loss associated with streptozotocin treatment. These findings provide *in vivo* evidence that standardized extract of *Picrorhiza kurroa* possess significant antidiabetic activity in streptozotocin-nicotinamide induced type-2 diabetes mellitus in rats.

**Keywords:** *Picrorhiza kurroa*, antidiabetic, streptozotocin, nicotinamide, diabetes mellitus

1. Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. It is characterized by elevated blood glucose concentration caused by insulin deficiency, often combined with insulin resistance. Type-2 DM is more prevalent and account for about 90% to 95% of all diagnosed cases of diabetes. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality in the near future (1).

The drugs currently available for treatment of diabetes have a number of serious adverse effects. As the knowledge of the heterogeneity of this disorder increases, there is a need to look for more effective agents with fewer side effects. This has led to the search for alternative therapies that may have a similar efficacy without potential adverse effects associated with conventional drug treatment. Ethnobotanical knowledge played a particularly important role in historical diabetes therapies, with over 1,200 species of medicinal plants recognized throughout the world for their ability to treat diabetic indications (2).

*Picrorhiza kurroa* (Family: Scrophulariaceae) is a small perennial herb that grows in northwest India on the slopes of the Himalayas between 3,000 and 5,000 meters. It is an important herb in the traditional Ayurvedic system of medicine, and has been used to treat liver and bronchial problems. Other traditional uses include dyspepsia, bilious fever, chronic dysentery, and scorpion sting. *Picrorhiza* has been shown to protect liver cells from a wide variety of toxins including amanita poisoning, carbon tetrachloride (3), galactosamine (4), ethanol (5), aflatoxin-B1 (6), acetaminophen (7), and thioacetamide (8), in both *in vitro* and *in vivo* experiments. *Picrorhiza kurroa* was found to be a potent immunostimulant, stimulating both cell-mediated and humoral immunity (9). *Picrorhiza kurroa* had been shown to possess anti-asthmatic and anti-allergic activity (10,11). *Picrorhiza kurroa* treatment reduced the cellular damage caused by hypoxia, indicating *Picrorhiza* constituents may protect against hypoxia/reoxygenation-induced injuries (12). Other reported activities of *Picrorhiza* include nitric oxide scavenging activity, cardioprotective effect, anti-cancer effect, and anti-viral effect (13). Recently, *Picrorhiza* extract has shown antidiabetic activity through alloxan-induced diabetic rat model (14), found effective in diabetic nephropathy (15) and possess hypolipidemic activity.
activity (16). In view of these findings and fact that dyslipidemia is the hallmark of type-2 DM, we have evaluated standardized extract of *Picrorhiza* in streptozotocin-nicotinamide induced type-2 diabetes and associated dyslipidemia.

### 2. Materials and Methods

#### 2.1. Animals

Adult Charles foster rats (180 ± 10 g) were obtained from Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The animals were housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1°C and 45-55% relative humidity, with a 12:12 h light/dark cycle. Unless stated otherwise, they were provided with commercial food pellets and water *ad libitum*. Animals were acclimatized to laboratory conditions for at least one week before using them for experiments and were subjected only once to the experimental conditions. Principles of laboratory animal care (NIH publication number 85-23, revised 1985) guidelines were followed.

#### 2.2. Plant extract

The standardized aqueous extract of *Picrorhiza kurroa* (standardized to contain 5.00% kutkin, HPTLC) was obtained from Promed Research Centre, Gurgaon, Haryana, India.

#### 2.3. Drug administration

Standardized extract of *Picrorhiza kurroa* was suspended in 0.3% carboxy methyl cellulose (CMC) and administered orally through oral gavage at the doses of 100 and 200 mg/kg of body weight per day. Doses are selected on the basis of available literature on the aqueous extract of *Picrorhiza kurroa* (3,16).

#### 2.4. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed to evaluate the peripheral glucose utilization. Albino rats of either sex were divided into four groups (*n* = 6), fasted overnight and administered as 0.3% CMC suspension, PkE (100 and 200 mg/kg) and glibenclamide (10 mg/kg), respectively. Glucose (2 g/kg) was orally administered 30 min after the drug treatments. Blood glucose levels were determined in blood samples collected at 0 min (prior to glucose administration), 30, 60, and 120 min after glucose administration.

#### 2.5. Induction of type-2 diabetes mellitus

Type-2 DM was induced in overnight fasted male rats by a single *i.p.* injection of 65 mg/kg dose of streptozotocin (Merck, Germany), 15 min after *i.p.* administration of 120 mg/kg nicotinamide (SD fine Chem, Mumbai, India) (17). Current pharmacology protocols (18) were followed for preparation and administration of streptozotocin solution. Hyperglycemia was confirmed by the elevated glucose level in the blood, determined at 72 h and then on 7th day after injection. The rats found with permanent diabetes were used for antidiabetic study.

#### 2.6. Experimental design

Animals were divided into five groups of six rats each viz. Group I: Normal control rats, administered 0.3% CMC for 14 days; Group II: Diabetic control rats, administered 0.3% CMC for 14 days; Group III: Diabetic rats administered PkE 100 mg/kg/day, *p.o.* for 14 days; Group IV: Diabetic rats administered PkE 200 mg/kg/day, *p.o.* for 14 days; Group V: Diabetic rats administered glibenclamide, 10 mg/kg/day, *p.o.* for 14 days. Blood samples were collected by retro-orbital puncture and fasting blood glucose levels were estimated on days 0, 7, and 14 with commercially available biochemical kit (Span Diagnostics Ltd., Surat, India) as in our previous study (19). On 14th day, plasma lipid profiles were estimated using biochemical kits (Span Diagnostics Ltd.) and liver glycogen levels were estimated using anthrone reagent (20). Body weight of rats was also measured periodically.

#### 2.7. Statistical analysis

All the values of the experimental results were expressed as mean ± standard error of mean (SEM). Statistical analyses were performed by one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. GraphPad InStat (version 3.06) software was used for all statistical analyses.

### 3. Results

#### 3.1. Effect on OGTT

Figure 1 shows blood glucose levels of normal control, PkE, and glibenclamide treated animals after oral administration of glucose (2 g/kg). Animals treated with PkE and glibenclamide showed a significant decrease in blood glucose levels at 30 and 60 min compared to vehicle control animals. The administration of PkE significantly prevented the increase in blood glucose levels without causing a hypoglycemic state. Maximum effect of PkE was observed 30 min after the oral glucose administration (*p* < 0.05 and 0.001 for PkE 100 and 200 mg/kg, respectively). Effect of higher dose of PkE (200 mg/kg) was found comparable to glibenclamide (10 mg/kg).
3.2. Effect on fasting blood glucose level and lipid profile

Table 1 illustrates the levels of blood glucose in the control and experimental groups of rats. Diabetic rats showed a significant increase in blood glucose compared with corresponding control rats \((p < 0.001)\). Oral administration of PkE (100 and 200 mg/kg) dose dependently and significantly reduced the fasting blood glucose levels on 7th \((p < 0.05)\) and 14th day \((p < 0.001)\) compared to diabetic control animals. Glibenclamide treatment also significantly reduced the increased blood glucose level of diabetic rats.

Table 2 shows the levels of plasma total cholesterol, triglycerides, and lipoproteins in the control and experimental groups of rats. The levels of plasma total cholesterol (TC), triglycerides (TG), and LDL-cholesterol (LDL-C) were significantly increased, whereas levels of HDL-cholesterol (HDL-C) were significantly decreased, in diabetic rats as compared to control rats. Oral administration of PkE and glibenclamide to diabetic rats significantly reversed all these changes to near normal level. The effect of PkE (200 mg/kg) was more significant than that of 100 mg/kg and was comparable with that of glibenclamide (10 mg/kg).

3.3. Effect on liver glycogen content

A significant decrease \((p < 0.001)\) in liver glycogen content was observed in diabetic rats compared to normal control group. PkE (100 and 200 mg/kg) showed a significant increase \((p < 0.001)\) in liver glycogen levels compared to the diabetic control rats. However, results of higher dose of PkE (200 mg/kg) were more significant than lower dose of PkE (100 mg/kg). Glibenclamide treatment also significantly increased \((p < 0.001)\) liver glycogen levels compared to diabetic control rats (Table 3).

3.4. Effect on body weight changes

The body weight changes in control, diabetic control and diabetic rats treated with PkE and glibenclamide are shown in Table 4. A significant decrease in body weight was observed in the diabetic rats \((p < 0.001)\) compared with the control group. Both doses of PkE as well as glibenclamide resulted in a significant increase in body weight gain \((p < 0.001)\) compared with diabetic control animals.

4. Discussion

Streptozotocin and appropriate protective dose of nicotinamide induce a diabetic syndrome with reduced pancreatic insulin stores that mimics some features of
Type-2 diabetes is associated with marked imbalance in lipid metabolism (28). Diabetic dyslipidemia is characterized by low level of HDL-C as well as elevated level of TG and LDL-C particles (29,30). A significant increase in plasma cholesterol and triglycerides along with a significant decrease in HDL-C, observed in diabetic rats in the present study, are consonant with the pathogenesis of diabetes.

Observed hypolipidemic activity of PkE in diabetic rats is consonant with the earlier studies conducted with *Picrorhiza* extract on different models of hyperlipidemia (16,31). The increase in alanine transaminase (ALT) activity in diabetes is almost always due to hepatocellular damage and is usually accompanied by an increase in aspartate transaminase (AST) activity (32). Several studies with liver tissues of streptozotocin induced diabetic rats indicate a trend towards increased activity of transaminases (33). The AST and ALT activities have been used as an indicator of liver function (34). *Picrorhiza* extract has been reported to reverse the increased AST and ALT activities towards near normalcy (31), which suggests prevention of cellular and tissue damages under diabetic conditions. Therefore, hepatoprotective activity of *Picrorhiza* extract may be partially responsible for the observed antidiabetic activity.

Diabetes is associated with a characteristic loss of body weight in animals. Several hypothesis have been proposed for the body weight loss in diabetic animals like increased muscle wasting (35,36) or loss of muscle proteins due to hyperglycemia (37). Rats treated with PkE extract or glibenclamide showed an increase in body weight as compared to the diabetic control rats suggesting a protective role of PkE on muscle wasting or due to better glycemic control.

**5. Conclusion**

Our findings have demonstrated for the first time through streptozotocin-nicotinamide induced type-2 diabetes model that standardized extract of *Picrorhiza kurroa* has an antihyperglycemic effect. Therefore, it may be potentially beneficial in type-2 diabetes and associated dyslipidemia.

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**References**


