

Antidiabetic activity of *Rheum emodi* in Alloxan induced diabetic rats.

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ABSTRACT

The present study was carried out to evaluate the antidiabetic effect of *Rheum emodi* rhizome extract and to study the activities of hexokinase, aldolase and phosphoglucosomerase, and gluconeogenic enzymes such as glucose-6-phosphatase and fructose -1,6-diphosphatase in liver and kidney of normal and alloxan induced diabetic rats. Oral administration of 75 % ethanolic extract of *R. emodi* (250 mg/kg body weight) for 30 days, resulted in decrease in the activities of glucose-6-phosphatase, fructose-1,6-disphosphatase, aldolase and an increase in the activity of phosphoglucosomerase and hexokinase in tissues. The study clearly shows that the *R.emodi* possesses antidiabetic activity.

KEY WORDS

R.emodi , gluconeogenic, alloxan, antidiabetic

INTRODUCTION

Diabetes mellitus is the most important disease involving the endocrine pancreas. Type 2 diabetes is a heterogeneous disease with both genetic and environmental contributory factors, involved multiple defects in insulin action and insulin secretion leads to hyperglycemia and effecting nearly 10% of the population all over the world. In modern medicine, the beneficial effects on glycemic levels are well documented; the preventing activity of these drugs against progressive nature of diabetes and its micro and macrovascular complications was modest and not always effective (1). Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death, and third when its fatal complications are taken in to account. Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities or as simple dietary adjuncts to existing therapies (2).

Herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects (3) *Rheum emodi* Wall. ex Meissn. (Polygonaceae) is a leafy perennial herb distributed in altitudes ranging from 2800 to 3800 m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India. Roots of *R. emodi* are reported to have antibacterial and antifungal activities (4-8). In addition several other biological activities such as laxative, diuretic, and *in vivo* inhibitory effect towards P388 leukemia in mice are also reported .It has the property of purgative, hemostatic, antipyretic, anthelmintic, laxative, atonic indigestion, constipation , diarrhea, dysentery, jaundice, liver disorder, antibacterial, antitumor, antifungal, diuretic, hemostatic, cholagogue, antihypertensive, lowers serum cholesterol, anti-inflammatory and antioxidant activity. The rhizomes ('roots') contain stilbene compounds (including rhaponticin) which seem to have diabetic property (9 - 11). Antioxidant and Anti-cancer Potentials of *R. emodi* rhizome extracts was recently reported by Rajkumar et al., (12). The aim of this study was to determine the antidiabetic effect of ethanolic extracts of *R. emodi* rhizome by various methods .

MATERIALS AND METHODS

Rheum emodi rhizomes were collected from Uttaranchal, India. Collected specimen were shade-dried, powdered and used for solvent extraction. The collected plant was identified and authenticated by a botanist. Department of Botany, Central Research for Siddha, Anna hospital, Chennai. The rhizome were dried at room temperature and coarsely powdered . Rhizome powder was extracted with 75 % ethanol using a soxhlet apparatus in a ratio of 1:6

[powder (in grams): solvent (in milliliters)]. The extract obtained was evaporated to dryness under reduced pressure in a rotary evaporator. The samples were stored in a vacuum dessicator at room temperature until further use.

Male albino rats of Wistar strain weighing about 150 – 200 g were used for the study. They were fed a standard rat pellet diet and water was provided *adlibitum* and maintained under standard laboratory conditions (Temperature 24-28°C, relative humidity 60 - 70%) Animals described as fasted were deprived of food for 16 hours but had free access to water. Animals were fasted for 24 hours and injected with freshly prepared aqueous solution of alloxan monohydrate (150 mg/ kg, i.p.). After a week, rats with marked hyperglycemia (fasting blood glucose >250 mg/dl) were employed for the study(13).

Experimental set up

The animals were divided in to 4 groups of 6 each.

Group I served as normal healthy control.

Group II (untreated diabetic control).

Group III diabetic rats given *R.emodi* rhizome extract (250 mg/kg body weight).

Group IV control rats given *R.emodi* rhizome extract (250 mg/kg body weight)

The crude extract was administered for a period of 30 days.

Collection of blood, kidney and liver from the rat

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected on decapitation and serum was separated by centrifugation (for 20 min at 2000 rpm). The liver and kidney were excised and thoroughly washed in ice – cold saline.

Estimation of biochemical parameters

Hexokinase (14), phosphoglucoisomerase(15), aldolase (16), glucose-6-phosphatase (17) and fructose-1,6-disphosphatase (18) were assayed in liver and kidney.

Statistical evaluation

Statistical evaluation was done using one-way analysis of variance (ANOVA)

followed by Duncan's Multiple Range Test (DMRT). Statistical significance was set at (P<0.05).

RESULTS AND DISCUSSION

The activities of glycolytic enzymes (hexokinase, aldolase, phosphoglucoisomerase) in the liver and kidney of control and experimental rats are shown in (Tables 1 and 2).The activity of hexokinase and phosphoglucoisomerase were seen significantly decreased, where as the activity of aldolase was seen significantly increased in diabetic rats, when compared with control rats (P<0.05) .Oral administration of *R.emodi* (250 mg/kg body weight) for 30 days significantly reversed these values to normal.

The activities of gluconeogenic enzymes (glucose 6-phosphatase and fructose-1, 6-diphosphatase) in the liver and kidney of control and experimental animals are shown in (Tables 3 and 4). The activity of gluconeogenic enzymes, glucose-6- phosphatase and fructose-1, 6-diphosphatase (liver and kidney) were found significantly elevated in diabetic rats when compared with control rats (P>0.05) . Oral administration of *R.emodi* (250 mg mg/kg body weight) for 30 days brought back the activity of the above enzymes to the near normal level. In our study, administration of *R.emodi rhizome* extract resulted in a significant reduction in blood glucose level, when compared with diabetic control animals.

Liver is the candidate organ involved in glucose homeostasis. It is the main site for glycolysis, a process where glucose is degraded and gluconeogenesis, where glucose is synthesized from lactate, amino acids and glycerol. These are the two important complementary events that balance the glucose load in our body (19).Hexokinase is the prime enzyme catalysing glucose phosphorylation. Impairment of hexokinase activity suggest the impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia.

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase and phosphofructokinase. Hexokinase is universally present in cells of all types. Hexokinase catalyses the conversion of

glucose to glucose 6-phosphate and plays a central role in the maintenance of glucose homeostasis. In the liver, this above enzyme is an important regulator of glucose storage and disposal (20).

The hexokinase activity was found to be decreased in diabetic rats which may be due to insulin deficiency. Treatment with *R.emodi rhizome extract* elevated the activity of hexokinase in the liver.

Activity of phosphoglucosomerase and ATP dependent phosphofructokinase enzyme are reported to be under regulation by citrate (21), which is a TCA cycle intermediate. Decrease in activity of phosphoglucosomerase might be expected to inhibit the proportion of glucose 6-phosphate metabolized via the glycolytic pathway(22).

Aldolase, another key enzyme in the glycolytic pathway, increases in diabetes and this may be due to cell impairment and necrosis . In experimental diabetes the cells are subjected to alloxan induced-damage and very often exhibit glycolysis after a period of increased oxygen uptake. Fructose-1,6-diphosphatase and glucose-6-phosphatase are important regulatory enzymes in gluconeogenesis. Liver being the main organ responsible for maintaining the homeostasis of the blood glucose. (23)

In diabetic animals the enzyme levels were observed to increase. The increased activities of glucose 6-phosphatase and fructose-1,6-diphosphatase in liver and kidney of the alloxan induced diabetic rats may be due to insulin insufficiency. Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase (24). In *R.emodi* treated rats, these two enzymes (glucose-6-phosphatase, fructose-1,6-diphosphatase) were seen significantly reduced in liver and kidney. This may be due to increased insulin secretion, which is responsible for the repression of the gluconeogenic key enzymes.

CONCLUSION

From the present study, it is concluded that *R.emodi rhizome* extract exhibited antidiabetic activity by enhancing the peripheral utilization of glucose by correcting the impaired liver and kidney glycolysis and by limiting its gluconeogenic formation similar to insulin.

Table 1
Effect of *R.emodi rhizome* extract on the activities of glycolytic enzymes in liver of control and experimental rats

Groups	Liver		
	Hexokinase #	Aldolase σ	Phosphoglucosomerase \$
Group I	402.67 \pm 46.62	155.13 \pm 5.48	47.78 \pm 0.91
Group II	83.80 \pm 1.14 a*	242.49 \pm 10.87 a*	24.42 \pm 1.25 a*
Group III	409.37 \pm 12.75 b*	160.61 \pm 5.25 b*	28.75 \pm 0.55 b*
Group IV	379.03 \pm 5.82cns	a*152.66 \pm 4.78cns	47.06 \pm 0.81cns

Values are expressed as Mean \pm SD (n=6).

Units :

Hexokinase # : nmoles of glucose -6-phosphate formed/min/mg/protein)

Aldolase σ : nmoles of glyceraldehyde formed/ min / mg protein)

Phosphoglucosomerase \$: nmoles of fructose formed /min/mg protein)

Stastical comparison:

a: Group I and Group II

b: Group II and Group III

c: Group I and Group IV

*P<0.05 ns- non significant.

Table 2
Effect of *R.emodi rhizome* extract on the activities of glycolytic enzymes in kidney of control and experimental rats

Groups	Kidney		
	Hexokinase #	Aldolase σ	Phosphoglucoisomerase \$
Group I	304.05 ± 16.87	172.23 ± 8.89	37.07 ± 1.28
Group II	67.84 ± 1.31 a*	221.94 ± 6.63 a*	15.94 ± 0.84 a*
Group III	287.48 ± 36.67 b*	205.13 ± 3.40 b*	38.08 ± 0.55 b*
Group IV	300.96 ± 8.80 cns	226.67 ± 27.48cns	37.36 ± 0.32cns

Values are expressed as Mean±SD (n=6).

Units :

Hexokinase # : nmoles of glucose -6-phosphate formed/min/mg/protein)

Aldolase σ : nmoles of glyceraldehyde formed/ min / mg protein)

Phosphoglucoisomerase \$: nmoles of fructose formed /min/mg protein)

Stastical comparison:

a: Group I and Group II

b: Group II and Group III

c: Group I and Group IV

*P<0.05 ns- non significant.

Table 3
Effect of *R.emodi rhizome* extract activities of gluconeogenic enzymes in liver of control and experimental rats

Groups	Liver	
	Glucose-6-phosphatase #	Fructose-1,6-diphosphatase \$
Group I	39.46 ± 0.85	26.44 ± 4.18
Group II	109.89 ± 4.71 a*	346.79 ± 17.02 a*
Group III	46.71 ± 2.44 b*	41.37 ± 8.57 b*
Group IV	38.59 ± 0.62ns	18.38 ± 1.38ns

Values are expressed as Mean±SD (n=6).

Units:

Glucose-6-phosphatase # : nmoles of Pi liberated / min / mg protein)

Fructose-6-diphosphatase\$: nmoles of Pi iberated / min / mg protein)Stastical comparison:

Stastical comparison:

a: Group I and Group II

b: Group II and Group III

c: Group I and Group IV

*P<0.05 ns- non significant.

Table 4
Effect of *R.emodi rhizome* on the activity of gluconeogenic enzymes in kidney of control and experimental rats

Groups	Kidney	
	Glucose-6-phosphatase #	Fructose-1,6-diphosphatase \$
Group I	49.06 ± 7.17	20.81 ± 1.78
Group II	133.88 ± 4.52a*	218.11 ± 16.14 a*
Group III	38.84 ± 1.73 b*	55.17 ± 10.92 b*
Group IV	38.07 ± 1.07ns	30.81 ± 3.61ns

Values are expressed as Mean±SD (n=6).

Units:

Glucose-6-phosphatase # : nmoles of Pi liberated / min / mg protein)

Fructose-6-diphosphatase\$: nmoles of Pi iberated / min / mg protein)Stastical comparison:

Stastical comparison:

a: Group I and Group II

b: Group II and Group III

c: Group I and Group IV

*P<0.05 ns- non significant.

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