JAMC, Vol. 2, Nos. 2&3, 1989

ANTIDIABETIC EFFECT OF POTERIUM SPINOSUM LINN. IN ALLOXAN DIABETIC RATS

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ABSTRACT

Hypoglycemic activity in many plant extracts is known to exist but the possible mechanism of action has not been worked out in most of the cases. The water extract of roots of Poterium spinosum has been known for its hypoglycemic activity in Arab countries. It is a bush like plant which grows abundantly and is called "Bullan" in Arabic language. The roots are washed and bioled in water which gives a brick red colour extract. Hypoglycemic activity was studied in normal and alloxan diabetic rats. The activity was compared with phenformin. The liver was rapidly removed and clamped in liquid nitrogen to determine the metabolites. A significant hypoglcemic effect in alloxan diabetic rats was observed and about 60% decrease in blood glucose level was found after 3 hours of administration of the extract. The extract was not found to be effective in normal rats. There was a simultaneous reduction in blood and liver glucose, in blood triglycerides level. While blood and liver lactate concentration increased significantly. The hypoglcemic activity of extract was compared with Phenformin and was found to be more potent. The extract of Poterium spinosum has a significant hypoglycemic activity which could be useful for oral treatment of Diabetes mellitus.

Key Words: Hypoglycemic, Poterium spinosum, alloxan, rats.

INTRODUCTION

Poterium spinosum is reputed for its antidiabetic properties in common man of Jordan. The use of this plant in these populations suggests the need for experimental assesment concerning its hypoglcemic effect in laboratory animals, in normal as well as in induced diabetic conditons. Poterium spinosum is repoted in "FloraEuropea" (1) as Sarcopoterium in family Rosaceae. The presence of antidiabetic properties in roots and other parts of plant does not seem to be repoted earlier, hence the present study was undertaken to investigate the hypoglcemic activity of Poterium spinosum extract on administration in normal and alloxanised rats and suggest the possible mechanism of action.

MATERIALS AND METHODS

Poterium spinosum was collected during the summer season in Jordan. It was air dried and washed of any dust. Whole roots were chopped to fine pieces and 100 g were immersed in 500 ml of distilled water for 30 minutes. It was then boiled for 30 minutes so that the volume of the water remained approximately one third of its original volume. Then the extract was filtered. The filtrate was brick red in

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colour and was further concentrated to 125 ml. Solid content was found to be 2.37 % on dried root basis in the extract. On qualitative analysis, the extract gave positive tests for the flavonoid, tannin and saponin, while it was negatiove for alkaloids.

Male albino rats weighing between 200-300 g of Wister Sprague Dawley strain were used troughout the study. They were kept in the animal house at about 27°C. In case death occured in the diabetic animals, the number was made up from a duplicate group of the same date.

Alloxan monohydride (obtained from sigma chemical co. USA) was prepared as 5% solution in double distilled water and 200 mg/kg body weight was injected animals fasted overnight for 14-18 hours (2). Food and water was supplied imediately after injection. Poterium spinosum water extract and phenformin hydrochloride solution in water were administerd through a stomach tube fitted to asyringe. The dose of poterium spinosum and phenformin hydrochloride was expresed in terms of solid present in the extract and the solution respectively.

Blood samples were collected as required from tail or by direct heart puncture. The animals were fasted for 12-16 hours before they were sacrificed. The animal were stunned by a blow over th head, blood was collected directly from the heart, inserting heparinized syringe. The blood was pooled from each group for lactate and pyruvate determinations. Rats were killed by dislocation of the neck. The liver was rapidly removed and pressed between metal clamps previously cooled in liquid nitrogen to determine the metabolites (3). The time elapsed between dislocation of the neck and deep freezing the tissue was not more than 10 seconds. The frozen liver was pulverized in a mortar to a fine powder with frequent addition of liquid nitrogen. The powder was transferred to a weighed plastic centifuge tube containing 2ml of frozen 30% (w/v) perchloric acid. After a rapid reweighing the tissue (1-2g) was mixed with perchloric acid, care being taken that no thawing occured. Ice cold 5 ml of distilled water was added the mixture was immediately homogenized in the centrifuge tube with a pestle driven by a low speed motor. This was continued for about 2 minutes untill homogenizing was complete. Protein was removed by centrifugation in a refrigerated centrifuge at 10000 g for 10 minutes. The supernatant fluid was adjusted to pH 5-6 with 20 % (w/v potassium hydroxide and after standing for 30 minutes in cold, the precipitate was centrifuged. The supenatant fluid was then shaken for 30 seconds with 0.1 g/ml florisil (60- 100) mesh (obtained from Merck, Darmstdt, W. Germany). This treatment removed flavins from the solution and decreased the slow enzymatic oxidation of NADH, while the recovery of the metabolites determination was not affected. The florisil was removed by centrifugation and supernatant fluid was used for the analysis.

Glucose was determined by the method of Nelson Somogyi (4), enzymatic assays were performed for lactate and pyruvate by Sigma Kit (5) and triglyceride was determined by sigma kit (6).

RESULTS

Figure 1 shows the hypoglycemic effect of Poterium spinosum in alloxan diabetic rats treated with 5, 10, 50 and 100 mg of the extract and normal rats treated with 10 mg of the extract. The maximum hypoglcemia was observed in all the alloxanized diabetic rats after 3 hours of treatment with Poterium spinosum. The group which was administerd oral dose of 10 mg/kg body weight of Poteruim spinosum extract showed the maximum hypoglycemia (60% blood glucose decrease as compared with zero hour) after 3 hours, when compared with other groups given different doses. Thus 10 mg/kg body weight was used in further experiments.

Effect of Poterium spinosum on blood metabolite concentrations is shown in (table 1). A significant increase was observed in diabetic rats in glucose, lactate and triglyceride levels as compared with control rats. When poterium spinosum was adminiterd to the diabetic rats , blood glucose and triglyceride levels were significantly decreased while lactate and pyruvate were increased as compared with diabetic control group. The treatment of diabetic rats with phenformin hydrochloride showed similar effect as the Poterium spinosum. There was a significant increase of liver glucose and lactae levels of diabetic rats as compared with that of normal control rats (table 1). In diabetic rats 3 hours after treatment with Poterium spinosum a significant in hepatic glucose concentration was observed when compared with diabetic control. Treatment with phenformin hydrochloride did not show any significant effect on hepatic metabolite concentration in diabetic rats as compared with diabetic control.

DISCUSSION

Apart from effects on hormonal status or hormonal reposiveness of tissue, pharmacological hypoglycemia may be produced by three main actions: Stimulation of glucose uptake and utilization by tissue, inhibition of glucose priduction by liver or inhibition of the supply of glucogenic precursors by peripheral tissues (7).

In our study glucose level decreased in both liver and blood and there was an increase in blood lactate (table 1) which may partly be due to increased peripheral glucose utilization and increased glycolysis or partial inhibition of hepatic gluconeogenesis. The hypoglycemia may not be due to diminished release of gluconeogenic precursor by peripheral tissue because blood lactate and pyruvate levels were increased (table 1). Reduced level of serum triglyceride in diabetic rats treated with Poterium Spinosum may be due to the oxidation of free fatty acids rather than due to esterification. The possible mechanism of action of Poterium spinosum may be either through increased glucose uptake and glycolysis with increased lactate production from periphery or by partial inhibition of hepatic gluconeogenesis, which is also observed in phenformin hydrochloride treatment.

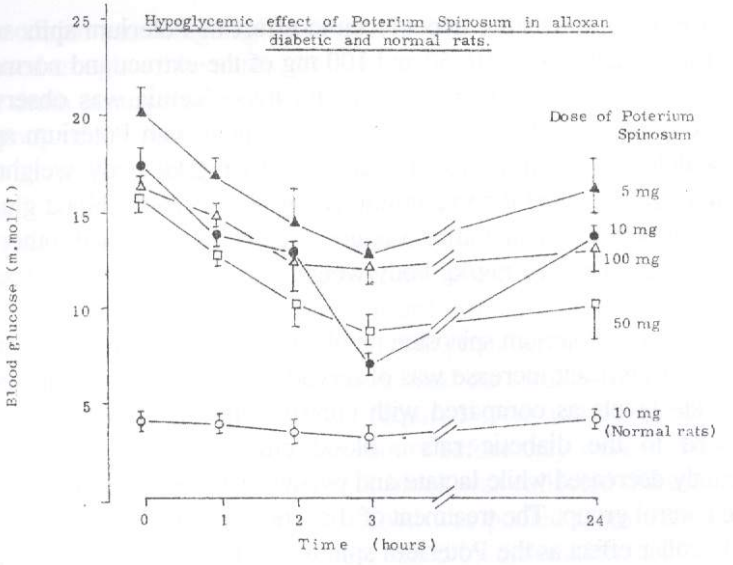

TABLE I

Effect of treatment of Poterium spinosum Extract and Phenformin on Body and Liver weights and Blood & Liver Glucose, Lactate and Pyruvate.

The values are expressed as mean ± s.e.m. The number of observations is given in parentheses.

			Glucose		Lactate		Pyruvate		Triglycerides
	Body weight (gm)	Liver weight (gm)	Blood (mmole /L)	Liver µmole /g)	Blood (mmole /L)	Liver µmole /g)	Blood (mmole /L)	Liver µmole /g)	Blood (mg / dl)
Normal Control	254.0 ± 9.0 (10)	5.3 ±0.2 (5)	4.1 ±0.3 (5)	6.5 ±0.7 (5)	1.6 ±0.1 (5)	1.5 ±0.2 (5)	0.05 ±0.004 (5)	0.02 ±0.002 (5)	30.0 ±4.8 (5)
Diabetic Control	227.7 9.8 (10)	5.7 ±0.1 (5)	20.1^{o} ±2.0 (5)	19.7^{o} ±1.3 (5)	2.6+ ±0.2 (5)	2.6^{x} ±0.2 (5)	0.05 ±0.003 (5)	0.03 ±0.007 (5)	332.0^{o} ±24.0 (5)
Diabetic rats treated with Poterium spino sum (10 mg/Kg)	229.0 ± 7.7 (10)	5.6 ±0.1 (5)	10.4** ±0.5 (5)	8.2** ±0.9 (5)	3.8** ±0.3 (5)	3.7 ±0.5 (5)	0.1 ±0.003 (5)	0.04 ±0.007 (5)	260.2* ±16.4 (5)
Diabetic rats treated with Phenformin (100 mg/Kg)	228.0 ± 9.5 (10)	5.4 ±0.1 (5)	14.3* ±0.9 (5)	17.0 ±0.5 (5)	3.9*** ±0.2 (5)	3.6 ±0.5 (5)	0.1** ±0.002 (5)	0.06 ±0.01 (5)	311.8 ±34.7 (5)

*P<0.05, **P<0.01 ***P<0.001, when compared with diabetic control
+P<0.05, x P<0.01 o P<0.001, when compared with normal control.

As Poterium spinosum extract was not found to be effective in normal rats (figure 1) but was effective in alloxan diabetic rats, its action is not dependent on insulin production insulin protection. In alloxan diabetic rats the insulin is decreased and therefore any fall in blood glucose by a hypoglycemic agent would be by its direct action or by helping in some way the action of poorly available endogenous insulin. The action of Poterium spinosum is comparable to that of phenformin (table 1).

Phenformin and other biguanide do not effect insulin secretion but increase glucose utilization apparently because they inhibit oxidative metabolism of glucose and cosequently increase anaerobic glycolysis within the cells. They also decrease glucose absorbtion.

The formation of insulin tannin complexes have been shown to be biologically active. Three flavonoid compounds were isolated from Ficus bengalensis, which possesed hypoglcemic activity (8). It is conceivable that probably Proterium spinosum contains some flavonoid and tannin compounds, which exert hypoglycemic action by complex formation with endogenous insulin. From the results it is concluded that Poterium spinosum is a potent oral antidiabetic agent and the exact mechanism of action is not known which could be useful for oral treatment of diabetes.

Acknowledgement:

The authors are grateful to Mr. Al. Qudah for providing roots of Poterium Spinosum from Jordan.

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