PRELIMINARY STUDIES ON THE HYPOGLYCEMIC EFFECT OF *PEGANUM HARMALA* L. SEEDS ETHANOL EXTRACT ON NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Peganum harmala L. (Zygophyllaceae) is a traditional medicine used for the treatment of variety of human ailments, including antidepressant, hallucination, antileishmaniasis etc. We report for first time the hypoglycemic activity of the ethanolic extract of this plant at two dose levels of 150 and 250mg/kg bw in sucrose challenged normal as well as in rats with streptozotocin induced diabetes. The oral administration of ethanolic extract causes maximum fall of blood glucose level to 22.9% ($p < 0.05$) and 29.4% ($p < 0.01$) respectively with the two doses in normal and 30.3% ($p < 0.01$) and 48.4% ($p < 0.001$) in diabetic rats. The standard drug metformin treated group showed 28.0% ($p < 0.01$) and 45.5% ($p < 0.001$) respectively in normal and diabetic rats. The above results show that the ethanolic extract of *P. harmala* is as effective as metformin in reducing the blood glucose levels of normoglycemic and streptozotocin-induced diabetic rats.

KEY WORDS

Peganum harmala, Hyperglycemic, Streptozotocin induced diabetic rats, Sucrose challenged.

INTRODUCTION

There are an estimated 143 million people in the world with diabetes and this number will probably double by the year 2030 (1). The use of medicinal plants has flourished as an alternative for the treatment of diabetes because modern medicines are tagged with several side effects and are also expensive. A multitude of herbs and medicinal plants and some compounds purified from them have been studied for the treatment of diabetes throughout the world as they might provide a basis of new synthetic antidiabetic analogues with potent activity (2- 4).

Peganum harmala L. (Zygophyllaceae) is a traditional medicine commonly known as "Harmal" in Hindi, "Isband" in Bengali, "Simagoronti" in Telgu, and "Simaiyalavinai" in Tamil, used for

the treatment of variety of human ailments (5). The β -carboline alkaloids isolated from this plant are reported to possess antidepressant (6), hallucinogenic (7) and antileishmanial effect (8). The present study was undertaken to study the hypoglycemic effect of ethanolic extract of *P. harmala* seeds in normal and streptozotocin (STZ) induced diabetic rats

MATERIALS AND METHODS

Plant material: The seeds of *P. harmala* were collected from Kashmir in the month of April 2005. Plant was identified and authenticated by Botany Division of the institute. The reference material and voucher specimen (No.6375) is deposited in the herbarium of the Central Drug Research Institute, Lucknow (India).

Preparation of ethanolic extract: Powdered seeds (6 kg) of *P. harmala* were placed in glass percolator with ethanol (25 liter) and are allowed to stand at room temperature for 16 hours (overnight). The percolate was collected. This process of extraction was repeated four times. The combined extract was concentrated under vacuum using rotavapour at 50°C. Weight of extract obtained was 305gm (yield 5.08%).

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Experimental animals: Male albino rats of Wistar / Sprague Dawley strain of 8 to 10 weeks of age and 160 ± 20 g of body weight were procured from the animal colony of the Institute. Research on animals was conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964. The institute has taken permission from the animal ethical committee for the work (No. 129/07/Biocmem/IAEC). Rats were always placed in polypropylene cages. The following norms were always followed for animal room environment: temperature $23 \pm 2^\circ\text{C}$; humidity 50-60%; light 300 lux at floor level with regular 12 h light cycle; noise level 50 decibel; ventilation 10-15 air changes per hour. The animals had free access to pellet diet (Dayal Industry Ltd. Barabanki Lucknow) and tap water unless stated otherwise.

Assessment of hypoglycaemic activity in normal rats: The hypoglycaemic effect of *P. harmala* seeds in normal rats was assessed by improvement of glucose tolerance. Overnight fasting blood glucose of Wistar strain male albino rats was measured and the animals showing fasting blood glucose level from 60 to 80 mg/dl were selected and divided into groups of five animals in each. The blood glucose measured at this time period was termed as baseline (0 min) blood glucose. Rats of experimental group were administered orally suspension of the ethanolic extract at 150 and 250 mg/kg dose prepared in 1.0% gum acacia. The standard drug metformin was given at 100 mg/kg dose level. Animals of control group received vehicle (1.0% gum acacia). An oral sucrose load of 10 g/kg body weight was given to rats of all groups exactly post 30 min administration of the test sample/vehicle. Blood glucose level was again measured at 30, 60, 90 and 120 min post administration of sucrose. Food but not water was withheld from the cages during the course of experiment.

Assessment of hypoglycemic activity in STZ-induced diabetic rats

Induction of diabetes in rats: Overnight fasted male albino rats of Sprague Dawley strain were made diabetic by intraperitoneal injection of streptozotocin at 60 mg/kg body weight dose prepared in 0.1M citrate buffer (pH 4.5). Fasting blood glucose level was measured after 48 hours and animals showing blood glucose level above 180 mg/dl were considered as diabetic(9).

Effect of single dose administration in diabetic rats: The diabetic rats with fasting blood glucose values (baseline at 0 min) from 180 to 270 mg/dl were included in this study. Animals were divided into four groups of five animals each. Rats of experimental group I and II were given orally, suspension of the ethanolic extract at 150 and 250 mg/kg b.w. dose, whereas group III were dosed with metformin at 100 mg/kg b.w. dose. Animals of control group were given an equal amount of vehicle (1% gum acacia). An oral sucrose load of 2.5 g/kg was given to all groups 30 min post administration of the test sample/ vehicle. Blood glucose levels of the animals of all groups were again measured at 1, 2, 3, 4, 5, and 6 hour after sucrose load. Food (not water) was removed from the cages during the experimental period.

Statistical analysis: Quantitative glucose tolerance of each animal was calculated by area under curve (AUC) method using Prism Software. Comparing the AUC of experimental and control groups determined the percentage antihyperglycaemic activity. Statistical comparison was made by Dunnett's test. Results were expressed as mean $\pm$ SE.

RESULTS

Effect of *P. harmala* on normoglycemic rats: The effects of *P. harmala* ethanolic extract (150 and 250mg/kg b.w.) and

Table 1: Effect of *P. harmala* ethanolic extract and metformin on glucose tolerance of normoglycemic rats

Group	Blood glucose concentration (mg/dl)					% reduction compared to control at 2 hrs
	0 min	30 min	60 min	90 min	120 min	
Control	67.8 $\pm$ 2.8	108.4 $\pm$ 3.9	118.3 $\pm$ 4.6	120.6 $\pm$ 6.1	117.0 $\pm$ 3.9	
150 mg/kg	67.5 $\pm$ 2.5	113.9 $\pm$ 2.8	105.1 $\pm$ 3.7	97.2 $\pm$ 2.3	90.1 $\pm$ 2.2*	22.9*
250 mg/kg	67.3 $\pm$ 2.5	96.6 $\pm$ 3.7	101.8 $\pm$ 1.8	91.6 $\pm$ 1.8*	82.6 $\pm$ 3.6**	29.4**
Metformin	67.6 $\pm$ 1.8	96.8 $\pm$ 3.4	99.3 $\pm$ 2.3	90.2 $\pm$ 1.2*	84.2 $\pm$ 2.5**	28.0**

Values are mean $\pm$ SEM, n=5 *p<0.05 and **p<0.01 vs control

Table 2: Effect of ethanolic extract of *P. harmala* seeds (150 and 250 mg/kg), metformin (100 mg/kg) on the blood glucose levels of streptozotocin-induced diabetic rats

Group	Blood glucose concentration (mg/dl)		
	Fasting	6 hour post sucrose load	% reduction compared to control
Control	251.3 ± 8.9	340.0 ± 16.8	
150 mg/kg	251.1 ± 10.3	237.0 ± 11.5**	30.3**
250 mg/kg	250.2 ± 9.7	175.4 ± 12.1***	48.4***
Metformin	252.2 ± 11.1	185.0 ± 12.0***	45.5***

Values are mean ± SEM, n=5, *p<0.01 and **p<0.001

metformin (100 mg/kg b.w.) on sucrose challenge in normoglycemic rats. The fasting blood glucose levels (mean ± SEM) of the test, standard drug and control groups were 67.5 ± 2.5, 67.3 ± 2.5, 67.6 ± 1.8 and 67.8 ± 2.8 mg/dl (Table 1) respectively. Two hours after administration of sucrose, the mean blood glucose concentrations of the *P. harmala* treated group (90.1 ± 2.2 and 82.6 ± 3.6 mg/dl) and the metformin treated group (84.2 ± 2.5 mg/dl), were significantly lower ($p<0.05$, $p<0.01$ and $p<0.01$) than that of the control group treated with 1% gum acacia (117.0 ± 3.9 mg/dl). The decrease in the blood glucose level at 2 hrs in the test group compared with that of the control was 22.9% and 29.4% whereas the decrease in the metformin treated group was 28.0%.

Effect of *P. harmala* on streptozotocin-induced diabetic rats: The effects of *P. harmala* ethanolic extract (150 and 250 mg/kg b.w.) and metformin (100 mg/kg b.w.) on sucrose challenged streptozotocin-induced diabetic rats. The fasting blood glucose levels (mean ± SEM) of the test, standard drug and control groups were 251.1 ± 10.3, 250.2 ± 9.7, 252.2 ± 11.2 and 251.3 ± 8.9 mg/dl (Table 2) respectively. Six hours after administration of sucrose, the mean blood glucose concentrations of the *P. harmala* treated group (237.0 ± 11.5 and 175.4 ± 12.1 mg/dl) and the metformin treated group (185.0 ± 12.0 mg/dl), were significantly lower ($p<0.01$, $p<0.001$ and $p<0.001$) than that of the control group treated with 1% gum acacia (340.0 ± 16.8 mg/dl). The decrease in the blood glucose level in the test group compared with that of the control was 30.3 % and 48.4% whereas the decrease in the standard drug treated group (metformin) was 45.5%, $p<0.001$.

DISCUSSION

Results of the present study clearly indicate that the ethanolic extract of *P. harmala* seed, significantly lowered ($p<0.001$) blood glucose level in normal and diabetic rats at variable dose levels (150 and 250 mg/kg body weight). The ethanolic

extract showed a significant improvement in their ability to utilize the external sucrose load. This effect is comparable to effect exerted by metformin. These preliminary data clearly show that an ethanolic extract of *P. harmala* is as effective as the known oral hypoglycaemic agent metformin in reducing the blood glucose concentration after a sucrose challenge in normal and streptozotocin-induced diabetic rats. But further studies are to be conducted to find out whether long term studies would bring the fasting blood glucose level to normal levels. Further studies on the mechanism of action whether it is an pancreatic insulin release or directly on absorption and utilization of glucose are underway.

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