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Study of the Hypoglycemic Activity of *Hedera helix* L. in Alloxan-induced Diabetic Rabbits

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The crude ethanolic extract of leaves of *Hedera helix* L. was orally administered to the alloxan-induced diabetic rabbits. Significant hypoglycemic activity ($p < 0.05$) was observed after 90 min. of the administration of the extract. The acute hypoglycemic activity was studied by the oral administration of the extract with a dose of 250 mg Kg⁻¹ body weight daily for seven days. It significantly decreased the concentration of glucose in blood ($P < 0.01$) without any visible physical-physiological changes. The oral administration of ethanolic extract has a beneficial effect on the diabetic state by lowering the blood glucose level through extra-pancreatic actions rather than by stimulated insulin release.

Key words: *Hedera helix* L., antidiabetic, rabbits

Introduction

Hedera helix L. (Araiaceae) is an evergreen woody climber widely distributed in Nepal, India, China and Pakistan (Stewart, 1972 and Bahijri *et al.*, 1984). The extract of this plant showed antifungal (Timon *et al.*, 1980) and antibacterial activities particularly against gram positive bacteria (Cioaca *et al.*, 1978). Saponins extracted from *Hedera helix* were found to be effective in mice infected with *Candida albicans* (Timon *et al.*, 1980).

Diabetes mellitus is characterized by hyperglycemia, glycosuria, polydipsia and ketosis, is caused by relatively or absolutely non-functioning of insulin activity. Many plants have been used for the treatment of the diabetes mellitus (Bahijri *et al.*, 1984). The hypoglycemic activity of some of these plants has been studied (Bahijri *et al.*, 1984 and Akhter and Iqbal, 1991).

Hedera helix is locally claimed to possess hypoglycemic properties and is used in the treatment of diabetes mellitus in the traditional system of medicine.

The aim of the present study was to evaluate the antidiabetic properties of the crude extract of the plant.

Materials and Methods

The study was carried out at the Department of Chemistry and Department of Pharmacy, University of Peshawar, during the months of October 1993 to February 1994.

Chemicals and instrumentation: Alloxan mono-hydrate ($C_4H_2N_2O_4 \cdot H_2O$), ethanol (C_2H_5OH) and methanol (CH_3OH) were obtained from BDH Laboratories (Pool England). Blood glucose level was measured using a kit method, Glucose GOD-PAP, (Thomas, 1992).

Hitachi U-2000, U.V. Visible Spectrophotometer was used to measure the absorption of samples at 450 nm in a 1 cm path length quartz cuvette.

Extraction in the second phase was carried out using a soxhlet apparatus. The extracts were evaporated under reduced pressure using a Rotavapor, Buchi RE 111.

Experimental animals: Healthy adult rabbits (*Oryctolagus cuniculus*) of either sex, weighing 1.0-1.6 kg were kept under observation for two weeks before and throughout the experiment, under the usual management conditions and fed with a normal diet (green vegetables). The rabbits were divided into group A, i.e. control (n = 10) and group B, i.e. alloxan treated group (n = 25). Animals in both groups were kept fasted overnight before the experiment.

Induction of diabetes in rabbits: Rabbits in group B were made diabetic artificially by the intravenous (i/v) administration of alloxan mono-hydrate (150 mg Kg^{-1} body weight) into the marginal ear vein. Blood samples were collected at zero time, just before injecting the drug. After eight days, the plasma glucose concentration of the surviving rabbits was determined. Animals with blood glucose levels above 140 mg dl^{-1} were considered to be diabetic. The blood samples were transferred to glass tubes containing ethylene diamine tetra-acetic acid (EDTA) and centrifuged at 3000 g for 10 min. The clear supernatant plasma was collected and assayed for glucose using the GOD-GAP method.

Plant material and extraction: The plant (leaves of *Hedera helix*) was collected from Murree (Punjab, Pakistan) in June-July 1993. The leaves were dried in the shade and pulverized to fine powder (120 g). Extraction in the first phase was carried out by maceration with ethanol (70 %) for one week. The ethanolic extract was filtered and the residue was re-extracted in the soxhlet extractor for eight hours using fresh solvent and then filtered. Both the filtrates were combined and evaporated under reduced pressure, a gummy extract (21 g) was obtained.

Preparation of the dose: The dose for each animal was calculated according to their body weights. The extract was then filled into

hard gelatin capsules (Size-2) and administered orally to the alloxan induced diabetic rabbits. The control-diabetic rabbits received only the empty shells of the hard gelatin capsules of the same size.

Precision and reproducibility of method: Precision of the method for determination of the blood glucose concentration was studied by measuring the plasma glucose level of five replicates of the same sample obtained from human blood. The reproducibility of the method was studied by measuring the blood glucose in five dilutions of the same blood sample.

Precision of the method for the analysis of the blood glucose concentration was determined by analyzing the five replicates of the same sample of human plasma and the reproducibility was studied on five separate analysis of the same sample of human plasma. Variation between the results were calculated using the equation, $\delta/M \times 100$, where δ is standard deviation ($\pm$ SD) and M is mean.

Statistical analysis: Data were statistically analyzed by using the paired t-test at 95% confidence interval. All the statistical calculations were conducted with the help of Minitab™.

Results and Discussion

Precision of the method: The low values of relative standard deviation (RSD) for the precision and repeatability i.e. 3.7 and 4.1 %, respectively showed that method is suitable for the analysis of glucose concentration in plasma.

Table 1: Mean ($\pm$ SD) weight (g) and blood glucose concentration (mg dl^{-1}) of rabbits before and eight days after the administration of the alloxan monohydrate (150 mg Kg^{-1} ; body weight)

	Controlled Group (A)		Alloxan treated Group (B)	
	Day 1	Day 8	Day 1	Day 8
Blood glucose	81.4 (8.6)	80.6 (6.8)	86.0 (9.2)	170.8** (15.8)
Weight	1563.0 (209.4)	1561.4 (205.0)	1550.0 (131.2)	1495.0 (113.7)

** : $P < 0.001$, Figures in parentheses represent 95% confidence interval

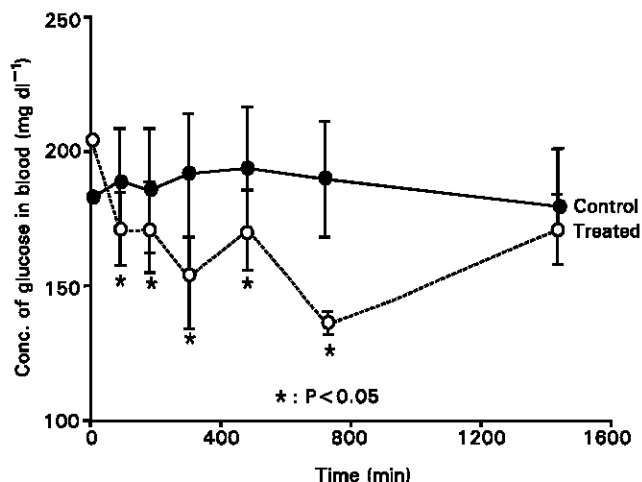


Fig. 1: Mean ($\pm$ SD) concentration of glucose in plasma of alloxan-induced diabetic, controlled and treated rabbits

Antidiabetic properties: Administration of alloxan (150 mg kg^{-1} body weight) to the rabbits in group-B showed the significant increase in blood glucose concentration ($P < 0.001$). While in control group the changes in the blood glucose level were not observed (Table 1). The elevation of blood glucose level was accompanied with the loss of weight of the animals that was not

significant ($P < 0.05$).

The crude extract (250 mg Kg^{-1} body weight) significantly ($P < 0.05$) reduced the blood glucose level after 90 min. of its administration compared with zero time level (Fig. 1). However a slight increase in blood glucose was observed between 300 and 480 min. post administration of the crude extract but still it was significantly lower ($P < 0.05$) compared with the zero time level. The significantly low blood glucose level, after administration was consistently maintained for about 720 min.

This study showed that the crude ethanolic extract of the *Hedera helix* significantly and consistently decreases the blood glucose concentration in alloxan induced diabetic rabbits for about 720 min (Fig. 1).

The acute hypoglycemic and possible visible physical-physiological change were studied by the oral administration of the same crude extract (250 mg Kg^{-1} body weight) to the diabetic rabbits ($n = 5$) for seven days. The animals were closely observed for about 8 hours a day throughout the study. All rabbits were active and no any visible sign of restlessness, diarrhoea or convulsions were observed.

Alloxan causes the selective degeneration of β -cells of the islets of the langerhans and induce diabetes mellitus (Bowman and Rand, 1980). The extract of *Myrcia uniflora* showed antidiabetic activities in streptozotocin diabetic rats mainly by improving the metabolic parameters of glucose homeostasis (Pepto *et al.*, 1993). The oral administration of Quei Fu DHW, a Chinese herbal mixture, decreases the plasma glucose level in diabetic rats lacking insuline (Cheng *et al.*, 2001). The blood glucose lowering effect of *Nigella sativa* oil in streptozotocin diabetic rats may be mediated by the extra-pancreatic action rather than stimulated insulin release (El-Dakhakhny *et al.*, 2002). Drugs like acetohexamide produce hypoglycemia by stimulating the release of insulin from the β -cells of the pancreas (Goth, 1985 and Larner, 1995). The blood glucose lowering effect of the ethanolic extract of *Hedera helix*, however, not paralld by the a stimulation of insulin release, as indicated by the other similar studies, and it may be mediated by the extra pancreatic action rather than by stimulation of insuline release.

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