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HYPOGLYCEMIC ACTIVITY OF *HEDERA HELIX* L. LEAVES AND THE POSSIBLE MECHANISM OF ACTION

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Abstract

The present study showed that both the aqueous and methanolic extracts of *Hedera helix* L. were hypoglycemic, reducing the blood glucose level in both the normal and alloxan-induced diabetic rabbits to significant levels. Trace element analysis of the leaves showed that *Hedera helix* L. leaves contain the *"hypoglycemic trace elements"* (Chromium, Manganese & Zinc) in sufficiently large amounts and these have played the main role in reducing the blood glucose level.

Introduction

Diabetes mellitus is a serious incurable, chronic disease that carries a high risk for a considerably short life span, renal failure, and disability, complications including blindness, imputation of organs, sexual impotence and early cardiovascular complications. Diabetes is one of the most prevalent diseases affecting mankind all over the world. In Mexicans and Anglo Americans, the prevalence of the disease is 12 and 3 per cent respectively (Rosental, 1984), while in India the prevalence of the disease is 18 per cent (Ahuja, 1978). In Pakistan, diabetes mellitus is common in both the sexes between 45-50 years, with slightly higher figures for females (Samad, 1992). Of the two main types of diabetes *i.e.* diabetes insipidus and diabetes mellitus, the latter one is the most common type usually occurring in the middle aged persons, mostly due to obesity and over- eating of rich caloric diet (Galloway *et al.*, 1988).

There are large numbers of indigenous medicinal plants, which are used as antidiabetic drugs. Akhtar (1992) has reviewed 26 indigenous plants for their hypoglycemic effect and observed that most of them possess hypoglycemic principles. *Hedera helix* L. is an important medicinal plant, growing wild in Kurrum, Swat, Hazara, Murree hills and Kashmir at 5-9 thousand feet (Nasir & Ali, 1975). In Swat, it is used as a folk drug for the treatment of diabetes mellitus. In the present study the hypoglycemic effect of both the aqueous and methanolic extracts of *H. helix* on normal and alloxan–induced diabetic rabbits were studied and the leaves were analyzed for the hypoglycemic trace elements.

Materials and Methods

Plant material: Leaves of *Hedera helix* were collected from Baragali, Abbottabad late in September (beginning of the blooming period). The leaves were washed with water to remove the adhering dirt and then dried in shade. The dried leaves were garbled, petioles being removed and grinned to a fine powder.

Preparation of aqueous and methanolic extracts: Aqueous extract was prepared by soaking 200 g of the powdered leaves in 1L distilled water for seven days at room

temperature, after which it was filtered and the filtrate was concentrated through rotary evaporator under vacuum to a semisolid mass (40.00 g). The methonolic extract was prepared using the same procedure and after removing the methanol under vacuum, 48.42 g of a semisolid mass was obtained.

Experimental animals: The 42 healthy rabbits (*Oryctolagus cuniculus*) of local strain weighing 1000-1500 g were kept under observation for one week before experimentation under usual management condition and fed with normal diet consisting of green grass during this period. The rabbits were then randomly divided into following three groups:

Group-I: 6 rabbits, control group
Group-II: 18 rabbits, normal, non-diabetic group. This group is further subdivided into following:
Group-IIa: 6 rabbits, tested for the aqueous extract.
Group-IIb: 6 rabbits, tested for the methanolic extract.
Group-IIc: 6 rabbits, tested for acetohexamide (Dimerol®), an oral hypoglycemic drug.
Group-III: 18 rabbits, alloxan-induced diabetic group. This group is also further subdivided into three groups:
Group-IIIa: 6 rabbits, tested for the aqueous extract.
Group-IIIb: 6 rabbits, tested for the methanolic extract.
Group-IIIc: 6 rabbits, tested for metformin (Glucophage®), an oral hypoglycemic drug.

The rabbits of group III were made diabetic by injecting alloxan monohydrate 150 mg/kg body weight in the marginal ear vein (Butt, 1962), one week before the blood testing. Rabbits with 250-400 mg/dl blood glucose level were considered as diabetic. The rabbits of group I were given 20 mL of 2 % gum tragacanth solution to serve as control, while the rabbits of group IIa and IIb received aqueous and methanolic extracts respectively at a dose equivalent to 4 g of powdered leaf per kg body weight in 20 mL of 2 % gum tragacanth solution. Rabbits of group IIc received acetohexamide, in 20 mL of 2 % gum tragacanth solution at a dose of 500 mg/kg-body weight. The rabbits of group IIIa and group IIIb received the same dose as that of IIa and IIb respectively, while the rabbits of group IIIc were given mitformin in a dose similar to the of IIc.

The blood glucose level of all the rabbits was recorded with the help of Accu-Check easy blood glucose monitoring glucometer (Cat. No. 00560, Boehringer Mannheim Corp., USA) at 0, 2, 4, 8, 12 & 24 hour time intervals.The hypoglycemic trace elements (Chromium, Manganese and Zinc) were determined by Wet digestion procedure and by Dry ashing (Issac & Johnson, 1975), using flame photometry, SP 1991 PYE UNICAM Atomic Absorption Spectrophotometer.

Results and Discussion

Both the aqueous and methanolic extracts of *Hedera helix* showed hypoglycemic effects. In case of normal rabbits (Table I), the aqueous extract started its hypoglycemic effect at 4 hours' time interval, and remained highly significant ($P<0.005$) at 8 and 12 hours' time intervals, whereas the hypoglycemic effect of methanolic extract started at 8 hours' time interval, but it was not as significant at 12 hours' time interval as that of the aqueous extract. The hypoglycemic drug, acetohexamide exhibited its effect ($P<0.005$) at 2 hours' time interval and remained up to 12 hours' time interval. In the alloxan-induced diabetic rabbits,

the hypoglycemic effect of both the aqueous and methanolic extracts appeared at 8 hours' time interval and sustained up to 12 hours' time interval (Table 2). But as compared to the methanolic extract ($P<0.05$), the aqueous extract exhibited highly significant effect ($P<0.005$) at these time intervals. The hypoglycemic dug, metformine exhibited its effect from 2 to 12 hours' time ($P<0.005$) intervals.

Table 1. Data of blood glucose level (BGL) of normal rabbits group-I (control) received 2 % gum tragacanth solution and group-IIa, group-IIb & group-IIc, received aqueous and methanolic extracts equivalent to 4 g/kg body weight of powered leaves of *Hedera helix* and acetohexamide, 500 mg/kg body weight in 2 % gum tragacanth solution, respectively.

Time Int. (hrs)	Group-I (Control)	Group-IIa Aqueous extract	Group-IIb Methanolic extract	Group-IIC Acetohexamide
0	94.17±0.60	92.00±0.57	91.83±0.87	92.16±0.83
2	94.00±0.58	90.00±0.57	91.00±0.51	79.66±1.76**
4	94.33±0.81	88.33±0.92*	90.83±0.80	76.50±1.82**
8	93.50±0.67	82.66±0.84**	87.83±0.79**	81.00±0.85**
12	91.67±0.50	83.33±1.12**	90.66±0.33*	87.16±0.75**
24	91.33±0.56	92.33±0.84	93.00±0.68	92.00±0.57

*Significant decrease as compared to zero hour level ($P<0.05$)

**Highly significant decrease as compared to zero hour level ($P<0.005$). Number of animals for each observation = 6

Table 2. data of blood glucose level (BGL) of alloxan induced diabetic rabbits (group-IIIa, group-IIIb & group IIIc) received aqueous and methanolic extracts equivalent to 4 g/kg body weight of powered leaves of *Hedera helix* and metformin 500 mg/kg body weight in 2 % gum tragacanth solution, respectively.

Time Int. (hrs)	Group-IIIa Aqueous extract	GROUP-IIIb Methanolic extract	Group-IIIC Metformin
0	257.17±3.14	260.50±2.86	261.50±2.40
2	255.00±2.61	260.00±2.59	238.00±2.83**
4	251.66±2.26	259.00±2.92	225.67±1.57**
8	245.66±2.15**	251.50±3.25*	217.83±3.63**
12	242.80±2.09**	254.16±2.24*	245.17±3.50**
24	262.50±5.33	262.00±2.45	262.17±2.80

*Significant decrease as compared to zero hour level ($P<0.05$)

**Highly significant decrease as compared to zero hour level ($P<0.005$). Number of animals for each observation = 6

The results (Tables 1 & 2) show that both the aqueous and methanolic extracts of *Hedera helix* are hypoglycemic, but the former is more effective than the latter. This might be due to the presence of fatty and colouring materials in the methanolic extract, leading to dose differences of the two extracts. It is evident from table 2, that at 8 and 12 hours' time interval the aqueous extract is as effective ($P<0.005$) as the oral hypoglycemic drug, Metformin, proving the efficacy of this plant as an antidiabetic drug in the folk medicine.

Table 3. Data of the trace elements present in the powdered leaf of *Hedera helix*.

Trace Elements	Concentration (ppm*)
Chromium (Cr+++)	15.50
Zinc (Zn++)	49.75
Manganese (Mn++)	53.25

* Average of the Wet Digestion and Dry Ashing methods.

Atomic Absorption Spectrophotometer analysis of *Hedera helix* leaves (Table 3) showed the presence of the trace elements (Cr, Mn and Zn), in sufficient amounts. These elements are known as "HYPOGLYCEMIC ELEMENTS" (Donsbach & Ayne, 1982), because these have a very important role in glucose metabolism. Chromium is a vital component of an organo-chromium complex, known as "GLUCOSE TOLERANCE FACTOR" (GFT), which potentiates the action of insulin through facilitating the attachment of insulin to cell membranes and ultimately enhances the uptake of glucose by the cells (Robenson & Hurly, 1985; Uusitupa, 1983). The deficiency of this complex in the body, due to lack of chromium or reduced ability to synthesize the specific complex, results in glucose intolerance and reduced efficiency of insulin. This fact is supported by the studies of Ottaway & Fell (1986) who demonstrated that intravenous supplementation of fluid with 150 mg chromium per day revised sever glucose intolerance, improved nitrogen balance and weight gain. Similarly the findings of Schorder & Balassa (1968), Mertz (1969) and Masironi & Mertz (1973) indicated that a severe chromium depletion led to fasting hyperglycemia, glucosuria and impaired growth rates in rats suggests an important role of chromium in glucose metabolism.

Zinc, which activates some 67 enzymes in the body (Schorder 1976) also plays an important role in glucose metabolism. Hurber and Gershoff (1973), Kirchagessner *et al.* (1976) and Wolman (1979) have reported that Zinc depletion leads to abnormalities in glucose utilization. Manganese also plays some important role in glucose metabolism. Donbach & Ayne (1982) have demonstrated that in the absence of manganese adequate diet the body does not utilize glucose.The hypoglycemic activity of *Hedera helix* might be due to the availability of the hypoglycemic trace elements in sufficient amounts and in proper form to certain enzymatic processes of the body which have either direct or indirect effect on glucose metabolism.

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