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In vivo antidiabetic and antioxidant potential of *Helichrysum plicatum* ssp. *plicatum* capitulums in streptozotocin-induced-diabetic rats

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Abstract

Helichrysum species (Asteraceae) are widely found in Anatolia. Decoction prepared from the capitulums of *Helichrysum plicatum* ssp. *plicatum* is used to alleviate the symptoms of diabetes mellitus in folk medicine. In the present study, the hypoglycaemic and antioxidant potential of *Helichrysum plicatum* ssp. *plicatum* was evaluated by using in vivo methods in normal and streptozotocin-induced-diabetic rats. After the oral administration of water and ethanolic extracts at doses of 500 mg/kg body weight prepared from the capitulums of plant, blood glucose levels were monitored at specific intervals. Tolbutamide was used as a reference drug at a dose of 100 mg/kg. The experimental data indicated that water and ethanol extracts of capitulums demonstrate significant antihyperglycaemic and antioxidant activity in streptozotocin-induced rats which confirmed the folkloric utilization. In order to assess the role of polyphenolic components in the relevant activity, phenolic and flavonoid contents of each extract were also determined in terms of total phenols: 113.5 ± 8.6 mg (gallic acid equivalent/1 g extract) and total flavanoids 50.5 ± 1.9 mg (quercetin equivalent/1 g extract) for ethanol extract, total phenols: 75.9 ± 3.7, flavonoids: 31.5 ± 2.3 for water extract using Folin-Ciocalteu reagent.

Keywords: *Helichrysum plicatum* ssp. *plicatum*; Asteraceae; Antidiabetic; Antioxidant; Hypoglycaemic

1. Introduction

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterised by hyperglycaemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin (Alberti and Zimmet, 1998). The number of people in the world with diabetes has increased dramatically over recent years. Indeed, by 2010 it has been estimated that the diabetic population will increase to 221 million around the world (Carter, 2004). The Diabetes Control and Complications Trial (DCCT) Research Group (1993) stated that tight control of blood glucose is an effective strategy in reducing clinical complications of diabetes mellitus significantly, but even optimal control of blood glucose could not prevent complications suggesting that alternative treatment approaches are needed.

Ethnobotanical field studies in Turkey revealed that a number of plant remedies were used to alleviate the symptoms of diabetes (Sezik et al., 1991; Tabata et al., 1993, 1994; Yesilada et al., 1993, 1995). However, only a few have been evaluated scientifically to confirm the claimed activity (Deliorman Orhan et al., 2005; Sezik et al., 2005).

The genus *Helichrysum* (Asteraceae) is represented by 20 species in Turkey and decoction prepared from various species have been widely used as folk remedy in Turkish folk medicine as diuretic, lithagogue and for stomachache (Fujita et al., 1995; Sezik et al., 1991, 2001). The capitulums of *Helichrysum plicatum* ssp. *plicatum* are also suggested to alleviate the symptoms of diabetes mellitus by herbalists in Turkey (Baser et al., 1986). However, the antidiabetic effect of any *Helichrysum* species growing in Anatolia have not been studied so far. The objective of this study is to evaluate hypoglycaemic, antidiabetic and

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antioxidant effects of capitulums of *Helichrysum plicatum* ssp. *plicatum*.

2. Materials and methods

2.1. Plant material

H. plicatum ssp. *plicatum* DC. (abbreviated as HPP) was collected in July 2003 from Palandöken Mountain (Erzurum, Turkey) and identified by one of the authors (MA). A specimen was deposited at Herbarium of Pharmacy Faculty of Gazi University (GUE 2355).

2.2. Preparation of the test samples

A 3% infusion prepared from the capitulum of plant is used as herbal tea to alleviate diabetic symptoms in Anatolia and 100 ml of water extract is drunk three times a day before meals. Accordingly, the capitulums were removed (100 g) and extracted with either distilled hot water (1000 ml) or ethanol 80% (1000 ml) on the shaker for 24 h at room temperature. Extracts were filtered and evaporated to dryness in a rotary evaporator to yield water (yield: 17.5%) and ethanolic (yield: 19.3%) extracts. A weighed portion of each extract (500 mg/kg, equivalent to 3 g crude drug) was suspended in 0.5% aqueous carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals (5 ml/kg, b.w.) [b.w.: body weight]. Animals in the control group received only the vehicle (5 ml/kg, b.w.). Tolbutamide (100 mg/kg, b.w.) was used as the reference drug.

2.3. Animals

Male Wistar-albino rats weighing 150–200 g, purchased from the Animal house of Gülhane Military Medical Academy (Ankara, Turkey), were used in the present study. All rats were kept at room temperature of 22 °C in the animal room of the Department of Pharmacognosy. Throughout the animal experiments were processed following the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food for 1 week in order to adapt to the laboratory conditions. Sixteen hours before the experiments, they were fasted overnight, but allowed free access to water. Seventy-two rats, included for the study, were divided into 12 groups, each consisting of 6 animals. The body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. Rats were divided into the following groups:

- *Group 1*: Normoglycemic control. Received only vehicle (0.5% CMC) (5 ml/kg)
- *Group 2*: Normoglycemic reference. Tolbutamide was given at a dose of 100 mg/kg
- *Group 3*: Normoglycemic. Water extract of HPP was given at a dose of 500 mg/kg
- *Group 4*: Normoglycemic. EtOH extract of HPP was given at a dose of 500 mg/kg
- *Group 5*: Diabetic control. Received only vehicle (0.5% CMC) (5 ml/kg)
- *Group 6*: Diabetic reference. Tolbutamide was given at a dose of 100 mg/kg
- *Group 7*: Diabetic. Water extract of HPP was given at a dose of 500 mg/kg
- *Group 8*: Diabetic. EtOH extract of HPP was given at a dose of 500 mg/kg
- *Group 9*: Diabetic control. Received only vehicle (0.5% CMC) (5 ml/kg) once a day throughout 8 days.
- *Group 10*: Diabetic reference. Tolbutamide was given once a day throughout 8 days at a dose of 100 mg/kg.
- *Group 11*: Diabetic. Water extract of HPP was given once a day throughout 8 days at a dose of 500 mg/kg.
- *Group 12*: Diabetic. EtOH extract of HPP was given once a day throughout 8 days at a dose of 500 mg/kg.

2.4. Determination of the blood glucose levels

Blood glucose concentration (mg/100 ml) was determined using an Ascensia-Elite commercial test (Bayer), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns.

2.5. Study on normoglycaemic and glucose-hyperglycaemic rats [NG-OGTT]

A combined methodology of Kato and Miura (1993) is preferred for the activity assessment of extracts and fractions in order to avoid wasting animals; there are some modifications incorporated in the time pattern for blood glucose level determination (Wohaieb and Godin, 1987). After overnight fasting (16 h) the blood glucose level of rats were determined and then were given test samples.

Test samples were given immediately after the collection of initial blood samples. The blood glucose levels were determined in the following time pattern: 30 and 60 min to assess the effect of the test samples on normoglycemic animals. The rats were then loaded orally with 2 g/kg glucose and the blood glucose concentrations were determined at 60, 90 and 210 min after the glucose load.

2.6. Study on diabetic rats (non-insulin dependent diabetes model—NIDDM)

2.6.1. Induction of diabetes

Diabetes was induced in rats by the intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 55 mg/kg b.w. dissolved in distilled water (1 ml/kg b.w.). Seven days after the injection, the blood glucose levels were measured. Each animal with a blood glucose concentration level above 250 mg/dl was considered to be diabetic and used in the experiments. To prevent the hypoglycemia which occurred during the first 24 h following the STZ administration, 5% glucose solution was orally given to the diabetic rats. In all experiments, rats were fasted for 16 h prior to STZ injection.