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Evaluation of antidiabetic and antihyperlipidemic activity of *Artemisia indica linn* (aeriel parts) in Streptozotocin induced diabetic rats

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ABSTRACT

Ethnopharmacological relevance: Diabetes mellitus is a major metabolic disorder affecting a huge population all over the world. *Artemisia* species have been extensively used for the management of diabetes in folkloric medicine. The present study is designed to investigate the antidiabetic and antihyperlipidemic effects of aeriel parts of *Artemisia indica*.

Materials and methods: Hydromethanolic crude extracts, chloroform, ethyl acetate and n-butanol fractions of aerial parts of *Artemisia indica* were tested for their antidiabetic potential in Streptozotocin (STZ) (50 mg/kg, *i.p.*) induced diabetic *Sprague-Dawley* rats. Blood glucose level, body weight, serum lipid profile and activities of liver enzymes were determined. The extracts were further subjected to preliminary phytochemical analysis.

Results: A daily oral dose of hydromethanolic crude extracts (200 and 400 mg/kg b.w.) and chloroform fraction (200 mg/kg b.w.) of *Artemisia indica* for 15 days showed a significant reduction in blood glucose level which was comparable to that of the standard antidiabetic drug, glibenclamide (500 μg/kg, p.o.). *Artemisia indica* extracts also showed reduction in total cholesterol, triglycerides and low density lipoproteins as well as serum creatinine level, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) in diabetic rats.

Conclusion: According to the results *Artemisia indica* possesses hypoglycemic, antihyperlipidemic and valuable effects on liver and renal functions in diabetic rats, which seems to validate its traditional usage.

1. Introduction

Diabetes mellitus is one of the most severe and incurable metabolic disorders characterized by increased blood glucose level as a result of an absolute or relative lack of insulin and failure of insulin to act on its targets tissue (Valiathan, 1998). The acute and chronic manifestations of hyperglycemia cause microvascular (retinopathy, neuropathy and nephropathy) and macrovascular complications (Coronary artery disease leading to myocardial infarction, diabetic ketoacidosis, nonketotic hyperosmolar coma and diabetic coma; Fowler, 2008). It is a leading factor of multiple disorders due to chronic hyperglycemia and causes abnormalities in glucose, lipids and protein metabolism (Kahn et al., 1976). India, China and United States are the largest countries presenting more than 30 million diabetic people and the incidence is increasing day by day (Wild et al., 2004). Several workers described the treatment of diabetes mellitus based on the interaction of nutritional agents. These agents limit the availability of fatty acids, inhibit gluconeogenesis (Moneva and Dagogo-Jack, 2003) and enhance lipolysis (Bebernitz and Schuster, 2002). Furthermore, they promote fatty acid oxidation in a futile cycle that does not yield metabolic energy and stimulate peripheral glucose utilization (Bebernitz and Schuster, 2002).

More than 800 plants have been reported to have antihyperglycemic effects with less adverse effects and low toxicity as compared to synthetic compounds (Kirithikar and Basu, 1995; Nadkarni, 1976). According to the World Health Organization (WHO), almost 70% of the diabetic patients use plants as a primary source of antidiabetic agents in order to satisfy their principal health needs (Bailey and Day, 1989). *Artemisia indica* also known as “mugwort” belongs to family Astereaceae. The plant is a perennial shrub with a height of 2–8 m and is found in northe rn areas of Pakistan, as well as in cold temperate zones of Asia. *Artemisia* species have been reported to exhibit antiplasmodial (Tran et al., 2003), antispasmodic and bronchodilator (Khan and Gilani, 2009), antihypertensive (Tigno et al., 2000), antiallergic

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(Rodrigues-Alves et al., 2008), hepato-protective (Gilani et al., 2005), antibacterial (Chen et al., 1989) and antinociceptive effects (Pires et al., 2009). Furthermore, various species of *Artemisia* have been traditionally used for their antihyperglycemic effects in Mexico (Andrade-Cetto and Heinrich, 2005). Therefore, keeping in view the medicinal importance of the plant and extensive folkloric use in rural Mexican communities as an antidiabetic plant, this study was planned to explore the antidiabetic activity of *Artemisia indica* on scientific grounds.

2. Materials and methods

2.1. Chemicals

Streptozotocin (Sigma-Aldrich), glibenclamide (Sanofi Aventis Pharma (Pvt.) Ltd., Pakistan) and glucose estimation kits (S.D Chek Gold Germany) were used in this study. The different organic solvents and chemicals used for extraction were purchased from local suppliers of Merck, Germany. Other reagents used in this study were Tween-80 (Scharlau chem., Spain), normal saline (Utsoka Pharma (Pvt.) Ltd., Pakistan), biochemical reagents for lipid profile, LFTs Kits (Human, Germany) and RFTs kit (Bioneed Germany diagnostic).

2.2. Animals

Adult *Sprague-Dawley* rats in the weight range of 150–200 g were purchased from the Department of Pharmacy, University of Peshawar. Animals were housed in the Department's animal house with fresh water and standard food available ad libitum. The animals were maintained at 12 h light and dark cycles and with room temperature maintained at 22–25 °C in the animal house. All animal procedures have been approved by the Departmental Animal Ethical Committee (DAEC/PHARM/2012/10) and were conducted according to the UK Animal Scientific Procedure Act, 1986.

2.3. Plant material

The aerial parts of the plant were collected from upper Dir (Sherin Gal) Khyber Pakhtunkhwa, Pakistan, in the month of July 2008 and were authenticated by a taxonomist. The plant specimen was deposited in the Department of Pharmacy, University of Malakand, Pakistan. A voucher specimen number (200300106) was assigned at the same institution.

2.4. Preparation of extracts

The shade dried aerial parts of the plant were coarsely ground with the help of a dry grinder followed by extraction with 70% methanol (three times) and filtered through a piece of cloth (plant material to solvent ratio was 1:5 w/v). The extract was concentrated to a semisolid mass using a rotary evaporator under reduced pressure at 45 °C for a final extract yield of 20% (w/w). The semisolid mass was fractionated with various organic solvents: ethyl acetate, chloroform and n-butanol. Each fraction was finally evaporated to semisolid masses by the rotary evaporator. The yields of ethyl acetate, chloroform and n-butanol were 3.8%, 5.5% and 4.3% w/w respectively. For antidiabetic activity, the extract and its fractions were formulated as suspensions in normal saline with 5% Tween-80 as the suspending agent.

2.5. Preliminary phytochemical tests

The crude methanolic extract, chloroform and ethyl acetate fractions of *Artemisia indica* were subjected to qualitative tests for identification of different constituents like alkaloids, flavonoids, terpenoids, glycosides, saponins and tannins by using standard qualitative methods described by Trease and Evans (1983).

2.6. Acute toxicity study of the crude methanolic extract of Artemisia indica

The acute toxicity of the crude methanolic extract of *Artemisia indica* was determined by using *Sprague-Dawley* rats (150–200 g), according to the method described by Irwin (1968) and described elsewhere (Karim et al., 2012). The animals were divided into six groups ($n=6$). One group served as a control and received Tween-80 suspension orally. The crude methanolic extract was given in a dose of 250, 500, 1000, 1500 and 2000 mg/kg b.w. to each rat orally. All the doses of the extracts were prepared by dissolving the extract in Tween-80 suspension prior to administration. The animals were observed at 0, 30 and 60 min, 24, 48 and 72 h and 1 week after administration for any kind of behavioral, physical and pharmacological toxic effects, respectively. Since the extract was found safe up to the dose level of 2000 mg/kg b.w., a dose of 200 mg/kg b.w. (1/10 of 2000 mg/kg b.w.) of the extracts was selected according to OECD guidelines for screening of the antidiabetic activity (Sharma et al., 2010).

2.7. Induction of hyperglycemia

Hyperglycemia was induced in *Sprague-Dawley* rats by a single intra peritoneal (*i.p.*) injection of 50 mg/kg of Streptozotocin (STZ) reconstituted in normal saline (0.9%) after overnight fasting. After 72 h of STZ administration, blood glucose levels were measured in blood samples collected from tail vein puncture with one touch Glucometer strips using an SD glucometer (Germany). Rats with fasting blood glucose levels more than 300 mg/dl were considered diabetic and selected for the study.

2.8. Experimental design

Rats that fasted overnight for 12 h were randomly divided into six groups (eight rats in each group). The first group served as normal control (non-diabetic) and received normal saline whereas the second group served as diabetic control and received 5% Tween-80 suspension. The third group received standard drug glibenclamide (500 μg/kg, p.o.). The fourth and fifth groups received hydromethanolic extracts of *Artemisia indica* at dose levels of 200 and 400 mg/kg (p.o.) respectively, whereas the sixth, seventh and eighth groups received chloroform, ethyl acetate and n-butanol fractions of *Artemisia indica* respectively, at a dose of 200 mg/kg orally.

The treatment of crude extract and fractions was continued once daily at 09:00 a.m. for 15 days. Body weight and blood glucose levels were estimated on the 0th, 4th, 7th, 10th and 15th day of treatment (Gupta et al., 2004).

2.9. Estimation of serum lipid and liver physiological profile

After completion of antidiabetic assay on the 15th day, all animals were anesthetized by pentobarbital sodium (35 mg/kg) and euthanized by cervical decapitation using the method described in schedule 1 of the Animal Scientific Procedure Act 1986 and blood samples were collected through cardiac puncture for biochemical parameters studies (Nagappa et al., 2003). Collected blood was centrifuged at 1500*g* for 10 min for serum separation. The serum sample was then analyzed by a spectrophotometer (Perkin-Elmer, Germany) for determination of serum SGPT, serum SGOT and serum alkaline phosphatase (ALP) using a standard IFCC kinetic Method (Bioneed kit, Germany). Total cholesterol (TC), triglycerides (TGs), low