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Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions

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ABSTRACT

We investigated the antidiabetic activity of 50% ethanolic extract of roots of *Ricinus communis* (RCRE) along with its bioassay-guided purification. Five-hundred milligram per kilogram body weight appeared to be the effective dose as it caused the maximum lowering of the fasting blood glucose, both in normal as well as type 1 diabetic animals. The maximum hypoglycemic effect was always observed at the 8th h up to which the study has been conducted.

Administration of the effective dose of RCRE to the diabetic rats for 20 days showed favorable effects not only on fasting blood glucose, but also on total lipid profile and liver and kidney functions on 10th and 20th day. RCRE was purified using silica gel column chromatography. Out of several different fractions tested, only one fraction (R-18) showed significant antihyperglycemic activity. RCRE seemed to have a high margin of safety as no mortality and no statistically significant difference in alkaline phosphatase, serum bilirubin, creatinine, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and total protein was observed even after the administration of the extract at a dose of 10 g/kg b.wt. Thus *R. communis* seems to have a promising value for the development of a potent phytomedicine for diabetes.

1. Introduction

Insulin-dependent diabetes mellitus or type 1 diabetes is an autoimmune disorder caused by destruction of insulin producing β-cells when auto aggressive T-lymphocytes infiltrate the pancreas. This leads to hypoinsulinaemia and thus hyperglycemia (Bach, 1995). Hyperglycemic condition causes increased glycosylation leading to biochemical and morphological abnormalities due to altered protein structure which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy (Arky, 1982).

Traditional medicines derived mainly from plants play major role in the management of diabetes mellitus (Ahmed et al., 2004; Karunanayake and Tennekoon, 1993; Patel and Srinivasan, 1997). World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy (Day, 1998). Recently, Mankil et al. (2006) have reviewed many medicinal plants possessing experimental and clinical antidiabetic activity that have been used in traditional systems of medicine. The present work was undertaken to explore the antidiabetic potential of a plant, *Ricinus communis* in type 1 diabetic animals.

R. communis L. is called as Erandah in Sanskrit, Amudam in Telugu and Erandi or Arandi in Hindi and is also known as castor oil bean, castor oil plant and wonder tree. It is a member of family Euphorbiaceae, cultivated all over India for its seed oil. Castor beans have been used in classical Egyptian and Greek medicine and their use has been described in the Susruta and Ayurveda as early as sixth century B.C. (Olsnes et al., 1976). The use of different parts of this plant for the treatment of various diseases in traditional or folk remedies throughout the world has been reviewed (Scarpa and Guerci, 1982). The root of this plant is also useful as an ingredient of various prescriptions for nervous diseases and rheumatic affections such as lumbago, pleurodynia and sciatica (Nandkarni, 1954). In the Indian system of medicine, the leaf, root and seed oil of this plant have been used for the treatment of inflammation and liver disorders (Kirtikar and Basu, 1991) as they have been found to be hepatoprotective (Yanfg et al., 1987; Visen et al., 1992), laxative (Capasso et al., 1994) and diuretic (Abraham et al., 1986). The antifertility activity of 50% ethanolic extract of *R. communis* has also been reported (Sandhyakumary et al., 2003). Roots and aerial parts are useful in the treatment of diabetes (Pullaiah and Naidu, 2003). Fifty percent of ethanolic extract of the root, stem and leaves of this plant showed hypoglycemic

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activity (Dhar et al., 1968). Except the initial report of Dhar et al. (1968), there is no report of systematic investigation of antidiabetic activity of *R. communis*. Fifty percent of ethanolic extract of roots of *R. communis* (RCRE) had shown significant hypoglycemic activity in normal animals and antihyperglycemic activity in diabetic animals in our initial screening studies. Thus, the aim of the present study was to perform detailed studies on the antidiabetic activity of RCRE and further to purify it.

2. Materials and methods

2.1. Reagents

Chemicals and reagents used in the present studies were of analytical grade, purchased from Sigma Chemical Co., USA, E Merck, Germany, Fine Chemicals, Fluka Chemicals, Lancaster, Spectrochem and SD Fine Chemicals, India. Organic solvents were freshly distilled prior to use. Thin layer chromatography (TLC) was carried out on commercially available flexible TLC silica gel (silica gel 60, F254) plates and compounds were visualized using short wave ultraviolet light. Alloxan monohydrate was purchased from Sigma–Aldrich Co., USA. One touch-glucometer (Accu-check sensor) of Roche Diagnostics, Germany and Uristix purchased from Bayer Diagnostics India Ltd. was used. Insulin from Abbot India was used. Insulin level of animals was measured using Rat Insulin Enzyme Linked Immunosorbent Assay (ELISA) kit form DRG Diagnostics, GmbH Germany. Total cholesterol (TC), high density lipoprotein (HDL) cholesterol, triglyceride (TG), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALKP), serum bilirubin (BIL), blood urea nitrogen (BUN) and creatinine (CRTN) was assayed using kits from Bayer Diagnostics-India Ltd. Total protein (TPR) was estimated using the Bradford kit purchased from Bangalore Genei. Roots of *R. communis* were collected from places nearby the campus of University of Delhi, Delhi.

2.2. Animals

Six to eight week old male Wistar rats (weighing 180 ± 20 g) obtained from Animal Research Facility of Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India were used. The animals were kept at 25–30° C and 45–55% relative humidity, acclimatized with free access to food (Golden feed, Delhi, India) and water ad libitum for 1 week under 12 h light, 12 h dark cycle. All animals were carefully monitored and all the experimental work with the animals was carried out after obtaining approval from the Institutional Animal Ethical Committee. For experimental purpose, animals were kept fasting overnight but had free access to water.

2.3. Preparation of 50% ethanolic extract

Roots of *R. communis* (2.5 kg) were washed with distilled water and shade dried. They were then cut into small pieces and ground in a grinder to be obtained in a powder form. The powder was extracted with 50% ethanol using a mechanical stirrer till exhaustion. The extract was filtered with Whatman filter paper (type 4) and the filtrate was concentrated under reduced pressure on rotavapor (BÜCHI, R-3000, Switzerland) at 40 °C temperature. The concentrated extract was further lyophilized. The lyophilized extract (300 g) was then used for the experiments.

2.4. Induction of experimental diabetes

Type 1 diabetes was induced by using alloxan monohydrate following the method of Sochor et al. (1985). Rats were starved for 24 h and diabetes was induced by a single subcutaneous injection of 150 mg/kg b.wt. alloxan monohydrate dissolved in freshly prepared 0.154 M sodium acetate buffer (pH 4.5). From next day onwards, the alloxan-induced rats were injected intraperitoneally with two units of insulin for the next seven days to reduce the mortality rate and stabilize the diabetic animals. The severity of diabetes was checked in alloxan diabetic rats by using urine glucose detection strips. Insulin was then withdrawn and the diabetic rats were randomly divided into different groups.

2.5. Determination of the effective dose

2.5.1. Normal animals

To find out the effective dose, six groups comprising of five animals each were used. Various doses (125, 250, 500, 750, 1000 and 2000 mg/kg b.wt.) of the root extract were administered orally to animals of groups I–VI, after drawing the initial blood sample. Results were compared with tolbutamide, which was administered orally to animals of group VII at a dose of 200 mg/kg b.wt. Further samples of blood were collected at 1, 2, 4, 6 and 8 h after the extract or drug administration (0 h). Animals of the control group were treated similarly but with distilled water instead of the extract.

2.5.2. Diabetic animals

Diabetic animals were similarly divided into eight groups of five animals each. Increasing doses of the root extract were administered to animals of group I–VI and tolbutamide was administered to group VII at a dose of 200 mg/kg b.wt. Additionally 500 mg/kg b.wt. tolbutamide was given to group VIII to observe the effect of increased dose of tolbutamide on FBG of diabetic animals. Animals of the control group were treated similarly but with distilled water instead of the extract/drugs.

2.6. Treatment of diabetic animals (for 20 days)

Three groups of six rats each were used in this experiment. Group I consisted of normal animals and served as normal healthy control group (NC). Groups II and III comprised of alloxan diabetic rats. Group II consisted of the untreated diabetic control (DC) animals, which were orally given distilled water daily for 20 days. Group III consisted of diabetic treated (DT) animals, which were treated daily with a single dose of 500 mg/kg b.wt. (effective dose) of the root extract for 20 days. FBG was determined on 0, 2nd, 5th, 7th, 10th, 15th and 20th day of the experiment. Also the effect of this dose on lipid profile was studied by collection of blood from tail on 0, 10th and 20th day of the experiment. TC was estimated by enzymatic method (Lopes-Virella et al., 1977), HDL–cholesterol by phosphotungstate method (Lopes-Virella et al., 1977; Allain et al., 1974; Richmond, 1973; Miller, 1977) and TG by enzymatic method (Trinder, 1969; Werner et al., 1981). VLDL (very low density lipoprotein) and LDL (low density lipoprotein) cholesterol were calculated by Friedewalds formula (Friedewald et al., 1972) as described below.

- VLDL: TG/5.
- LDL: TC–(HDL+VLDL).

ALKP, BIL, BUN, CRTN, SGOT, SGPT, TPR were also estimated on 0, 10th and 20th day to study the effect of the extract on chronic administration. ALKP was estimated by PNPP method (Klin, 1972; Saligman et al., 1950), BUN by the UV method (Talke and schubert, 1965), BIL by Jendrassik and Grof method (Jendrassik and Grof, 1938; Gambino, 1965) and CRTN by picrate method (Henry et al., 1974). SGOT and SGPT were measured by UV kinetic (Expert panel of the IFCC on Enzymes, 1976) method while TPR was measured by Bradford Macro method (Bradford, 1976). Body weight and urine sugar were estimated on 0, 10th and 20th day. At the end of the experiment, the rats were sacrificed and their liver and kidneys were weighed. The weight of liver and of two-kidneys expressed as percentage body weight was also calculated.

2.7. Estimation of serum insulin levels

Three groups (1–3) of six rats each were used in this experiment. Group 1 served as normal healthy control (NC), groups 2 and 3 were served as untreated diabetic control (DC) and diabetic treated (DT) animals, respectively. Treated diabetic and untreated diabetic control animals were given RERC at a dose of 500 mg/kg b.wt. and distilled water, respectively for the period of 20 days. Blood was collected from the tail vein and the serum insulin levels were determined on 0, 7th, 15th and 20 day of the experiment using rat insulin ELISA kit.

2.8. Phytochemical investigation of 50% ethanolic extract

Phytochemical tests were carried out for various constituents of RCRE using the following chemicals and reagents. Dragendoff's reagents were used for alkaloids, frothing test for saponins, Liebermann–Burchard test for sterols, $FeCl_3$ for tannins, Molisch reagent for carbohydrates, ninhydrin for proteins and vanillin/sulfuric acid for terpenoids (Wagner et al., 1984; Evans, 2002; Cannell, 1998; Barbehenn, 1995).

2.9. Purification of RCRE

RCRE was purified (275 g) using silica gel (60–120 mesh size) column chromatography. The column was packed in petroleum ether (60–80 °C) and was eluted using solvents of increasing polarity, such as petroleum ether, ethyl acetate, methanol and water. Fractions of 500 ml were collected and similar fractions were combined on the basis of their TLC pattern.

2.10. Testing the activity of different fractions

For testing the antihyperglycemic activity of the fractions obtained from the 50% ethanolic extract, 21 groups of diabetic animals with five animals in each group were taken. Different fractions were administered orally at a single dose of 500 mg/kg b.wt. to the overnight fasted rats in group I–XX. Blood was collected at 0, 1, 2, 4, 6 and 8 h after the administration of each fraction. Animals in the control group were given distilled water only.