Journal of Traditional and Complementary Medicine 7 (2017) 421–427

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original Article

Antidiabetic activity of extracts of *Anacardium occidentale* Linn. leaves on *n*-streptozotocin diabetic rats

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ARTICLE INFO

Article history:
Received 21 June 2016
Accepted 29 November 2016
Available online 29 December 2016

Keywords:
Anacardium occidentale
Neonatal-induced diabetic rats
Type 2 diabetes
Streptozotocin
Leaves

ABSTRACT

Anacardium occidentale L. (Anacardiaceae) is used in South Cameroon as well as in other tropical countries by traditional practitioners as a folk remedy for treatment of diabetes mellitus. We demonstrated the antidiabetic potential of the plant extracts in *n*-streptozotocin diabetic rats. The aim of the current study was to investigate the antidiabetic effects of ethanol extract of leaves of *A. occidentale* on neonatal streptozotocin diabetic rats.

Two day old neonates were injected with 100 mg/kg of streptozotocin. At the end of the experimental period of 30 days, reduction in the fasting blood glucose levels, serum insulin, glycated hemoglobin levels, serum lipid parameters, and renal function biomarkers were estimated in the control and treated rats. Histopathological examination of liver, kidney and pancreas were also carried out.

On administration of 100 mg/kg of plant extract, blood glucose levels of the rats showed 8.01% and 19.25% decrease in the fasting blood glucose levels on day 15 and day 30, respectively. The administration of extract showed that the effects of extract treatment are comparable to treatment with the standard drug Pioglitazone.

These results demonstrate significant antidiabetic potential of the ethanol extract of leaves of *A. occidentale*, justifying the use of plant in the indigenous system of medicine. Further studies for investigating the specific compound(s) responsible for such beneficial role in diabetes would open new outlook in the therapy of type 2 diabetes.

1. Introduction

Since past few decades type 2 diabetes has become a global health problem. As estimated by the World Health Organization more than 176 million people are suffering from this disease globally.[1] The common cause of chronic morbidity and disability among the working population is the complications which are caused due to diabetes. Type 2 diabetes mellitus begins with a period of insulin resistance with increased pancreatic insulin secretion. As the disease advances, pancreatic functions are decreased and are no longer able to meet peripheral requirements. Thus, insulin levels fail to sustain with the body requirements.[2] Experimental animal models of diabetes mellitus (DM) have been useful in gaining insights of the complex pathogenesis of DM. Streptozotocin (STZ) when injected in neonates of rats leads to the key features depicted in diabetic patients in a small period. Diabetes mellitus is a result of low insulin sensibility and dysfunction of the pancreatic beta-cell. Before the development of the disease, the condition is characterized by a symptomless pre-diabetic phase. Practitioners of traditional system of medicine in South Cameroon use *Anacardium occidentale* L. (Anacardiaceae) as a folk remedy for treating diabetes mellitus. Hence, we made an attempt to study the validity of this folk remedy by investigating the effects of ethanol extract of leaves in neonatal STZ diabetic rats.

The Cashew tree, known by the Latin name *A. occidentale*, is a member of the Anacardiaceae family. *A. occidentale* is grown widely in tropical countries like Malaysia, India and Brazil and occurs widely in Senegal and is known as *Darkassou*.[3] Practitioners of Traditional system of medicine in South Cameroon and other

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2016.11.007

tropical countries use *A. occidentale* L. (Anacardiaceae) as a folk remedy for treating diabetes mellitus.[4,5]

A. occidentale commonly known as Cashew is also ethno pharmacologically known to be used in treatment of diarrhea, skin diseases, and various inflammatory conditions such as arthritis.[6–10] It is also used for treatment of fevers, aches and pains.[11–13] Literature reports reveal that the studies of acute, subacute toxicity and genotoxic effect and hypoglycemic effect of Cashew in mice and rats (*A. occidentale* L.) have been reported.[14–16] There are numerous reports on the phytochemical studies of *A. occidentale* and they reveal the presence of various compounds, such as glucosides and glucose and flavonoids.[17–19]

The *n*-STZ rat model is adequate for testing type 2 diabetes.[20] The (n5-STZ) rat model show signs of a clear basal hyperglycemia with glucose intolerance, a strong reduction of pancreatic insulin stores, high HbA1c values, a lack of plasma insulin response to glucose and a decreased (50%) basal plasma insulin level. A single dose of STZ when given to neonates of rats induces injury of beta-cell which is then followed by limited regeneration (short-term normalization of glycemia), at about 6–15 weeks period, significant beta-cell secretory dysfunction (type 2 diabetes) and an impaired glucose disposal rate is observed.[20]

2. Materials and methods

2.1. Plant material

Cashew leaves were collected from Tungareshwar forests of Vasai Taluka, Dist. Thane in the state of Maharashtra, India. The plant specimen was authenticated at the Botanical Survey of India, Pune; (M.S), India. A herbarium of the plant specimen (specimen voucher number no. YOGA1/No.BSI/WC/Tech/2008/69) was submitted at the Botany Department of BSI, Pune, M.S., India.

2.2. Preparation of the extract

Fully matured shade dried leaves of Cashew were collected, cleansed and ground to coarse powder form. The samples were extracted by using Soxhlet extractor, with ethanol with a mass to volume ratio of 1:6 (g/mL). The ethanol extract was evaporated to dryness on the rotary evaporator and the residue stored in a refrigerator at 2–8 °C for use in subsequent experiments.

2.3. Acute oral toxicity studies

Animals were procured from Haffkine's Research Institute, Mumbai, India and acclimatized with free access to food and water for at least 1 week. Female Albino mice (Wistar strain) were selected for the study. Healthy young animals, 2 months old and 220–250 g weight range of commonly used laboratory strains were employed in the study. Females used were nulliparous and nonpregnant. The study group used 6 animals in each group.[21] Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. The body weights and food intake of animals were recorded. All observations were systematically recorded with individual records being maintained for each animal. Additional conditions like that of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and lethality were observed.

2.4. Animals and induction of experimental diabetes

Two day old neonates were injected with optimized dose of 100 mg/kg of Streptozotocin (Sigma, no. 242-646-8) in acetate buffer 0.1 M, pH 4.5. At 4 weeks of age, rats were separated from their mothers and acclimatized with free access to food and water in an air-conditioned room (23 °C with 55% humidity) under a 12 h light: dark cycle. The animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum. When animals were 12 weeks old, an oral glucose tolerance test was performed. All the animals were fasted overnight before experiments. Animals which were intolerant to glucose by OGTT and fasting blood glucose levels >120 mg/dL were selected for the study with ethanol extract of leaves. The study protocol was approved by Institutional Animal Ethics Committee of C. U. Shah College of Pharmacy, Mumbai, India (Approval No: CUSCP/IAEC/29/09-10). The administration of the drugs was done orally for 30 days. At the end of the experimental period, the rats were fasted overnight and blood samples were withdrawn from the retro orbital plexus. Serum samples were used for the various biochemical estimations.

2.5. Experimental groups

The rats were divided into 4 groups for study of neonatal streptozotocin-induced (n-STZ) model containing six animals each. Group 1 non-diabetic control received 1.5 ml of physiological NaCl-solution (Vehicle), group 2 was treated with the standard oral hypoglycemic agent Pioglitazone (2 mg/kg) in the same vehicle, group 3 diabetic control, treated with streptozotocin (100 mg/kg i.p) also received 1.5 ml of physiological NaCl-solution (Vehicle), group 4 received ethanol extract of cashew leaves 100 mg/kg. The extract was redissolved in 1.5 ml of physiological NaCl-solution and administered orally by a canule.

2.6. Collection of blood and determination of blood parameters

The effects of administration of ethanol extract of Cashew leaves to normal and diabetic rats were determined by measuring fasting plasma glucose levels, serum insulin levels, serum lipid profiles, liver glycogen levels (Nicholas, 1956), glycated hemoglobin levels and initial and final changes in body weight. Fasting plasma glucose, serum triglycerides, total cholesterol, were estimated on days 1, 15, and 30 of extract administration. Body weights were determined on day 1, 10, 20 and 30 of extract administration. All other biochemical parameters were determined on day 30 after the animals were sacrificed by decapitation. Serum insulin levels were estimated using a radio immunoassay kit issued by the Board of Radiation and Isotope Research, Bhaba Atomic Research Centre (BARC), Mumbai, India. On day 30, when the animals were sacrificed, the pancreas, liver and kidney of one animal from each group was excised and stored in 10% formalin after washing with normal saline and histopathological parameters were studied. The tissue was washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial sections of 5 μm thickness were cut using a rotary microtome. The sections were de-paraffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to hematoxylin for 10 min, followed by rinsing with water. These were examined and later counter stained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted.

2.7. Statistical analysis

All statistical analyses were made using the software InStat for windows. All results were expressed as mean ± SEM. Post hoc Dunnett's test was used to determine statistical significance. The values were considered statistically significant when $p < 0.05$.