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Evaluation of *in vivo* anti-hyperglycemic and antioxidant potentials of α-santalol and sandalwood oil

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ABSTRACT

Sandalwood finds numerous mentions across diverse traditional medicinal systems in use worldwide. The objective of this study was to evaluate the *in vivo* anti-hyperglycemic and antioxidant potential of sandalwood oil and its major constituent α-santalol. The *in vivo* anti-hyperglycemic experiment was conducted in alloxan-induced diabetic male Swiss albino mice models. The *in vivo* antioxidant experiment was performed in D-galactose mediated oxidative stress induced male Swiss albino mice models. Intraperitoneal administration of α-santalol (100 mg/kg BW) and sandalwood oil (1 g/kg BW) for an week modulated parameters such as body weight, blood glucose, serum bilirubin, liver glycogen, and lipid peroxides contents to normoglycemic levels in the alloxan-induced diabetic mice. Similarly, intraperitoneal administration of α-santalol (100 mg/kg BW) and sandalwood oil (1 g/kg BW) for two weeks modulated parameters such as serum aminotransferases, alkaline phosphatase, bilirubin, superoxide dismutase, catalase, free sulfhydryl, protein carbonyl, nitric oxide, liver lipid peroxide contents, and antioxidant capacity in D-galactose mediated oxidative stress induced mice. Besides, it was observed that the beneficial effects of α-santalol were well complimented, differentially by other constituents present in sandalwood oil, thus indicating synergism in biological activity of this traditionally used bioresource.

Introduction

Diabetes mellitus (DM) is one of the most widespread chronic, metabolic, hereditary, heterogeneous group of diseases in the planet with highest rates of mortality. Diabetes is one of the key leading causes of global mortality, long-term complications, disability, hospitalization and a risk factor for cardiovascular diseases. It is characterized by hyperglycemia (the fasting plasma glucose cut-off level being 7 mM/l) (WHO 1999) and glycosyuria due to an impaired carbohydrate "glucose" resulting from a defective or deficient insulin secretory response (either absolute or relative lack of it, reduced circulating concentration, poor insulin sensitivity, insulin resistance or poor glucose tolerance resulting in high sugar in plasma), *per se* impairs insulin secretion (Davis and Granner 2001). Other characteristics include, alterations in carbohydrate, fat, and protein metabolism associated with complications such as atherosclerosis, neuropathy, and cataract formation as well as abnormalities in serum lipids (West 2000). Hyperglycemia induced by decreased cellular glucose uptake and metabolism, increased utilization and decreased storage of proteins, for energy, instead of glucose, leads to reduction of body weight by depletion of the body proteins (Guyton and Hall 2000).

Oral hypoglycemic agents and clinical grade insulin are synthetic ones having certain serious adverse side effects as well not suitable for use during pregnancy. Thus promoting alternative therapeutic approaches and to find safer hypoglycemic agents, the ethnopharmacological knowledge of plants which have proved beneficial due to their effectiveness, safety, lower toxicity and fewer side effects compared to synthetic drugs and age old practices as folklore medicine (Pari and Umamaheswari 2000) have become important. However, there is an ever increasing demand for complementary and alternative medicine with higher achievable antidiabetic activities, in the form of anti-hyperglycemic or hypoglycemic principles.

Plants possessing hypoglycemic activities, do so by reducing blood sugar, similarly as in sulfonylurea drugs like glibenclamide, effecting hypoglycemia in normal animals by stimulating insulin release from pancreatic β-cells, besides reducing hepatic clearance of insulin hormone and like the biguanides i.e., metformin where they target hyperglycemic but not hypoglycemic condition in normal state and others show insulin sparing action (Davis and Granner 2001). Metformin-like activity of plants augments insulin action by increasing the number of glucose transporters, inhibiting gluconeogenesis, reducing absorption from the intestine but

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increasing glucose metabolism in liver (Zhang and Tan 2000). Natural α-amylase inhibitors reduce post-prandial hyperglycemia by slowing down the digestion of carbohydrates and, consequently, the absorption of glucose. However, only a few comprehensive studies on scientific validation of traditional antidiabetic medicinal plants are known.

Natural antioxidants of foods derived from fruits, vegetables, spices and cereals (known as *nutraceuticals*) are effective, amenable to defense mechanism of body, safe, nutritional and therapeutic (Ajila et al. 2007). Vitamins C and E, α-tocopherol, β-carotene, glutathione and flavonoids prevent the formation of excess free radicals, scavenging them or by repairing damaged molecules (Sánchez-Moreno 2002). Many Indian plants showing ethnopharmacological usages, with antioxidant potential have been reviewed extensively (Govindarajan et al. 2003).

The East Indian sandalwood tree, *Santalum album* L., finds numerous mentions in traditional medicinal systems such as Ayurveda, as antiseptic, antipyretic, antiscabietic, diuretic, expectorant, stimulant, and for the treatment of bronchitis, dysuria, urinary infection, and in gonorrheal recovery (Dikshit and Hussain 1984). The hydrolyzed exhausted sandalwood powder (HESP) possesses anti-remorogenic, anti-inflammatory, anti-mitotic, antiviral, anti-cancerous, anti-hypertensive, anti-pyretic, and sedative properties (Desai et al. 1991). Recently, apoptotic (Bommareddy et al. 2012), cytotoxic (Matsuo and Mimaki 2012), antiviral (Paulpandi et al. 2012), and anti-*Helicobacter pylori* (Takaishi et al. 2005) properties were reported for α-santalol [$C_{15}H_{24}O$, 220.35 Da, CAS No. 11031-45-1, FEMA No. 3006], the major constituent of sandalwood oil (Demole et al. 1976) and hence, have generated enormous interests.

However, there are no scientific corroborations available in literature for the *in vivo* antidiabetic or antioxidant efficacies of the sandalwood oil and its ingredients in scientific literature. Hence, this study was undertaken to investigate the *in vivo* anti-hyperglycemic and antioxidant potential of sandalwood oil and α-santalol in experimentally induced mice models using alloxan and D-galactose, respectively.

Materials and methods

Collection of test samples

Authentic sandalwood oil was obtained from Cauvery™ (Government of Karnataka, Bangalore, India) for use in this investigation.

Gas chromatography (GC) based profiling of sandalwood oil

The sandalwood oil sample used in the study was analyzed by GC following protocols as described (Howes et al. 2004). Briefly sandalwood oil was diluted to 1% (v/v) in spectroscopy grade pyridine or diethyl ether. A Chemito 100™ (Chemito Technologies Pvt. Ltd., India) work station was used in this entire study. Briefly, gas chromatography was performed on either a BP 5 or BP 21 column [dimensions: 30 m (*l*) × 0.25 mm (*i.d.*) × 0.25 μm, (SGE, Melbourne, Australia)] using an oven program of 40–200 °C at 2 °C min^{-1} or 3 °C min^{-1}. In both cases, the carrier gas used was helium (He) at a column flow rate of 1 ml min^{-1}. Injections of 1–3 μl (with split of 1:10 or 'none', respectively for the 2 columns) were made with injector temperatures set at 220–250 °C. Detection was done by flame-ionization detection (FID) at 250 °C, 45 ml min^{-1} H_2, and 450 ml min^{-1} air. For BP 5 column, the oven program was held for 1 min at 40 °C, then ramped at 7.5 °C min^{-1} to 250 °C and was further held for 10 min. For BP 20 column, the oven temperature programs were as follows: held for 3 min at 40 °C, then 3 °C min^{-1} to 110 °C, 10 °C min^{-1} to 180 °C, and a final ramp of 15 °C min^{-1} to 240 °C where this temperature was held for 5 min. Data extraction and analysis were performed using the Iris™ software ver. 4.1.1. Sesquiterpenoid constituents were expressed as percentages from peak area normalization, assuming that the total injection was 100% of 10–100 μg of essential oil. The Kovat's indices (KI) were determined relative to the retention times of a series of n-alkanes with linear interpolation based on available standard compounds, an in house custom library and literature.

Bulk purification of α-santalol by high performance thin layer chromatography (HPTLC)

Alpha-santalol was bulk-purified by preparative HPTLC (Camaag, Switzerland) as described (Misra and Dey 2012).

Experimental animals

Healthy, adult male Swiss albino mice of approximately 4 months in age, weighing 20–30 g were used for the study. The mice were housed in the Departmental Animal Facility and were kept under controlled conditions in polypropylene cages lined with husk, renewed every 24 h, and with temperature maintained at 22 °C on a 12 h light: 12 h dark cycles. Mice were fed a balanced pellet feed prepared in-house. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), New Delhi, India, and was permitted by the Animal Ethical Committee of Indian Institute of Technology Kharagpur, Kharagpur.

In vivo anti-hyperglycemic assay in alloxan-induced diabetic mice model

Administration of compounds to normal mice

Animals were divided into different test groups, i.e., diabetic mice treated with α-santalol (100 mg/kg BW) and sandalwood oil (1 g/kg BW), one control group administered with the vehicle 5% Tween 80, one positive control group treated with glibenclamide (1 mg/kg BW) and one group treated with alloxan only, with each group comprising a minimum of six mice ($n = 6$). Test compound doses were administered intraperitoneally by injection in 5% Tween 80 as the carrier/vehicle and glucose levels were monitored at different time intervals up to 15 days following the administration. Food, but not water was withheld during test period not exceeding 24 h. Body weights were monitored for 2 weeks after administration of the extract.

Induction of experimental diabetes

Animals were administered intraperitoneally with alloxan monohydrate (150 mg/kg BW) prepared in 5% Tween 80 in saline. Preceding administration, mice were fasted overnight but given water *ad libitum*. Alloxanised animals were then kept under observation for a week following administration and blood glucose levels were subsequently determined. Mice with more than 3–4 fold increased in their blood sugar levels were considered diabetic and used for further tests.

Estimation of blood glucose level

Blood samples were collected by puncturing the tail veins and were analyzed for glucose levels employing a commercial glucometer. The EZ Smart™ glucometer (Tyson Bioresearch, Taiwan) technically measured electrical current generated by blood glucose oxidation (enzymatic). The EZ Smart™, Blood Glucose Test Strips had a detection range for glucose, i.e., 1.1–33.3 mM/l or 20–600 mg/dl with test time of 10 s with a sampling volume of 1.5 μl of blood in the capillary for a result.