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In-vitro and *in-vivo* antiadipogenic, hypolipidemic and antidiabetic activity of *Diospyros melanoxylon* (Roxb)

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ABSTRACT

Ethnopharmacological relevance: The plant *Diospyros melanoxylon* (Roxb) belongs to the family Ebenaceae that is native to India and Sri-lanka. This is a medium-sized tree, reaching a height of 15 m and is well known for its beedi making leaves throughout the world. The purpose of the present study is to assess the effect of *Diospyros melanoxylon* leaves petroleum ether extract on blood glucose level, lipid level, insulin level, body weight, water and food intake in Streptozotocin (STZ) induced diabetic rats.
Materials and methods: Two different doses of extract AK001 (250 mg/kg) and AK002 (500 mg/kg) of *Diospyros melanoxylon* leaves were taken to evaluate different activities. The animals were divided into five groups namely normal control, diabetic control, reference group, AK001 and AK002 each containing six animals for *in-vivo* study. *In-vitro* study for antiadipogen activity was performed on 3T3-L1 cell line.
Results: The extract showed dose dependent fall in Fasting Glucose Level (FSG) in experimental diabetic animals with significant reduction in food and water intake and increase in body weight. The extract exhibited hypocholesterolemic and hypotriglyceridemic effects while increased level of HDL in diabetes induced rats. *In-vitro* activity showed more than 75% viability of cells and significant inhibition in differentiated cells as compared to non-differentiate cells in 3T3-L1 cell line. The extract exhibited the concentration-dependent inhibitory effect with an IC_{50} value of 689.22 μg/ml.
Conclusions: The extract exhibited significant results for antiadipogenic, antidiabetic and hypolipidemic activity both *in-vivo* and *in-vitro* and it may prove to be effective for the treatment of both types of diabetes, *i.e.* Insulin Dependent Diabetes Mellitus (IDDM) and Noninsulin Dependent Diabetes Mellitus (NIDDM).

1. Introduction

Diabetes is a disorder of carbohydrate, fat and protein metabolism attributed to diminished production of insulin or mounting resistance to its action; and obesity is also an increasing problem worldwide and induces many diseases like diabetes, atherosclerosis and other metabolic syndromes. So the knowledge about the process of adipogenesis and formation of adipose tissue is very important. For better understanding of these processes at least the study should be conducted on *in vitro* and *in vivo* models. Few cell lines can undergo adipocyte differentiation of the mouse 3T3-L1 cell which is well characterized cell line for adipogenic assay. In normal condition 3T3-L1 preadipocyte cells have fibroblastic phenotype (Gregoire et al., 1998). When this cell is treated with differentiation media they accumulate lipid droplet inside the cell and achieve adipocyte phenotype.

Craving for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Concurrently, phytochemicals identified from traditional medicinal plants are presenting exciting opportunities for development of new drug therapies. It was reported that treatment of diabetic animals with medicinal plant extracts resulted in activation of β cells and granulation returned to normal showing an insulinogenic effect (Kedar and Chakrabarti, 1982, Gray and Flatt, 1999). The plant *Diospyros melanoxylon* (Roxb.) belongs to the family Ebenaceae (Orwa et al., 2009). This is used in an Indian cigarette product known as beedi to wrap the

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tobacco together to be smoked. The genus *Diospyros* consists of 240 species, 59 of which are distributed in the northern part of India (Bihar, Madhya Pradesh, Chhattisgarh, Himachal Pradesh, West Bengal, Mumbai, *etc.*) and also in Tamil Nadu (Coimbatore, Dharmapuri, Salem, *etc.*). This is a medium-sized tree, reaching a height of 15 m and is well known for its beedi making leaves throughout the world (Rath et al., 2009).

In the present study the plant *Diospyros melanoxylon* (Roxb.) was selected for screening of antihyperglycemic activity on the basis of its ethnopharmacological information that the tribe of Chotta Nagpur region (Orrisa) use it extensively as antidiabetic (Gupta et al., 2009). The traditional medicinal importance of the plant is ascertained as diuretic, carminative, laxative, styptic, good in epitaxis and night blindness, improves the eyesight, used in opthalmia, trichiasis, burns, tuberculosis glands, scabies, and old wounds (The Wealth of India, 2006; Kirtikar and Basu, 2006; Nadkarni, 2007).

The preliminary phytochemical screening of *Diospyros melanoxylon* (Roxb) shows the presence of steroids, triterpenoids in petroleum ether extract and flavonoids, tannins, phenolic compounds, sterols, triterpenoids in ethyl acetate extract (Parmar et al., 2012). Flavonoids, tannins, phenolic compounds, steroids in alcoholic extract and carbohydrates, proteins, amino acids, flavonoids, tannins, phenolic compounds, and tartaric acid as an organic acid in aqueous extract are also present (Gupta et al., 2013). The active phytoconstituents present in petroleum ether extract of *Diospyros meloxylon* (Roxb) furnished were ceryl alcohol, lupeol, betulin, β-sitosterol and a triterpene alcohol, $C_{30}H_{50}O$ (Gupta and Rao, 1964). The new compounds have been characterized by a combination of chemical and spectroscopic analysis as 4,6-dihydroxy-2-[α,α-2-(4-hydroxyphenyl) hydroxy] methylene-3-(2H)-benzofuranone(2), 3,3′,5,7-tetrahydroxy-4′-O-{α-2,4,6-trihydroxy-2-[3,4-dihydroxy phenyl methyl-3-(2H)-benzofuranone}-4H-benzopyran-4-one(6) and 2,3-dihydro-2,3-oxymethylene-3-methoxy-5-hydroxy-7-O-β-D-glucopyranosyl-2-[4-methoxy phenyl]-4H-benzopyran-4-one(7) found on methanolic extract of *Diospyros melanoxylon* leaves (Mallavadhani and Mahapatra, 2005).

A large number of bioactive triterpenoids have shown multiple biological activities with apparent effects on glucose absorption, glucose uptake, insulin secretion, diabetic vascular dysfunction, retinopathy and nephropathy. The versatility of the pentacyclic triterpenes provides a promising approach for diabetes management and antiadipogenic activity (Alqahtani et al., 2013, Velusami et al., 2013). Steroids have the ability to release insulin by the stimulation of regeneration process and revitalization of the remaining β cells. Lupeol (triterpenoid) and β-sitosterol (steroid) cause decrease in glycated hemoglobin, serum glucose and nitric oxide with a concomitant increase in serum insulin level. (Gupta et al., 2011, 2012). Lupeol minimizes the lipid abnormalities and possesses cardioprotective effects which will be beneficial in hypercholesterolemic condition (Sudhahar et al., 2007).

Diospyros melanoxylon is used in the management of diverse diseases and treatment of diabetes, anemia, inflammation of spleen (Parmar et al., 2012) dyspepsia, diarrhea, scabies, hypotensive and used as carminative, laxative, diuretic and astringent. The beneficial effects of flavonoids have been studied in relation to diabetes mellitus which may act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms of insulin signaling (Van Dam et al., 2013).

2. Materials and methods

2.1. Collection, authentication, extraction of plant

The leaves of *Diospyros melanoxylon* were collected from the forest of Korba, Chhattisgarh, India, authenticated by S. Prakash Rao, Department of Pytochemistry and Pharmacognosy, Columbia Institute of Pharmacy, Raipur and voucher specimen, *i.e.* dried plant part with voucher number 0114, was deposited in the herbarium of the Institute. The powdered air-dried leaves (1 kg) of *Diospyros melanoxylon* were extracted with petroleum ether (b.p. 40–60) by cold percolation. The extract was concentrated to dryness in a rotary evaporator (Buchi type) under reduced pressure and controlled temperature (37–40 °C) and percentage yield was found to be 1.82%. The dried extract was used to evaluate antiadipogenic, antidiabetic and hypolipidemic activity. The extract was named as Extract L (light) and Extract D (dark) due to two different exposures of the sample in UV and dark condition of *in vitro* study respectively. However, *in vivo* study mentioned extract as AK001 (250 mg/kg) and AK002 (500 mg/kg) due to two different dose groups of extract in experimental animals.

2.2. Phytochemical investigation

Phytochemical investigation revealed the presence of triterpenoids and steroids in petroleum ether extract of *Diospyros melanoxylon* (Roxb). Phytochemical investigation was performed as per WHO guidelines (WHO, 2001).

2.3. Animal used

Male Wistar rats of weight 160–200 g were taken. Experimental protocols used in the experiment were approved by the Institutional Animal Ethic Committee (IAEC reg. no. 67/99/CPCSEA/REG) of Indian Institute of Integrative Medicine, IIIM (CSIR) Jammu. The animals were housed in polycarbonate cages in a room with a 12 h day–night cycle, temperature of 22 ± 2 °C, and humidity of 45–64%. During the whole experimental period, animals were fed with a balanced commercial pellet diet (Ashirwad Industries, Mohali, India) and water *ad libitum* and normal saline. Animals were divided into five different groups as Normal control, Diabetic control, Reference group, AK001 (250 mg/kg) and AK002 (500 mg/kg) each containing six animals for *in-vivo* antidiabetic and hypolipidemic activity.

2.4. Cell line

Original stock of 3T3-Ll cell lines was received in frozen state (dry ice) in cryovials from the National Centre for Cell Science (NCCS), Pune, India.

2.5. Chemicals used

Streptozotocin (45 mg/kg for induction diabetes), Dexamethasone (1 μM), 3-Isobutyl-1-methylxanthine (0.5 mM for adipocyte differentiation), Insulin (10 μg/m), and Dulbecco's Modified Eagle Medium (DMEM) (a widely used basal medium for growth of mammalian cells) all were purchased from Sigma-Aldrich Co. (USA); glibenclamide and simavastatin were used as standard drugs for anti-diabetic and hypolipidemic action respectively; 10% FBS and 60 mg/L Amikacin, Gentamycin, Phosphate Buffer Saline, Oil Red O Dye, Trypsin, Dimethyl sulfoxide were purchased from Sigma Chem. Co., (USA). Rat Insulin ELISA kit was purchased from Mercodia, Sweden.

2.6. Pharmacological screening methods

2.6.1. In-vitro experimental method

2.6.1.1. In-vitro cytotoxicity assay. 3T3-L1 cell line was cultured in DMEM media with 10% bovine calf serum (BCS) and 60 mg/L amikacin at 37 °C, 5% CO_2 and above 90% relative humidity. The *in vitro* toxicity of petroleum ether extract in a pane of cell line