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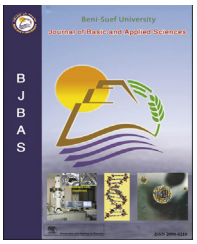

Hypoglycemic and hypolipidemic effects of ethanolic and aqueous extracts from *Ziziphus oenoplia* (L) Mill on alloxan-induced diabetic rats

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ABSTRACT

The objective of present study was to investigate hypoglycemic and hypolipidemic effect of ethanolic and aqueous extracts of *Ziziphus oenoplia* (L) stem bark against Alloxan induce hyperglycemia in rats. Hyperglycemia was induced by an injection of alloxan monohydrate 150 mg/kg (i.p.). After 72 hr, the rats having Blood Glucose Level (BGL) above 150 mg/dL were selected for the investigation. At two different doses (200 mg/kg and 400 mg/kg b.w.) of aqueous and ethanolic extracts were observed antidiabetic effect for 12 consecutive days. BGL was monitored after 1, 3, 6 and 12 days and compared with Metformin (250 mg/kg). Alpha amylase and alpha glucosidase activity of both extracts were also determined. Phytochemical study revealed the presence of glycosides, flavonoids, alkaloids and terpenoids in ethanol extract and flavonoids, carbohydrates and proteins found in aqueous extract of *Z. oenoplia* bark. Oral administration of both extracts showed significant ($P < 0.05$) antihyperglycemic activity in dose dependent manner in alloxan induced diabetic rats. The diabetic rats had significant ($P < 0.01$) reduction in blood glucose; serum liver enzyme level (AST, ALT, and ALP) and lipid profile were compared with normal rats. Significant effects of aqueous and alcoholic extract in alpha amylase and alpha glucosidase activity were observed in rats. The ethanolic and aqueous extract reveals the reduction in the blood glucose level, inhibition of alpha amylase and alpha glucosidase enzymes which support antidiabetic effect (reduce postprandial glucose levels) of *Z. oenoplia* and this may be due to presence of flavonoids constituents.

1. Introduction

Diabetes mellitus is a syndrome resulting from a variable interaction of hereditary and environmental factors, characterized by damaged of β-cells from pancreas and complication of vascular disease. Diabetes is a major and one of the most common chronic diseases in the world (Vinuthan et al., 2007). It is a group of metabolic disease characterized by hyperglycemia, altered metabolism of lipids, carbohydrate and protein; this may be

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due to defects in insulin secretion, insulin action, or both (Mourya et al., 2014). Type 2 diabetes mellitus is by far the commonest form of the diseases globally, with developing countries being at the forefront as far as the epidemic is concerned (Pepato et al., 2005; Tiwari and Rao, 2002). There are different types of oral hypoglycemic agents existing along with insulin for the treatment of diabetes mellitus, but due to some complications there is an increasing demand by the patients to use natural products for lowering blood glucose level (Hara and Honda, 1990). Lower level of insulin in diabetic patients leads to increased amino acid level in the blood. It results to increase in transaminase activity (increased level of AST and ALT) that will result in ketogenesis and gluconeogenesis (Amraie et al., 2015). In type 2 diabetes, postprandial hyperglycemia plays an important role in developing stage. There are two major carbohydrate hydrolyzing enzymes i.e. α-amylase and α-glucosidase which are responsible for postprandial hyperglycemia. Alpha-amylase begins the carbohydrate metabolism by hydrolysis of 1, 4-glycosidic linkages of polysaccharides to disaccharides and alpha-glucosidase catalyzes the reaction in which disaccharides convert into monosaccharides, which promote to postprandial hyperglycemia (Shukla et al., 2011).

The use of herbal medicines has a long tradition for the management of blood glucose abnormalities. Therefore the researchers continue looking for more effective and safer hypoglycemic agents from natural source (Bhati et al., 2014).

Ziziphus oenoplia (L) Mill is also known as *Rhamnus oenoplia* or Jackal Jujube (family Rhamnacae) distributed throughout the tropical region of India, and Australia. A straggling shrub often semi scan dent by its prickles, and young branches are rusty. Leaves numerous, 2.5–6.5 by 2–2.5 cm, ovate, acute to mentose tips, galabarous, densely silky with hair, petioles 6–8 mm long flowers 12–20 sub sessile calyx hairy outside, petals obviate. Fruits are edible. The bark, fruits, leaves and stems of plant are extensively used in the rural area for stomach, hypotensive, diuretic, wound healing, antibacterial, anti-inflammatory and analgesic (Kirtikar and Basu, 2005). The stem bark of *Z. oenoplia* was reported for antioxidant activity (Sameera et al., 2015). *Z. oenoplia* reveals the presence of alkaloids, flavonoids, phenolic compounds and terpenoids which may be responsible for its medicinal efficacy. Chemical investigation of *Z. oenoplia* roots has shown the presence of cyclopeptide alkaloids such as Ziziphine (Suksmrarn et al., 2005; Shukla et al., 2016). Bark and fruits of *Z. oenoplia* have been used traditionally for antidiabetes mellitus (Prabhavathi and Vijayalakshmi, 2015; Subramoniam, 2016). It is reported that a formulation containing *Z. oenoplia* along with other species is used for treatment of diabetes and its complications (Krishnan, 2011). The aim of the present study was to investigate the effects of *Z. oenoplia* stem barks in type 2 diabetes model against alloxan induced diabetic rats to ascertain the traditional claim of plant.

2. Material and methods

2.1. Plant collection and preparation

The plants *Z. oenoplia* (L) mill were collected from Salem district, Tamil Nadu, India. The plant was identified and authenticated by the botanist Mr. Balsubramanyam, Director of ABS botanical garden, Salem, Tamil Nadu. Voucher samples were deposited in the herbarium for reference (ZE/221). The fresh stem barks were dried under shade sliced into small pieces and pulverized into coarse powder with mechanical grinder. The powder was passed through sieve no.10 and kept in polythene bags at room temperature for extraction.

2.2. Extraction of plant material

The dried coarse powder of *Z. oenoplia* (L) mill stem barks were defatted with petroleum ether (60–80 °C) in a Soxhlet apparatus by continuous hot extraction. The defatted powder material (marc) thus obtained was further extracted with ethanol (95% v/v) with same method and fresh powder was used with chloroform water extraction by cold maceration method up to complete extraction for 24 hrs. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. Qualitative analysis of different extracts was carried out to find out the presence of various phytoconstituents (Kokate et al., 2010).

2.3. Determination of total phenolic content

The total phenolic content in aqueous and ethanolic extracts was determined by colorimetric method with Folin–Ciocaletu reagent (Bozin et al., 2008). A reaction mixture contain 500 µL of 0.1% aqueous dilution of both extracts, 2.5 mL of freshly prepared 0.2M FC reagent and 2 mL of sodium carbonate solution. The mixture was kept in the dark under ambient conditions for 30 min to completion of reaction. Absorbance of the resulting solution was measured at 760 nm in a UV–vis spectrophotometer (Shimadzu, USA). The total phenolic content was expressed as mg of gallic acid equivalents per gram of extracts, using a standard curve of gallic acid (Sigma Aldrich Chemicals Pvt Ltd, Mumbai, India).

2.4. Total flavonoids content

Total flavonoids content from ethanol and aqueous extract was determined by aluminum chloride colorimetric assay method (Park et al., 2008). A test tube containing 0.3 mL of extract, 3.4 mL of 30% methanol, 0.15 mL of $NaNO_2$ (0.5 M) and 0.15 mL of $AlCl_3.6H_2O$ (0.3 M) was shake up to complete mixing. One milliliter of NaOH (1 M) was added after 5 min, with mixing well and the absorbance was measured at 510 nm. The standard curve of quercetin (Sigma Aldrich Chemicals Pvt Ltd) was made and the total flavonoids content was expressed as milligrams of quercetin equivalents per 100 gm of dried extract.

2.5. Acute toxicity study

Healthy Wistar albino rats of either sex were divided into two groups, each consisting of five rats. The animals were orally fed extracts in increasing dose levels of 100 to 4000 mg/kg body weight. The study was carried out according to OECD guidelines–423. All animals were observed continuously for behavior changes up to 2 h (Bhandarkar and Jain, 2015; OECD Guideline, 2001).

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