Anti-hyperglycemic and Anti-hyperlipemia Effects of Syzygium Cumini Seed in Alloxan Induced Diabetes mellitus in Swiss Albino Mice (Mus musculus)

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Abstract

Context: Syzygium cumini commonly known as jamun, a tree grown throughout India is used in traditional medication for the treatment of different diseases and ailments of human beings.

Objective: In this study, the seed extract of Syzygium cumini was evaluated for anti-hyperlipidemic activity in alloxan induced diabetic mice.

Materials and methods: LD_{50} and acute toxicity studies of Syzygium cumini (SC) were done. Hyperglycemia was induced in mice by alloxan. Three days after alloxan induction, the hyperglycemic mice were treated with Syzygium cumini orally at the dose of 150 and 250 mg/kg body weight daily for 21 days. After 21 days of treatment, the animals were sacrificed and serum glucose and lipid profiles were estimated in the serum.

Results: The LD_{50} was found to be 1000 mg/kg; 150 and 250 mg/kg doses were selected as no toxic symptoms were observed at both doses. SC significant (p<0.05) reduced serum glucose level, CH, TG-C, LDL-C, VLDL-C and increased HDL-C, body weight and liver, kidney weight.

Conclusion: The above results prove potential of hypoglycemic and anti-hyperlipidemic activity Syzygium cumini.

Keywords: Alloxan; Aqueous extract of Syzygium cumini seed; Anti-hyperlipemia; Anti-hyperglycemic

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that affects a significant portion of the population worldwide [1]. DM is a group of metabolic diseases characterized by hyperglycemia, hypertriglyceridemic and hypercholesterolemia, resulting from defects in insulin secretion, its action or both [2]. Both type 1 and type 2 diabetes are known to be multifactorial diseases caused by a combination of genetic (inheritance) and environmental (diet and lifestyle) factors [3]. Non-insulin dependent Diabetes mellitus (NIDDM) is a multifactorial disease, which is characterised by hyperglycemia and lipoprotein abnormalities [4]. These traits are hypothesised to damage cell membranes, which results in excess generation of reactive oxygen species. NIDDM has also been associated with an increased risk for developing premature atherosclerosis due to an increase in triglycerides (TG) and low-density lipoproteins (LDL), and decrease in high-density lipoprotein levels (HDL) [5]. Two groups of oral hypoglycemic drugs, sulphonylurea and biguanides, have been used in the treatment of DM. They act by lowering blood glucose thereby delaying or preventing the onset of diabetic complications [6]. The pathogenesis of diabetes and its management by oral hypoglycemic agents has stimulated great interest in recent years. Despite considerable progress in the management of diabetes mellitus by synthetic drugs, the search for indigenous natural anti-diabetic agents is still going on [7]. Before the development of modern pharmaceutical treatments, therapeutic capacity was completely dependent on the use of medicinal herbs for prevention and treatment of diseases [8]. Ethno botanical information also indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes throughout the world [9]. However, there is still an unmet need for scientific proof of the antidiabetic activity of medicinal plants and phytopharmaceuticals with fewer side effects. In view of this, the present study was taken up to explore antidiabetic potential of Syzygium cumini seeds and also to reduce the risk of late complications and negative outcomes of diabetes mellitus which requires not only to control blood glucose level but also to control lipid profile.

Syzygium cumini Skeels (Myrtaceae) (Syn Eugenia jambolana) is a large evergreen tree up to 30 m high [10]. It is widely distributed throughout India, Sri Lanka and Australia and it is known as Jamun, Jam, and Jambut in India. It has been valued in ayurveda and unani systems of medication for possessing a variety of therapeutic properties. The therapeutic value of Syzygium cumini has been recognized in different system of traditional medication for the treatment of different diseases and ailments of human beings. It contains several phyto-constituents belonging to the category of alkaloids, glycosides, flavonoids and volatile oil. In the literature it has been reported as a digestive, astringent, blood purifier and anthelmintic, antibacterial, analgesic, anti-inflammatory, antioxidant, ulcers, diabetes as well as gastro protective agents. Several studies using modern techniques have authenticated its use in diabetes and shown promising results [11,12].
Materials and methods

Plant Material

The basic plant material of *Syzygium cumini* seed used for the investigation was obtained from local garden (Allahabad, Uttar Pradesh). The plant species was authenticated by Department of Botany, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad, Uttar Pradesh (India).

Chemicals

All chemicals were obtained from the following sources: alloxan was purchased from the Loba chemie (Batch no: G204207) Mumbai. Commercially available kits for chemical analyses such as glucose, cholesterol, triglycerides and HDL-cholesterol were used with crest Coral clinical system, Goa, India.

Preparation of plant extract

The fresh seeds of *Syzygium cumini* were collected, washed with distilled water and shadow dried. The shadow dried seeds of *Syzygium cumini* were subjected to pulverization to get coarse powder. Aqueous extract was made by dissolving it in distilled water using by mortar and pestle. The dose was finally made to 150 mg/kg and 250 mg/kg body weight for oral administration after the LD50 estimation.

Animal care and monitoring

Healthy Swiss albino mice (*Mus musculus*) (4-6 months old, weighing 28-32 g) were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India. They were maintained in an air-conditioned experimental room at 12 hour light:dark cycles. The animals were randomized into experimental and control groups and were housed in a polypropylene cage. Standard pellets were used as a basal diet during the experimental period. The control and experimental animals were provided with purified drinking water ad libitum. The animals were maintained in accordance with the CPCSEA guidelines for laboratory animal facility (Committee for the Purpose of Control and Supervision on experiments on animals) and the approval number is (CPCSEA Regd. No. 1129/by/07/CPCSEA, dated 13/02/2008).

Acute toxicity

The acute oral toxicity of *Syzygium cumini* (SC) in male Swiss albino mice was studied as per reported method [13].

Induction of diabetes

We used Alloxan as a diabetic model to maintain a mild and stable form of diabetes during the experimental period. Alloxan was freshly dissolved in distilled water and immediately injected intraperitoneally with a single dose of 150 mg/kg in overnight fasted mice. The animals were allowed free access to 5% glucose solution to overcome the drug induced hypoglycaemia. After 72 hours of alloxan injection, diabetes was confirmed by blood samples collected from the tip of the tail using a blood glucometer (Accu Sure, Tai Doc Technology Corporation, Taiwan). The mice with a fasting blood glucose level above 200 mg/dl were considered diabetic and were used in the experiment [14]. The normal group served as non-diabetic control mice.

Study groups & Sampling:

Four groups of mice, six mice in each received the following treatment schedule.

- **Group I**: Normal control
- **Group II**: Alloxan treated control (150 mg/kg body weight Lp)
- **Group III**: Alloxan (150 mg/kg body weight Lp)+ *Syzygium cumini* (seed extract at the dose of 150 mg/kg body weight).
- **Group IV**: Alloxan (150 mg/kg body weight Lp)+ *Syzygium cumini* (seed extract at the dose of 250 mg/kg body weight).

At the end of the experimental period, the mice were sacrificed following an overnight fast by anaesthetized. Blood samples were collected by orbital sinus puncture method [15]. Serum was prepared following procedure. Briefly, blood samples were withdrawn from orbital sinus using non-heparinised capillary tubes, collected in dried centrifuge tubes and allowed to clot. Serum was separated from the clot by centrifuged at 3000 rpm for 15 minutes at room temperature. The serum was collected carefully and kept at -20°C until analysis.

Estimation of serum biochemical parameter

Collected blood was used for the estimation of serum biochemical parameters, viz glucose content was estimated by the method of Trinder [16]. Total cholesterol was estimated by the method of Richmond [17] and triglycerides were estimated by the method of Fossati and Principe [18]. HDL was estimated by the method of Farmitalia Carlo Erba [19]. VLDL-C and LDL-C were calculated by Friedwald [20]

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\text{VLDL} = \frac{\text{TG}}{5} \\
\text{LDL} = \frac{\text{TC} - (\text{VLDL} + \text{HDL})}{2}
\]

Body weight, liver and kidney weights

The body weight of mice of each group were measured just before and 21 days after SC treatment. Liver and kidney weights of all mice were measured after post treatment sacrifice.

Statistical analysis

Results were presented as mean ± S.D and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among means had been analysed by applying Tukey’s multiple comparison test at 95% (p<0.05) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

Results

Acute toxicity

The oral LD50 value of the *Syzygium cumini* (SC) in mice is 1000 mg/kg body weight.

Body weight, liver and kidney weights

The body weight, liver and kidney weights of mice from diabetic control group (after 21 days) were significantly (p<0.05) decreased when compared with normal control group. SC at 150 and 250 mg/kg body weight significantly (p<0.05) maintained the body weight, liver and kidney weights toward normal in a dose related manner as compared with diabetic control group as shown in Figure 1-4.

Blood glucose level

The glucose levels in normal control, diabetic control and treated mice are summarized in Figure 5. Alloxan at the dose of 150mg/kg body weight produced marked hyperglycemia as evident from significant (p<0.05) elevation in serum glucose level in alloxanized diabetic control as compared with normal control group. Administration of
SC (Syzygium cumini) in alloxanized diabetic mice at the dose of 150 mg/kg body weight and 250 mg/kg body weight produced significant (p<0.05) fall in glucose level when compared with alloxanized diabetic mice. The serum glucose level reducing effect by SC at the dose of 250 mg/kg body weight was found to be more potent.

Serum lipid profile parameters

Biochemical parameters like serum cholesterol, TG, LDL-C and VLDL-C in diabetic control group were significantly (p<0.05) elevated as compared with the normal control group. Treatment with SC at the dose of 150 mg/kg b.w and 250 mg/kg body weight significantly (p<0.05) brought the cholesterol, TG, VLDL-C and LDL-C toward the normal values in a dose dependent manner. The HDL-C was found to be significantly decreased in the diabetic control group as compared to normal control group (p<0.05). Oral administration of SC in diabetic animals significantly (p<0.05) increased the HDL-C content as compared with the alloxan control group as shown in Figure 6-10.

Discussion

The world is facing an explosive increase in the incidence of Diabetes mellitus and cost effective complementary therapies are needed. Although insulin has become one of the most important therapeutic agents known to medicine, there is a continuing effort to find insulin substitutes, secretagogues, for sensitizers from synthetic or plant sources for the treatment of diabetes [21,22]. Animal models of diabetes are increasingly being used for patho-physiology and pharmacological studies of diabetes mellitus. Advantages of animal studies in the examination of alternative medicines and their efficacy include the ability to define experimental conditions more tightly and to undertake more detailed studies of the biologic effects of the agents being used [23].

In the present study, alloxan (150 mg/kg body weight) was used for making a perfect diabetic model, after induction of alloxan the level of blood glucose was higher than normal value. This elevated level, due to the alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of largerhans and thereby induces
hypoglycemia [24]. Another finding on alloxan is selectively toxic to β-cell because they preferentially accumulate in β-cell as glucose analogues through uptake via GLUT-2 glucose transporter. Alloxan in the presence of intracellular thiols, especially glutathione, generates reactive oxygen species (ROS) in cyclic reaction with its reduction product dialuric acid. The β-cell toxic action of the alloxan is initiated by free radicals formed, in this redox reaction autoxidation of dialuric...
acid generates superoxide radicals (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and final iron catalysed reaction step, hydroxyl radicals (OH$^-$). These hydroxyl radicals are ultimately responsible for the death of β-cell with their particularly low antioxidative defence capacity and ensuing state of insulin-dependent alloxan diabetes [14].

On the other hand, treatments of seed aqueous extract of Syzygium cumini (150 mg/kg body weight & 250 mg/kg body weight) on alloxanized diabetic mice, the elevated level of glucose were significantly decreased. This result agree with another finding of [25] indicating that the blood glucose lowering effect of S. cumini bark extract in oral glucose fed hyperglycaemic mice occurs within 30 min from the onset of S. cumini bark extract treatment. Phyto-chemical examinations of this plant have indicated the presence of flavonoids and other polyphenolics such as acetyl oleanolic acid, tannin, gallic acid, ellagic acid, quercetin, isouqueretin, kaempferol, myricetin, flavonol glycoside, triterpenoids, saponins and anthocyanid in different concentrations [26–28]. Most of these compounds isolated from different plants have previously been suggested to be the active anti-diabetic ingredients of various plant remedies. These natural compounds could act separately or synergistically to cause the hypoglycaemic effect [29].

As one of the complications that followed diabetic hyperglycemia is dyslipidemia. In our study alloxan (150 mg/kg, i.p) induced diabetic mice exhibited clear-cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum total cholesterol, triglycerides, LDL-C, VLDL-C and reduction of HDL-C levels, during this study period, which is in well agreement with previous reports [30,31]. The abnormal high concentration of serum lipids in diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that characterised the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [32,33]. According to the Framingham Heart study, dyslipidemia which can range from hypercholesterolemia to hyper lipoproteinemia is one of the many modifiable risk factors for coronary artery diseases, stroke, and peripheral vascular disease [34]. In the present study, the elevated level of serum CH, TG-C, VLDL-C and LDL-C in diabetic mice was normalized after oral administration of Syzygium cumini. This suggests that the extract may potent capability of inhabit the pathway of lipid synthesis in diabetic mice.

High density lipoproteins (HDL) are very important as possess anti-atherosclerotic and anti-inflammatory properties [35]. In the present research work, the level of HDL-C significantly decreased as compared to normal. Furthermore, the treatment of extract (150 and 250 mg/kg body weight for 21 days) increased the level of HDL-C, which denotes protective effect of atherosclerosis. The extract may cause increase in HDL-C level by inducing ApoA-1 production. In this respect Lahoz et al., [36] suggested that increase in HDL levels after treatment extract may be due to the induction of ApoA-1 production. Another reported by Rubin et al. [37] direct evidence for a protective effect of HDL in atherosclerosis has come from transgenic mice in which high levels of expression of apoprotein A-1 (apoA-1) increased the concentration of HDL and protected mice against diet induced atherosclerosis. These ameliorating effects clearly denote the anti-hyperlipidaemic potential of Syzygium cumini [38–40]. It could also be suggested that this anti-hyperlipidaemic effects of Syzygium cumini pass through a decrease in intestinal cholesterol absorption or a decrease in the biosynthesis of cholesterol specifically by decreasing the activity of HMG-CoA reductase inhibitors.

Conclusion

The beneficial effects on the regulation of serum glucose levels and hyperlipidemia in alloxan induced diabetic mice is evident with Syzygium cumini seed extract. Syzygium cumini at the dose of 150 and 250 mg /kg body weight appears to be effective for further studies, leading to possible drug development for diabetes. Development of phytomedicines is relatively inexpensive and less time consuming than allopathic drug development which is more expensive and spread over several years. In conclusion, the results from this study give scientific support to the use of Syzygium cumini in folklore medicine for the treatment of diabetes and show the potential role of anti diabetic activity.

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References


