Original Article

Liver protective effects of aqueous extract of Syzygium cumini in Swiss albino mice on alloxan induced diabetes mellitus

Bhaskar Sharmaa,*, Md. Sufiyan Siddiquia, Shiv Shunker Kumara, Gurudyal Ramb, Manisha Chaudharyc

a Department of Biochemistry, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad 211008, U.P, India
b Jacob School of Biotechnology & Bioengineering, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad 211008, U.P, India
c Department of Biological Science, Rani Durgavati University, Jabalpur 482001, MP, India

ABSTRACT

Background: Diabetes mellitus is one of the most common endocrine disorders accompanied with many metabolic syndromes. Use of herbal medicines has always been an option to treat a great number of diseases such as diabetes and its complications. The aim of the present study is to investigate the liver protective effects of Syzygium cumini seed extract in alloxan induced diabetic Swiss albino mice.

Methods: Eighteen Swiss albino mice (weighing 28–32 g) were randomly divided into control, alloxan treated and S. cumini treated mice group. Diabetes was induced in mice by injecting intraperitoneally alloxan monohydrate at dose of 150 mg/kg body weight. Aqueous extracts of S. cumini seed at dose of 250 mg/kg body weight were given orally in diabetic mice daily for three weeks after established LD50 value.

Results: In diabetic mice, the SGOT, SGPT, Bilirubin and serum glucose levels were significantly increased in comparison with the control groups. Statistical analysis ($p < 0.05$) of the data indicated that aqueous extract of S. cumini were significantly decrease serum contents of liver enzymes (SGOT, SGPT and Bilirubin) as well as serum glucose in treated groups.

Conclusion: The results suggested that aqueous extracts of S. cumini seed possesses liver protective effect against alloxan induced diabetic mice.

Copyright © 2013, JPR Solutions; Published by Reed Elsevier India Pvt. Ltd. All rights reserved.

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by decreased responsiveness of the organs to secreted insulin. Diabetes mellitus is a syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired
metabolism of glucose and other energy yielding fuels such as lipids and proteins. DM is a leading cause of end stage kidney disease, cardiomyopathy and heart attacks, strokes, retinal degeneration leading to blindness and non-traumatic amputations. Dyslipidemia, quite common in diabetic patients, is the main risk factor for cardiovascular and cerebrovascular diseases. DM is currently one of the most costly and burdensome chronic disease and is a condition that is increasing in epidemic proportions throughout the world. Diabetes is a serious illness with multiple complications and premature mortality, accounting for at least 10% of total health care expenditure in many countries. The prevalence of diabetes of all age groups worldwide is projected to rise from 171 million in 2000 to 366 million in 2030. Reason of this rise includes increase in sedentary life style, consumption of energy rich diet, obesity, higher life span, etc. DM is a major and growing health problem in most countries. It causes considerable amount of disability, premature mortality, and loss of productivity as well as increased demands on health care facilities. As diabetes aggravates and β-cell function deteriorates, the insulin level begins to fall below the body’s requirements and causes prolonged and more severe hyperglycemia. Hyperglycemia induces long term complications of diabetes such as cardiovascular complications and micro vascular complications such as retinopathy, nephropathy and neuropathy and foot ulcer.

Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicine are important. The attributed antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels. Most of these plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently implicated as having antidiabetic effects.

Alloxan was one of the most widely used chemical diabetogen during initial research work on experimental diabetes. It is a cyclic urea analogue of chemical composition 2,4,5,6-tetraoxo-hexa hydropyrimidine. Alloxan induces diabetes in animals and impairs glucose induced insulin secretion from β-cells of Islets of Langerhans of Pancreas. It has been reported that alloxan rapidly and selectively accumulates in β-cells and in comparison with non-b cells. Several reports directly or indirectly indicate that alloxan affects the membrane potential and ion channels in β-cells.

Syzygium cumini also called Eugenia jambolana (EJ) has been reported to have hypoglycemic effects both in experimental models and clinical studies. S. cumini seed apart from hypoglycemic activity has been reported to have anti-inflammatory, neuro psychopharmacological, antibiotic, anti-oxidant and ant diarrhoeal effects. In the present investigation, aqueous extract of seeds of S. cumini was used to evaluate the antidiabetic activity and liver protective effect in alloxan induced diabetic Swiss albino mice.

2. Materials and methods

2.1. Animals

Healthy Swiss albino mice of both sexes, weighing approximately (28–32 g) were used in the pharmacological studies. Before and during the experiment the animals were maintained in well-ventilated room at room temperature with natural day–night cycle in polypropylene cages lined with husk in standard environmental conditions (temperature (22 ± 2) °C, relative humidity (55 ± 10) % and 12:12 (light:dark cycle). The mice was fed on a standard pellet diet ad libitum and had free access to water. The experiments were performed after approval of the protocol by the (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008).

2.2. Preparation of plant material

The seed of S. cumini were procured from local market (Allahabad, U.P). The identity of the seeds of S. cumini was confirmed by Botanist, Department of Botany, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad, UP (India). The seeds were washed with distilled water and dried completely under the mild sun and crushed with electrical grinder coarse powder. Aqueous extract was made by dissolving it in distilled water using by mortar and pestle. The dose was finally made to 250 mg/kg body weight for oral administration after the LD_{50} estimation.

2.3. 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, SGOT, SGPT, bilirubin was purchased from Crest Coral Clinical Systems, Goa, India. Analytical grade ethanol was purchased from Merck Company (India).

2.4. Induction of hyperglycemia with alloxan

The selected mice were weighed, marked for individual identification and fast for overnight. The alloxan monohydrate at the rate of 150 mg/kg body weight were administered intraperitoneal (i.p) for making the alloxan induced diabetic mice model. Blood glucose level of these mice were estimated 72 h after alloxan administration, diabetes was confirmed by blood samples collected from the tip of the tail using a blood glucometer (Accu Sure, Taiwan). Animals with blood glucose level equal or more than 200 mg/dl were declared diabetic and were used in entire experimental group.

2.5. Experimental design

Mice were divided into three groups, with six mice in each group, as follows:

(i) group I – control mice, (ii) group II – alloxan-induced diabetic control mice, (iii) group III – diabetic mice given S. cumini seed extract (250 mg/kg) in aqueous solution daily for 21 days through Gavage’s method.
2.6. Animal sacrifice and sample collection

After the last dose, animals were fasted for 12 h and sacrificed. Blood samples were collected by orbital sinus puncture method. 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 and centrifuged at 3000 rpm for 15 min. at room temperature. The serum was collected carefully and kept at −20 °C until analysis. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) activities were measured according to the method described by Reitmann and Frankel while bilirubin activity was measured.

2.7. Histopathological studies

A portion of the liver was cut into two to three pieces of approximately 6 mm³ sizes and fixed in 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5 μm thickness of liver tissue were cut and stained with haematoxylin–eosin. The thin sections of liver were made into permanent slides and examined under high resolution microscope with photographic facility and photomicrographs were taken as shown in Figs. 5–7.

2.8. 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).

3. Results

3.1. Serum glucose level

The effect of aqueous extract of S. cumini seed on blood glucose levels is shown in Fig. 1. The mean level of glucose in the control group of mice was evaluated to be 74.33 ± 7.31 mg/dl (range 65–85) whereas it was 222.5 ± 22.52 mg/dl (range values 198–250) in alloxanized group. After the treatment of mice with the seed extract of S. cumini the glucose level decreased down to 91 ± 7.82 mg/dl having a range of 82–99 mg/dl. These variations in glucose concentrations are evident from Fig. 1. The significant increase in glucose concentration in the diabetic animals than that of the control mice is evident on alloxanization. However, the oral administration of aqueous extract of S. cumini significantly reduced the glucose level in serum when compared with alloxan induced diabetic mice.

3.2. Serum glutamate oxaloacetate transaminase (SGOT)

In Control group of mice SGOT activity was found to be 25 ± 5.06 IU/ml having the range of 20–32 IU/ml. In diabetics, its activity got raised to 50 ± 6.87 IU/ml with values ranging from 40 to 59. However, extract treatment of this group for three weeks resulted in decrease of SGOT activity to 35.83 ± 5.98 having values ranging from 25 to 41 IU/ml. These variations are depicted by the box-plot in Fig. 2.

3.3. Serum glutamate pyruvate transaminase (SGPT)

In Control group mouse SGPT activity was found to be 20.71 ± 4.96 having range values between 15 and 26.54 IU/ml which got raised to 53.83 ± 6.70 (range values 45–63) IU/ml in diabetic mice. However, after the treatment of mice with the seed extract of S. cumini, the activity decreased down to 30.83 ± 4.87 (ranging between 25 and 38) IU/ml. These values are compared by the box-plot as evident in Fig. 3.

3.4. Bilirubin levels in various groups

Bilirubin level of control mice was observed to be 0.53 ± 0.054 mg/dl (values ranging between 0.44 and 0.60) which got increased to 0.82 ± 0.093 mg/dl in alloxan induced
diabetic mice. Bilirubin contents ranged from 0.70 to 0.90 in diabetic mice. However, after the treatment of diabetic mice with the seed extract of *S. cumini*, the bilirubin level decreased down to the mean value of 0.65 ± 0.053 having values ranging from 0.59 to 0.72 mg/dl. These variations along with statistical significance are depicted by box-plot as shown in Fig. 4.

### 4. Discussion

Diabetes mellitus patients in India are increasing day by day probably due to change in life style change in food pattern i.e. from traditional fibre rich diet to sugary fast food diet and also because of genetic basis. The disorder being chronic in nature needs long term treatment to prevent the complications arising due to persistent high blood glucose level. Pharmacotherapy available for the treatment of diabetes in modern healthcare system includes insulin and oral 16 hypoglycemic drugs. However due to economic constraints, it is not possible for majority of the diabetic patients in developing countries like India to use these drugs on regular basis. Moreover these synthetic antidiabetic drugs are associated with large number of adverse effects. Hence there is increase in the trend to use traditional indigenous plants widely available in India for the treatment of diabetes mellitus. Over 150 plant extract and some of their active principles including...
flavonoids, tannins, alkaloids etc are used for the treatment of diabetes.25

During the present investigation, alloxan (150 mg/kg i.p) was used to induce diabetes in mice and their serum glucose levels were found to be significantly elevated as compared to normal mice. The increased levels of serum glucose may be due to the partial damage of the pancreatic β-cells. Alloxan, a β-cytotoxin, induces "chemical Diabetes" in a wide variety of animal species including rats by damaging the insulin secreting β-cells.17,26 Similar results reported by Vuksan & Sievenpiper,27 shows that the administration of alloxan significantly increases the level of glucose when compared to control, which might account for the cytotoxic effect of alloxan on beta cells. Alloxan is relatively toxic to insulin producing pancreatic β-cells because it preferentially accumulates in β-cells through uptake via the GLUT-2 glucose transporter. This cytotoxic action is mediated by ROS source of generation of ROS is dialuric acid, a reduction product of alloxan. These radicals undergo dismutation to H2O2. The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells, thereby decreasing the secretion of insulin, which in turn increases the blood glucose level. Another result of alloxan, a β-cytotoxin, was preferred to produce the diabetic state in mice as it induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cell resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues.28

On the other hand, treatment of extract (250 mg/kg b.w) for 21 days, the elevated level of serum glucose level was significantly decreased. Our results are similar to previous reports.29,30 The antidiabetic activity of aqueous extract of S. cumini may be its promote insulin secretion by closure of K+-ATP channels, membrane depolarization and stimulation of calcium influx, an initial key step in insulin secretion. In this context, number of other plants has also been reported to have antidiabetic and insulin stimulatory effects.31 Flavonoids sterols, triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles.32 Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats.33 Phenolics are found to be effective antihyperglycemic agents. On this basis we have selected the glucose induced hyperglycemic model to screen the antihyperglycemic activity of the plant extracts.

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin. Hepatocellular damage causes release of these enzymes into circulation. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, infarction, and muscular damages. ALT, which mediates conversion of alanine to pyruvate and glutamate, is specific for liver and is a suitable indicator of hepatic injuries.24 In the present study, the level of SGOT, SGPT and bilirubin level were significantly increased.35 Increased level of serum marker enzymes due to directly conversion of amino acids to keto acids are AST and ALT. Inflammatory hepatocellular disorders results in extremely elevated transaminase levels.36 The increase in the activities of plasma AST and ALT indicated that diabetes may be induced hepatic dysfunction. Supporting our findings it has been found by Larcan et al.37 that liver was necrotized in diabetic patients. Chronic mild elevation of amino transferase is frequently found in type 2 diabetic patients. Therefore, an increase in the activities of AST and ALT in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream.38 Further that, our results on the recovery after treatment with S. cumini seed extract are in parity with findings with other plants reported by other workers.30–41

In conclusion, the present study demonstrated that the treatment of diabetic mice with S. cumini has exerted a considerable hypoglycemic effect. In addition, these herbs could be liver damage associated with alloxan diabetes. However, further biochemical studies should be conducted to promote using of these herbs as antidiabetic agents.

Conflicts of interest

All authors have none to declare.

Acknowledgements

Authors are thankful to Director, Mahavir Cancer Sansthan & Research Centre, Patna, Bihar (India) for providing required facilities for the current study. We also thank Head of the Department for providing the animals for the present work.