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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIDIABETIC ACTIVITY OF LEAF EXTRACTS OF *SYZYGIUM CUMINI* IN ALLOXAN AND DEXAMETHASONE INDUCED DIABETES IN RATS.

Pharmaceutical Science

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ABSTRACT

Objective: This study aimed to evaluate the antidiabetic effects of the leaf extracts of Syzygium cumini in Alloxan and Dexamethasone induced diabetes in rats. The present investigation indicated that the leaf of Syzygium cumini possessed significant antihyperglycemic potential which may prove the claimed use of the plant in amelioration of diabetes. **Methods:** Healthy young male wister Rats weighing from 160-180 mg of 8 – 12 weeks old were selected prior to the induction of diabetes. Experimental diabetes was induced by a single intraperitoneal injection of 150 mg/kg of alloxan monohydrate and Dexamethasone (10mg/kg, S.C.) freshly dissolved in 0.1 M citrate buffer (pH=4.5). Then, the solution was immediately administered intraperitoneally to each young male Wister Rats. After 48 Hrs, rats with marked hyperglycemia (fasting blood glucose >300mg/ dl) were selected to determine the efficacy of extracts of the plant. After successfully developing the diabetes animals were divided into seven groups and each group contains six rats. Group I: Normal control mice administered vehicle only; Group II: Diabetic control rats administered vehicle only; Group III: Tested rats administered glibenclamide 5 mg/kg; and Group IV–VII: Tested rats administered Syzygium cumini at doses of 250, and 500 mg/kg of ethanolic and aqueous extracts respectively. The effect of the extracts on body weight also performed for initial day and final day. **Results:** The effect of Syzygium cumini having significant ($p<0.05$ and $p<0.001$) reduction in BGL starting 1 hr when compared to the negative control. Administration of Alloxan monohydrate (150mg/kg, i.p.) and freshly prepared aqueous solution of Dexamethasone (10mg/kg, s.c.) to the rats produced significant ($p<0.001$) increase in BGL 30 min following 1 h after glucose loading, confirming the induction of hyperglycemia. The extracts with two doses (250 and 500 mg/kg) showed a significant reduction in BGL. The phytochemical screening of the leaves extract was done for the presence of alkaloid, saponin, terpene, carbohydrate, steroid, protein, cholesterol, flavonoids. **Conclusion:** It is concluded from this study that the ethanolic and aqueous extracts of Syzygium cumini leaves possess significant antihyperglycemic effects.

KEYWORDS

INTRODUCTION

Hyperglycemia is a long term metabolic related disorder evident in the form of diabetes as a defect of insulin secretion and/or insulin action along with an imbalance in the liver metabolism of lipids, proteins and mainly in carbohydrates. The incidence of hyperglycemia has reached epidemic proportions predominantly due to obesity, intake of food especially carbohydrates rich foods, cystic fibrosis, and mitochondrial defects [1]. hyperglycemia is a serious metabolic disorder with micro and macrovascular complication that results in significant morbidity and mortality [2]. Chronic diabetes mellitus causes glycation of body proteins that in turn leads to secondary complications affecting eyes, renal, nerves and arteries [3]. Modern medicines like Biguanides, Sulphonylureas and Thiozolidinediones are available for the treatment of hyperglycemia. But they also have undesired effects associated with their uses [4]. The epidemiological status of diabetes has stimulated the researchers for new innovations and targets for the eradication of this incurable disease. Search for safer and effective hypoglycemic agents is a continuous challenge in managing hyperglycemia [5].

Syzygium cumini Linn. (Syn. Eugenia jambolan) is a large evergreen tropical tree. It is belongs to the family Myrtaceae. S. cumini is also known as Jamun or black plum or jambolan. S. cumini is very well known for their pharmacological properties ancient age. The native home of the Syzygium is India and East Indies. It is found throughout in India up to an altitude of 1800 meters and its habitat starts from Myanmar and extended to Afghanistan. Syzygium is also found in other countries like Thailand, Philippines, Madagascar, extensive work were carried out on S. cumini for their pharmacological properties. The medicinal value is due to presence of malic acid. [6,7].

METHODS

Chemicals And Reagents

All chemicals, reagents and solvents used in the study were of analytical grade.

Collection, Identification and Authentification of the Plant.

The leaves of Syzygium cumini, were collected from the malappuram distric, kerala, India, during the month of October 2013. The plant materials were identified and authenticated by Dr. Pradeep, Botanist, Calicut University, Kozhikode. The voucher herbarium specimen (Syzygium cumini Linn .2013/7) has been deposited in the department of botany.

Preparation Of The Ethanolic Extract Of Dried Leaves Of *Syzygium Cumini*:

The granulated dried leaves of *Syzygium cumini* (500 g) was packed in a Soxhlet apparatus and subjected to continuous hot percolation for using 450 ml of ethanol (95 % v/v) as solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 75 g, 15 % w/w). The extract was suspended in 5 % gum acacia and used for further experiments.

Preliminary Phytochemical Screening

The alcoholic and aq. extracts of the leaves of *Syzygium cumini* were screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides etc.

The both extracts results were tabulated in the Table No.1

Experimental Animals

Animals were selected as per the OECD guidelines. Healthy young male wister Rats weighing from 160-180 mg of 8 – 12 weeks old were selected. There were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (CPCSEA/IAEC No.-25/50/2013/09 Ministry of social justice and empowerment, Government of India) after scrutinization. The animals received the drug treatments by oral routs.

Experimental procedure:

Alloxan Induced Antidiabetic Activity[8-10]

Procedure

The alloxan induced diabetic rats were divided into 7groups of 6 each.

Group 1 - Normal untreated rats
Group 2 - Diabetic control
Group 3 - Diabetic rats treated with 5 mg/kg of Glibenclamide
Group 4 - Diabetic rats treated with 250mg/kg of Ethanol extract

Group 5 - Diabetic rats treated with 500mg/kg of Ethanol extract
Group 6 - Diabetic rats treated with 250 mg/kg of Aqueous extract
Group 7 - Diabetic rats treated with 500 mg/kg of Aqueous extract

Blood samples were collected for the measurement of blood glucose level from the tail vein at 0, 1, 2 and 3 hrs The blood glucose level was monitored by using electronic glucometer (one touch Horizon). The blood samples were collected in morning 1 hr after drug administration of extracts on day 1, 3,6,14.

The results were recorded and presented in Table no: 2

Dexamethasone Induced Antidiabetic Activity [11]
The Dexamethasone induced diabetic rats were divided into 7groups of 6 each.
Group 1 - Normal untreated rats
Group 2 - Diabetic control
Group 3 - Diabetic rats treated with 5 mg/kg of Glibenclamide
Group 4 - Diabetic rats treated with 250mg/kg of Ethanol extract
Group 5 - Diabetic rats treated with 500mg/kg of Ethanol extract
Group 6 - Diabetic rats treated with 250 mg/kg of Aqueous extract
Group 7 - Diabetic rats treated with 500 mg/kg of Aqueous extract

At the end of the experimental period, i.e. on day 11, the overnight fasted animals were anaesthetized with ether and blood was collected by retro-orbital puncture method. Serum was separated by using centrifuge machine at 5000 rpm for 5 min and stored at 4-8° C until use. The results were recorded and presented in Table no: 3

Statistical Analysis
Statistical evaluation was done by using one-way analysis of Variance (ANOVA) followed by Dunnett's test. P values <0.05 were Considered significant.

RESULTS AND DISCUSSION

Table 1: Screening Of Preliminary Phytochemicals Of *Syzygium Cumini*

Constituents	Test	*Ethanolic extract*	*Aq. extract*
Carbohydrates	• Molisch Test	+	+
	• Fehling's Test	+	+
	• Benedict's test:	+	-
	• Barfoed's test:	+	+
Alkaloids	• Dragendroff's Test	+	+
	• Wagner's test	+	+
	• Mayer's Test	+	+
	• Hager's Test	+	+
Steroids and Sterols	• Liebermann Burchard test	-	-
	• Salkowski test	+	+
Glycosides	• Legal's test	+	+
	• Baljet test	+	+
	• Borntrager test	+	+
	• Killer Killani test	+	+
Saponins	• Foam test	+	+
Flavonoids	• Shinoda test	+	+
Tri-terpenoids	In the test tube, 2 or 3 granules of tin+2ml of thionyl chloride solution and test solutionis added.Pink color	+	+

+ Present, - Absent

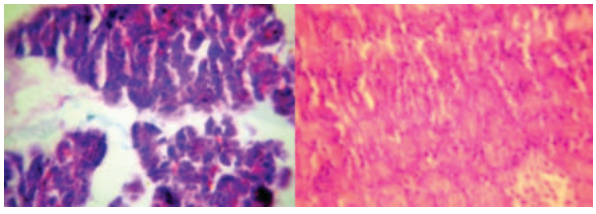
Table 2: Effect Of Ethanolic And Aqueous Extracts Of Leaves Of *Syzygium Cumini* On Blood Glucose Level On Alloxan Induced Rats

S. No	Treatment	Blood glucose level in mg/dl				
		Initial	3st day	6th day	9th day	14th day
1	Normal control 5% cmc solution 10 ml/kg	80.05±31.833	82.8±11.32	76.53±15.28	78.83±21.39	85.5±15.79
2	Diabetic control 1 50mg/kg	304.5±23.1	321.16±10.9	323.33±19.35	331.66±15.38z	338.16±19.51
3	Glibenclmide (5mg/kg)	300.50±6.01	261.88±3.32*	234.0±9.02*	161.7.±3.88*	131.0±13.39*
4	Alloxan+Alcoholic Ext.(250 mg/kg)	307.66±4.26	271.58±6.82*	242.0.±40.93*	214.0±11.68*	154.56±7.09*
5	Alloxan+Alcoholic Ext(500 mg/kg)	311.166±3.86	264.83±6.17*	242.44±12.50*	204.0±5.87*	142.75±10.48*
6	Alloxan+Aqueous Ext. (250 mg/kg)	313.66±11.13	276.56±10.53*	254.16±51.74*	211±12.62*	156.16±10.63*
7	Alloxan+Aqueous Ext. (500 mg/kg)	302.0±10.36	271.66±5.88*	245.16±9.52*	88.730±9.77*	143.33±13.87*

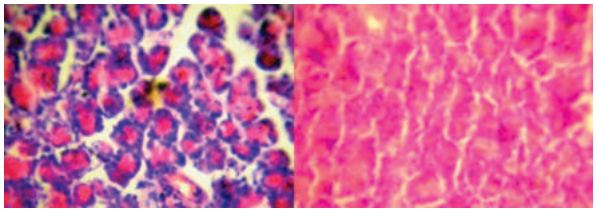
Table 3: Effect Of Ethanolic And Aqueous Extracts Of Leaves Of *Syzygium Cumini* On Blood Glucose Level On Dexamethasone Induced Rats After 11 Days.

S. No	Groups	Serum glucose concentration (mg/dl) Mean ± SEM
1	Normal control	87.392±602
2	Diabetic control	181.922±3.716
3	Standard treated glibenclamide (5mg/kg)	114.34±2.74*
4	Ethanol Ext. 250 mg/kg	151.37±3.09*
5	Ethanol Ext. 500 mg/kg	145.185±3.14*
6	Aqueous Ext.250 mg/kg	148.47±1.96*
7	Aqueous Ext.500 mg/kg	145.1823±2.51*

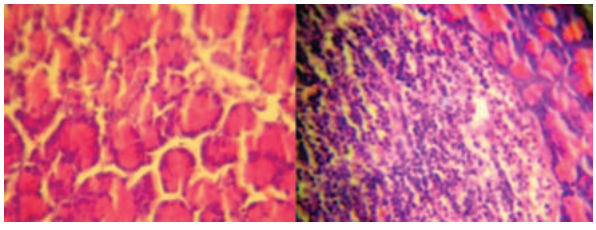
Histopathological Studies



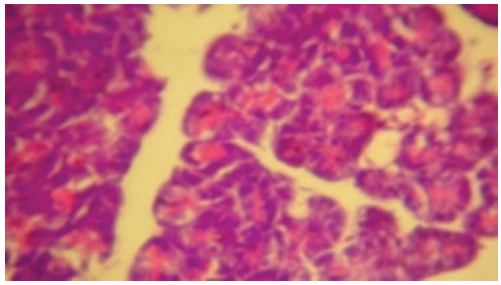
A. Normal Control B. Diabetic Control



C. Standard D. Ethanolic. Ext 250mg/kg



E. Ethanolic.Ext 500mg/kg F.Aqueous.Ext 250mg/kg



G. Aqueous.Ext 500mg/kg

Fig no. 36 Histopathology of Rat Pancreas [Alloxan induced]

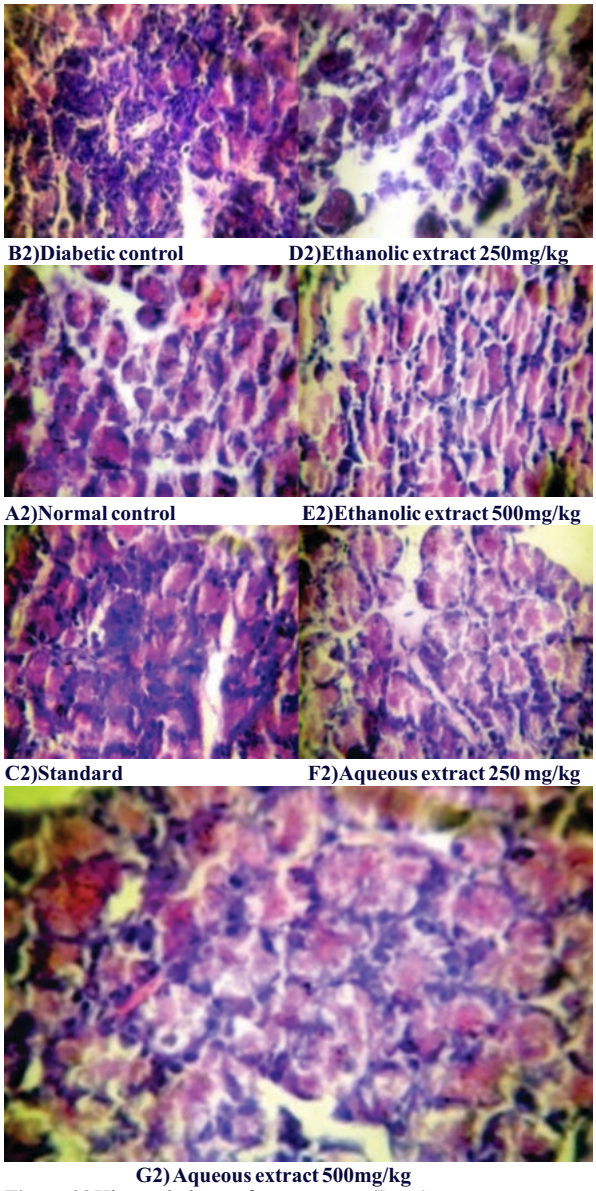


Fig no; 38 Histopathology of rat pancreas (Dexa)

DISCUSSION

Regardless of that, there are many antihyperglycemic drugs available in the market for the treatment of hyperglycemia, but this metabolic disorder with its complications is serious socioeconomic burden both for developed and for developing countries. There are many plants that possess antidiabetic properties and these are used in the treatment of hyperglycemia globally. More than 400 plant species having antidiabetic activity are reported, but research for new active antidiabetic plants is necessary because natural compounds have not well-reported side effects on the body. The majority of secondary metabolites used for the treatment of hyperglycemia include carotenoids, flavonoids, terpenes, and glycosides. These secondary metabolites are commonly concerned for having antidiabetic activity [12] Frequently, the plant's medicines are given for therapeutic purposes. [13,14]

Alloxan is a chemical that is harmful to living body because it damages the pancreatic β cells. Alloxan decreases the secretion of insulin from pancreatic β cells, leading to extracellular hyperglycemia Numerous studies confirmed that many of the plant drugs effectively decrease the GL in alloxan-induced diabetic rats. The mean (SD) of blood GLs of rats after IP administration of various doses of studied plant extracts at different time period are shown in Figure. Results revealed that administration of *Syzygium cumini* at dose concentrations 250, 500 mg/kg body weight decreased the blood GL from above 200 mg/dL respectively. The given dose of *Syzygium cumini.*

Dexamethasone is a synthetic glucocorticoid whose chronic exposure to high doses causes insulin resistance [15] In the present study, experimental induction of insulin resistance by dexamethasone at dose of 1 mg/kg administered continuously for 8 days led to profound alterations of metabolic parameters characterized by high levels of total cholesterol, LDL cholesterol, and triglyceride and low levels of HDL cholesterol, total protein, and body weight in rats. Insulin resistance promotes the increase of hormone sensitive lipase activity of adipose tissue and the decrease of lipoprotein lipase activity. .is leads to an increase in fatty acids mobilization from adipocytes and an increase in hepatic synthesis of triglycerides, which are released into the bloodstream as VLDL cholesterol [16] In the present study, the aqueous extract of *Syzygium cumini* protected the rats against the significant reduction of total protein levels induced by dexamethasone. .is effect would reflect an insulin sensitizing effect of these extracts and, consequently, a decrease in proteolysis decrease in body weight and relative liver weight of rats observed was not affected by the extract. From the results of this study, dexamethasone did not modify significantly the blood glucose of rats. However, in animals treated with the aqueous extract at dose of 500 mg/ kg, there was a significant decrease in blood glucose. The results suggest that *Syzygium cumini* extracts may also act by stimulating insulin secretion and thus have hypoglycemic effects. These hypoglycemic effects could be related to the presence of flavonoids and flavonols in the plant extract. showed that these compounds possess antidiabetic properties. In normal and dexamethasonetreated rats, the aqueous extract of *Syzygium cumini* was effective in preventing the impaired glucose tolerance observations suggest that *Syzygium cumini* would be able to prevent dexamethasone induce insulin resistance.

AUTHORS CONTRIBUTION

I, Mr. Bhagwant singh, assistant professor, has done the above the work including data search, data collection, interpretation to conclude the whole work under the guidance and supervision of Professor Dr. Rajasekaran.S Department of Pharmacology, Institute of Pharmaceutical sciences and Research centre, Bhagwant University, Sikar road, Ajmer, Rajasthan, India, under whom I am pursuing my present research work as Ph.D. candidate.

Conflicts Of Interests
No conflict.

Source Of Funding
Nil.

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