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Evaluation of Anti-Diabetic Activity of Various Extract of Syzygium Cumini (Seed) and Ficus Benghalensis (Bark) in Streptozotocin Induced Rat

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ABSTRACT:
The Antidiabetic effects of ethanolic extract of Syzygium cumini (Seed)and Ficus benghalensis (bark) was examined and evaluated in Diabetes mellitus induced in Male Wistar Albino rats using Streptozotocin which was also compared with hypoglycemic and hypolipidemic effects of Metformin. It is generally used in Diabetes mellitus treatment in humans as well as canines. The drugs studied systematically, analyzed and compared with plant extracts. For induction of diabetes, Male Albino Wistar rats of average weight about (160-180)gm were intraperitoneally administered with Streptozotocin a crystal pink powder, dissolved in cold NS, at the dose rate of 50 mg/kg BW. Diabetes induction was confirmed by evaluating the blood glucose levels. The blood glucose level of selected rats for this study was about 160 mg/dL. Streptozotocin induced diabetic rats of Group II showed increase in the levels of total cholesterol, serum triglycerides, serum creatinine, blood urea nitrogen and blood glucose till the end of this study. Group IV, treated with STZ+ S.c and F.b at a dose 200 mg/kg BW significantly reduced the elevated blood glucose level, total cholesterol, serum triglycerides, blood urea nitrogen (BUN); and serum creatinine level was restored near to the normal range.In this study, results showed that Syzygium cumini(seed) and Ficus benghalensis (bark) of ethanolic extract showed both hypoglycemic and hypolipidemic activities, which can further be studied for the advancement of the drug discovery for the Diabetes mellitus treatment.
KEYWORDS: Hypoglycemic, hypolipidemic, Metformin, streptozotocin, Ethanol.

I. INTRODUCTION:

The pancreas secretes the hormone insulin, which facilitates the uptake of glucose by our cells which is utilized as a source of energy. Diabetes mellitus is taken from the Greek word diabetes, meaning siphon-to pass through and the Latin word mellitus meaning sweet. A metabolic condition known as hyperglycemia in which there are high levels of sugar in the blood. Diabetes increases the chance of harming the heart, kidneys, nerves and eyes. Diabetes is connected to certain cancerous form. An examination of history reveals that the term "diabetes" was initially used by Memphis's Apollonius between 250-300 BC. The term Diabetes mellitus originated with its dissemination by the ancient Greeks, Indians, and Egyptians. At the University of Toronto, Mering and Minkowski [1889] identified the function of cow's pancreas, which resulted in the development of a successful diabetic treatment in 1922.

Diabetes is a chronic, metabolic disease characterised by elevated levels of blood glucose [or blood sugar], which leads overtime to serious damage to the heart, blood vessels, eyes, kidney and nerves.

1.1 TYPES OF DIABETES MELLITUS:

There are several types of diabetes. The most common form include

1. Type 1 Diabetes [or] insulin dependent diabetes mellitus
2. Type 2 Diabetes [or] non -insulin dependent diabetes mellitus
3. Gestational Diabetes.

1.2 TYPE 1 DIABETES MELLITUS:

This illness is autoimmune-related. Our body produce very little or no insulin. Our immune system targets and kills the insulin-producing cells in our pancreas. Although it can manifest at any age, type 1 diabetes is typically diagnosed in children and young people. Insulin must be taken daily by people with type 1 diabetes in order to

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survive. Up to 10% of diabetics have type 1 diabetes.

1.3 TYPE 2 DIABETES MELLITUS:

Our body's cells don't use insulin as it should. Insulin resistance is the result of the pancreas producing insufficient insulin, even if it producing enough to maintain our blood glucose levels within normal limits. Diabetes type 2 is the most prevalent kind of the disease. Although it primarily affects adults, children.

1.4 GESTATIONAL DIABETES:

This type of Diabetes develop in some people during Pregnancy. Gestational diabetes goes away after pregnancy. However, if you have gestational diabetes, you're at a higher risk of developing type 2 diabetes later.

1.5 OTHER TYPES:

- Latent autoimmune diabetes in adults [LADA]
- Maturity onset diabetes of the young [MODY]
- Neonatal diabetes
- Brittle diabetes

Insulin promotes the entry of glucose and amino acids into muscle cells in muscular tissue. Glycogen, a storage molecule that can be broken down to provide energy for muscular contraction during exercise and for fasting, is where the glucose is stored. Protein synthesis is carried out by the amino acids that are carried into muscle cells in response to insulin stimulation. On the other hand, in the absence of insulin, muscle cell protein is broken down to provide the liver with amino acids, which it then converts into glucose. Diabetes mellitus is a disorder caused by insufficient insulin synthesis. Insulin injections are periodically needed for severe diabetes. Hormone extracts from sheep, cattle, and pigs were used in the first insulin injections, but by the early 1980s, some bacterial strains had been genetically altered to generate human insulin. Today, human insulin produced by recombinant DNA technology is the mainstay of treatment for Diabetes mellitus.

II. PLANT PROFILE:

TABLE NO: 1 BOTANICAL CLASSIFICATION OF SYZYGIUM CUMINI:

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	cumini

FIG. NO: 01: SEED OF SYZYGIUM CUMINI

International Journal of Pharmaceutical Research and Applications
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TABLE NO: 2 BOTANICAL CLASSIFICATION OF FICUS BENGHALENSIS

Kingdom	Plantae
Sub Kingdom	Tracheobiota
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Hamamelidae
Order	Urticales
Family	Moraceae
Genus	Ficus
Species	benghalensis

FIG. NO: 02: BARK OF FICUS BENGHALENSIS

III. MATERIALS AND METHODS:

3.1 CHEMICALS:

All the chemicals and reagents used in the study were of analytical grade and procured from reputed Indian manufactures.

3.2 COLLECTION AND AUTHENTICATION OF PLANT:

The seed of Syzygium cumini and bark of Ficus benghalensis were collected from the natural habitat in and local area, Tamilnadu and the Plant material were authentified by Dr.KN Sunil kumar., research officer/Sci-II HOD, Department of Pharmacognosy and by Dr.P.Elankani., research officer [siddha], Sci-IV/Incharge, SIDDHA CENTRAL RESEARCH INSTITUTE [central council for research in siddha ministry of AYUSH, Government of India],Arumbakkam,Chennai-601106.

3.3 PREPARATION OF EXTRACTS BY MACERATION METHOD:

The seeds of Syzygium cumini and bark of Ficus benghalensis were shade dried for a week. The dried plant material was powdered, and 10g of Syzygium cumini seed + 10g of Ficus benghalensis bark powder mixed well and subjected to different methods of extraction. 99% of ethanol were macerated for 72 hours. Various chemicals like acetone, aqueous were macerated for 72 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in refrigerator for further use.

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FIG. NO: 03: PREPARATION OF EXTRACTS BY MACERATION METHOD

3.4 METHODOLOGY:

METHODS:

The anti-diabetic activity of plant extracts can be determined by the following methods

1. In-vivo Methods
2. In-vitro Methods

3.5 IN-VIVO METHODS:

Experimental Diabetes mellitus is generally induced in laboratory animals by several methods that include: chemical, surgical and genetic (immunological) manipulations. Most of the experiments in diabetes are carried out in rodents, although some studies are still performed in larger animals.

3.6 STREPTOZOTOCIN:

Streptozotocin is naturally occurring chemical, used to produce Type- I diabetes in animal model and Type-2 diabetes with multiple low doses. It is also used in medicine for treating metastatic cancer of Islets of Langerhans. Streptozotocin was originally identified in the late 1950s as an antibiotic. The drug was discovered in a strain of the soil microbe Streptomyces achromogenes. The soil sample in which the microbe turned up had been taken from Blue Rapids, Kansas, which can therefore be considered the birthplace of streptozotocin.

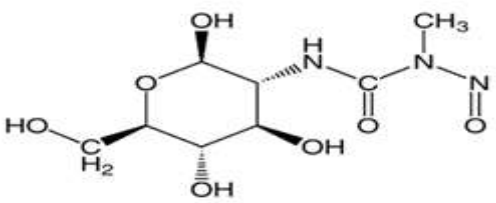

FIG. NO: 04: STUCTURE OF STREPTOZOTOCIN

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Mechanism of Streptozotocin:STZ is a broad-spectrum antibiotic that is toxic to the insulin producing ß cells of pancreatic islets. It is currently used clinically for the treatment of metastatic Islet cell carcinoma of the pancreas and has been used in a wide variety of large and small animal species. The method of STZ action in ß cell depletion has been studied extensively over the years. Streptozotocin prevents DNA (Deoxyribonucleic acid) synthesis in mammalian and bacterial cells. In the bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The streptozotocin enters the pancreatic cell via a glucose transporter- GLUT2 (Glucose transporter 2) and causes alkylation of DNA. Further STZ induces activation of Poly adenosine diphoshate ribosylation and nitric oxide release, as a result of STZ action, pancreatic -cells are destroyed by necrosis and finally induced insulin dependent diabetics.

Objective: The diabetogenic activity of the antibiotic Streptozotocin (STZ) is cytotoxic especially to Beta cells of the pancreas. Diabetes induction in laboratory animals, mostly in rats, by STZ has become a valuable tool in diabetes research being used by many investigators. Parp-deficient mice are almost resistant to STZ-induced diabetes.

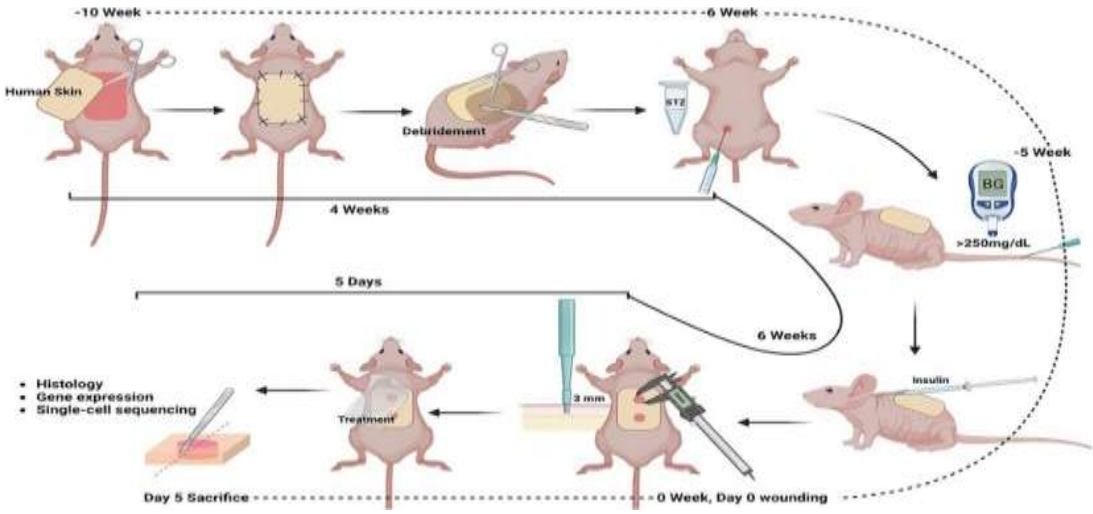

FIG. NO: 05: MECHANISM OF STREPTOZOTOCIN

Method: Male Wistar Albino rats weighing 150-220 g are used. STZ (60 mg/kg) is injected intravenously. The serum insulin values decrease up to 4 times, after six to eight hours of injection, resulting in a hypoglycaemic phase after persistent hyperglycaemia. Diabetic symptoms severity and onset depend on the dose of STZ. After the dose of 60 mg/kg i.v., symptoms occur already after 24- 48 h with hyperglycaemia up to 800 mg%, glycosuria and ketonemia. Histologically degranulation of the beta cells is seen. After 10-14 days a steady state is reached allowing using the animals for pharmacological tests.

3.7 EXPERIMENTAL DESIGN:

- **Animal:** MaleWistar Albino Rat
- **Sex:** Male
- **Age:** 6-8 weeks
- **Animal number:** 25Nos
- **Inducing agent :** STZ (60 mg/dl) administered subcutaneously (s.c.)
- **SCFBE** = Ethanolic extract of Syzygium cumini (seed) and Ficus benghalensis (bark)
- **Low dose** (200mg/kg) and
- **High dose** (400 mg/kg)

3.8 INVITRO STUDY:

The various in-vitro testing methods for anti-diabetic activity of plant extracts are

3.8.1 ENZYME INHIBITION ASSAY FOR ANTI-DIABETIC ACTIVITY:

α- AMYLASE INHIBITION ASSAY: The assay was performed with slight modification of a previously reported method. The test sample was prepared in dimethyl sulfoxide from 1 µg/mL-1 stock solution and the sample was added to a 0.5 mg/ml α-amylase solution and incubated for 10min at room temperature. 1.0% starch solution (500µL) was added and incubated at room temperature at

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar – Apr 2025, pp: 838-846 www.ijprajournal.com ISSN: 2456-4494

10min. After that I ml of di-nitro-salicylic acid was added to the reaction mixture and heated in a boiling water bath for 5 minutes. After cooling, tested samples were diluted with 10 ml of distilled water. The absorbance was measured at 540nm. The percentage of enzyme inhibition activity of the bioactive fractions were calculated using the following formula shown in Equation (1)

Inhibition activity (%) = Abs Sample – Abs Control / Abs Sample × 100

Where, Abs Sample is absorbance of test samples and Abs Control is the Absorbance of Control reactions (contains all reagents except the test sample). All the experiments were carried out in triplicates.

3.9 ANTI-DIABETIC EVALUATION:

3.9.1 EXPERIMENTAL INDUCTION OF DIABETES :

After fasting for 18hrs, rats were injected intraperitoneally with a single dose of 50 mg/kg streptozotocin after dissolving it in freshly prepared ice-cold citrate buffer (pH 4.5). After the injection, they had free access to feed water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock. The development of diabetes was confirmed after 48h of the streptozotocin injection. The rats having fasting blood glucose level more than 200 mg/dL were selected for experimentation.

3.9.2 BLOOD SAMPLE COLLECTION:

Collection of blood samples by end tail vein cutting method and blood glucose level was determined by using one touch electronic glucometer using glucose strips.

3.9.3 EVALUATION OF PARAMETERS:

General parameters Body weight: 5, 10, 15 and 21 day to determine the blood glucose level by electronic glucometer. The group I consist of 6 normal control animals. The remaining each group consists of 7 Streptozotocin (STZ) induced diabetic rats. Group I–Normal control animals received saline orally for 15 days; Group II–STZ induced diabetic animals received , per orally for 15 days; Group III–STZ induced diabetic animals received Metformin (5mg/kg) orally daily for 15 days; Group IV–STZ induced diabetic animals received Ethanolic extract at the dose of 200 mg/kg daily per orally for 15 days; GroupV–STZ induced diabetic animals received Ethanolic extract at the dose of 400 mg/kg daily per orally for 15 days. Blood samples were collected one hour after drug administration on the day 1.

IV. RESULTS:

4.1 PRELIMINARY PHYTOCHEMICAL SCREENING:

TABLE NO: 3 PRELIMINARY PHYTOCHEMICAL SCREENING:

S.NO	CHEMICAL CONSTITUENTS	REPORT
1.	Alkaloids	+
2.	Flavonoids	+
3.	Phenols	+
4.	Glycosides	+
5.	Saponins	+
6.	Carbohydrates	+
7.	Tannins	+
8.	Steroids	+
9.	Protein and amino acids	+
10.	Gum and Mucilage	+
11.	Tritepenoids	+

4.2 ESTIMATION OF BODY WEIGHT:

The Effect of the different doses of Ethanolic extract of Syzygium cumini (seed) and Ficus benghalensis (bark) on body weight

GROUP	BODY WEIGHT
Group I Normal saline	180.16 ±6.93
Group II STZ (60mg/kg)	160.06 ± 5.31***

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Group III STZ + Metformin (5mg/kg)	**190.31 ± 5.30****
Group IV STZ + SEFBE (200mg/kg)	**178.56 ± 5.29***
Group V STZ + SEFBE (400mg/kg)	**188.10 ±7.30**

TABLE NO: 4 ESTIMATION OF BODY WEIGHT

The values were expressed as Mean ± S.E.M (n=5animals in each group)
*,**,***, indicates significance p<0.05, p<0.01 and p<0.001 when p<0.05 and p<0.01 Standard (Group III) and Syzygium cumini and Ficus benghalensis (200mg/kg) and (400mg/kg) showed statistically increase in body weight when compared to Diabetic control (Group II)

4.3 ESTIMATION OF BLOOD GLUCOSE LEVEL:

The effect of different doses of Ethanolic extract of Syzygium cumini (seed) and Ficus benghalensis (bark).

Group	Blood glucose level (mg/dl)		
	Initial stage	2nd week	3rd week
Group I (saline)	**82.15±15.76**	**81.11±13.23**	**82.13±14.05**
Group II STZ(60 mg/dl)	**320.27±26.31**	**336.28±32.08**	**352.27±36.10*****
Group III STZ+ Metformin(5 mg/kg)	**300.15±11.18**	**256.10±12.06**	**135.01±15.02****
Group IV STZ + SEFBE (200mg/kg)	**285.17±18.31**	**186.12±7.03**	**126.08±13.15**
Group V STZ + SEFBE (400mg/kg)	**226.10±13.20**	**122.17±12.08**	**118.22±10.0**

TABLE NO: 5 ESTIMATION OF BLOOD GLUCOSE LEVEL

The values were expressed as Mean ± S.E.M (n=6 animals in each group)
*,**,***,Indicates significance p<0.05, p<0.01 and p<0.001 when compared to Diabetic control, p<0.05 and p<0.01 Significance between diabetic control as indicates Non-significant comparison were made (a)–Initial day Vs 2nd week and 3rd week. (b)–Group II Vs Group III, IV and V

4.4 SERUM INSULIN, Hb, HbA1c, AND TP LEVELS CHANGES IN TYPE 2 DIABETIC RATS:

Groups	Dose (mg/kg)	Hb (g/dL)	HbA1c (%)	Serum insulin (μIU/mL)	TP (g/dL)
Control	**Vehicle**	**12.88 ± 0.13**	**5.71 ± 0.21**	**10.06 ± 0.15**	**7.25 ± 0.26**
Diabetic control	**Vehicle**	**7.22 ± 0.25[a]**	**11.96 ± 0.35[a]**	**4.33 ± 0.13[a]**	**4.93 ± 0.18[a]**
SEFBE	**200**	**10.45 ± 0.25[b]**	**6.31 ± 0.20[b]**	**6.98 ± 0.22[b]**	**6.35 ± 0.22[b]**
SEFBE	**400**	**11.77 ± 0.31[b]**	**5.86 ± 0.15[b]**	**8.11 ± 0.22[b]**	**5.77 ± 0.21[c]**
Metformin	**5**	**12.16 ± 0.31[b]**	**6.02 ± 0.16[b]**	**7.45 ± 0.22[b]**	**6.83 ± 0.16[b]**

TABLE NO: 6 SERUM INSULIN, Hb, HbA1c, AND TP LEVELS CHANGES IN TYPE 2 DIABETIC RATS

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STZ-NIC-mediated diabetes induction in rats increases HbA1c levels and reduces serum insulin, Hb, and TP significantly ($P < 0.001$) when compared to normal control rats (Table 10). Subcutaneous route of administration of both doses of SCFBE and standard drug Metformin to the type 2 diabetic rats showed significant ($P < 0.001$) reduction of HbA1c levels and increase in Hb, TP, and serum insulin levels than diabetic control rats. SCFBE at 200 mg/kg dose showed significant ($P < 0.001$ and $P < 0.01$) higher efficacy than SCFBE 100 mg/kg dose on normalization of Hb and HbA1c levels in type 2 diabetic rats.

4.5IN-VITRO ACTIVITY:

Inhibition of α-Amylase Activity:

Sample	Concentration (μg/mL)	% inhibition of enzyme activity	IC_{50} (μg/mL)
SCFBE	1	09.54 ± 0.65	4.32
	2	41.99 ± 0.22	
	4	53.41 ± 1.30	
	6	66.72 ± 0.95	
	8	75.13 ± 1.31	
	10	88.24 ± 1.02	
Acarbose	50	13.52 ± 0.48	198.20
	100	26.17 ± 0.51	
	200	48.92 ± 0.93	
	400	71.22 ± 0.68	
	800	83.82 ± 0.33	
	1000	95.53 ± 0.21	

TABLE NO: 7 INHIBITION OF α-AMYLASE ACTIVITY

The data represented as mean ± SD (n = 3).

V. CONCLUSION:

The response with dose of 400 mg/kg was found to be better than 200mg/kg oral dose. The hypoglycemic effect of Syzygium cumini and Ficus benghalensis in diabetic rats was more powerful.These results confirmed the use of Syzygium cumini seed and Ficus benghalensis bark in traditional system of medicine to treat diabetes in India. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of seed of Syzygium cumini and bark of Ficus benghalensis.

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