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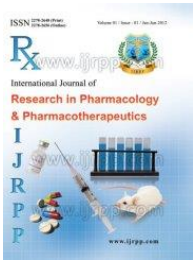

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Evaluation of anti-diabetic activity of *syzygium cumini* on streptozotocin induced diabetes in rats

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ABSTRACT

Aim and objectives

To evaluate the anti-diabetic activity of *Syzygium cumini* seed powder extract on streptozotocin induced diabetes in rats.

Materials and Methods

Wister Albino rats weighing 230-250gm were divided in to 5 groups. Diabetes induced in four groups by administration of streptozotocin (45mg/kg/i.p). G-I severs as control and normal saline was administered. G-II diabetic control, G-III Diabetic control (Streptozotocin 45mg/kg/i.p/0day) + Glibenclamide (5mg/kg/orally/120 days), G-IV: Diabetic control (Streptozotocin 45mg/kg/i.p/0day)+ Aqueous extract of *Syzygium cumini* seeds (250mg/kg/orally/120 days) and G-V: Diabetic control (Streptozotocin 45mg/kg/i.p/0day) + Aqueous extract of *Syzygium cumini* seeds (500mg/kg/orally/120 days). All the drugs were respective groups for 120 days. Blood was collected from the groups at different time intervals (0day. 15th day, 30th day, 60th day and 120th day). Plasma was separated and used for estimation of glucose.

Results

Comparison of glucose values of control group with other groups showed that there was significant decrease in glucose values in other groups $p<0.05$. But 15th day group-III and V showed significant decrease in glucose level compared to group-IV. G-V showed decrease glucose levels compared with other groups at 30th, 60th and 120th day. Comparison on 0 day between groups showed insignificant difference in blood glucose level ($P>0.05$).

Conclusion

High dose of *S.C* seed extract showed results like standard oral hypoglycemic drug. There is a requirement of more studies on this plant seed to find active phytochemicals.

Keywords: Diabetes, Glucose, Hypoglycemia, Oral Hypoglycemics, Streptozotocin, *Syzygium cumini*,

INTRODUCTION

Diabetes mellitus has been recognized as a growing world-wide epidemic by many health's groups like World Health Organization (WHO). It as has estimated that diabetes will be one of the world's leading cause of death and disability within the next quarter century. The statistics are alarming; 30 million people were diagnosed with diabetes world-wide in 1985, by 1995 the number had risen to 135 million, and at the current rate there will be 300 million by the year 2025 as predicted by the WHO [1]. Diabetes is a metabolic disorder of the endocrine system. It is considered to be one of the most serious endocrine syndrome. It's broadly classified in to two types Type-I IDDM (Insulin Dependent Diabetes Mellitus) and Type-II NIDDM (Non Insulin Dependent Diabetes Mellitus) [2]. Type-I caused by destruction of pancreatic beta cells leads to decrease in the secretion of insulin. Decreased insulin section leads increase glucose levels in the body [3]. In modern medicine no satisfactory effective therapy is still available to cure the diabetes mellitus. Administration of insulin is used for the management of type-I diabetes mellitus [4]. Insulin therapy causes various adverse effects like hypoglycemia, insulin resistance, fatty liver and muscle atrophy. Drawbacks of modern medicine can overcome by use of plant products in the treatment of diabetics [5]. According to literature drugs derived from plants are frequently considered to be less toxic with fewer side effects compared to synthetic drugs. Therefore the search for more effective and safer antidiabetic agents has become an area of active research [6]. *Syzygium cumini*, locally known as Jam, is a tree grows up to 18 meters tall and bears thorns and fragrant flowers, stem and branches are light brown to green, leaves are alternate, pale green, belonging to the family Martaceae. This plant seeds, fruit juice showed antidiabetic activity but not leaves. According to previous studies *Syzygium cumini* were used in treating diabetes, diarrhoea, ringworm, teeth and gum problems, liver problems and infections [7, 8, 9]. The present study was conducted to screen anti-diabetic activity of aqueous extract of Syzygium *cumini* seed powder against streptozotocin induced diabetes in rats.

MATERIALS AND METHODS

Animals

According to criteria outlined in the guide for the care and use of laboratory animals prepared by the national academy of Science and published by the national institute of health. 230-250gm of rats was included in the study. The animals understudy was maintained at temperature of 25 ± 1^0C in a well-ventilated animal house under natural photoperiod conditions. They were provided with balanced commercial diet and water ad libitum [10].

Syzygium cumini seed collection

Syzygium cumini seeds were collected from rural areas around Chidambaram in the province of Tamil Nadu, India. The seeds were authenticated with the help of botanist at the Annamalai University.

Preparation of aqueous extract of Syzygium cumini seed powder

The *S.C* seeds were dried and powdered and a suspension of 100gm in 200ml distilled water was stirred magnetically overnight at room temperature. This was repeated three consecutive times. The extract was evaporated to dryness under reduced pressure in a rotary evaporate. The residual extract was dissolved in Saline and used in the study [11].

Induction of Diabetes in rats

Adult Wister Albino rats weighing 230-250gm were used in the study. Experimental animals received freshly prepared solution of Streptozotocin (45mg/kg) in 0.1ml citrate buffer p^H 4.5 solution imtraperitoneally in a volume of 0.1ml/kg. The animals allowed drinking 5% glucose solution over night to overcome the drug induced hypoglycemia [12]. 72h after streptozotocin administration the rats with moderate diabetes having persistant glycosuria and hyperglycemia (blood glucose 200-300mg/dl) were considered as diabetic rats and used for the experiments. The animals were fasted over night and blood samples were collected from the retro orbital vein [13].

STUDY DESIGN

Total 30 rats were selected and divided in to five groups each of 6 rats.

Group-I: Normal control (Normal Saline)

Group-II: Diabetic control (Streptozotocin 45mg/kg/i.p) [14]
Group-III: Diabetic control (Streptozotocin 45mg/kg/i.p/0day) + Glibenclamide (5mg/kg/orally/120 days) [15]
Group-IV: Diabetic control (Streptozotocin 45mg/kg/i.p/0day)+ Aqueous extract of *Syzygium cumini* seeds (250mg/kg/orally/120 days)
Group-V: Diabetic control (Streptozotocin 45mg/kg/i.p/0day)+ Aqueous extract of *Syzygium cumini* seeds (500mg/kg/orally/120 days) [16]

The test and standard drugs were administered orally to the diabetic rats by using polythene tubing sleeved on 18-20 gauge blunted hypodermic needle. Streptozotocin was administered by using 1ml syringe.

Method of blood collection and estimation of glucose

Blood samples for glucose estimation were collected from the retro orbital vein procedure. In a well restrained rat the eyelid were retracted properly and with the help of capillary tubes retro-orbital vein was punctured and a drop of blood was collected directly on the strip placed in the glucometer. This device estimate glucose by oxidase peroxidase method (Glucose oxidase specific strips). They waok on a principle called as "Rflectance photometry" and they are called strip reading or reflectance meter. They are reliable, easy to use and quick to perform. There is a reasonable correlation between the results obtained with the glucometers and the laboratory results. The blood samples were placed on strip within 15sec results was appeared [17].

STATISTICAL ANALYSIS

The data analysed by SPSS (20th version) to find statistical significant between the groups. ANOVA (Post hoc test) followed by Sheffs t test applied to find statistical significant at 95% confidence interval. P value less than 0.05 considered statically significant [18].

RESULTS

Group-I showed euglycemic throughout the study. Standard and *S.C* seed extract (500mg/kg) administered groups showed decreased in glucose levels compared with group-IV on 30th, 60th and 120th day It was observed that high dose of *S.C* seed extract (500mg/kg) administered group showed significant decrease in blood glucose levels compared standard drugs at different time periods (Table-1). Within g-III, IV and g-V decreased glucose levels observed on 30th, 60th and 120th day compared to 15th day (Table-2). Comparison of blood glucose levels between the groups and within the group on 0 day showed insignificant results. The comparisons showed p value significant is showed in the tables.

Table-1 Comparison of *Syzygium cumini* seed extract effect on blood glucose levels at different time periods between the groups (MEAN±SEM)

Groups	0 day	15th day	30th day	60th day	120th day
G-I	67.83±2.79	67.83±2.79	67.83±2.79	67.83±2.79	67.83±2.79
G-II	70.83±4.79				
G-III	73.00±4.73	255.83±11.14*	233.33±11.69*	171.33±5.35*	157.67±2.25*
G-IV	70.33±4.80	263.83±13.13*	233.50±7.58*	176.67±3.88*	163.83±3.92*
G-V	72.00±6.48	245.17±8.16*,#,$	204.33±4.27*,#,$	164.83±5.15*,#,$	139.33±3.78*,#,$

(*G-I compared with II, III, IV and V, #G-III compared with I, IV and V, $G-IV compared with I, III and V. The designated symbols indicate significant comparison (p<0.05). Non significant comparisons are not showed in the table)

Table-2: Comparison of *Syzygium cumini* seed extract effect on blood glucose levels at different time periods within the groups

Time	Group-I	Group-III	Group-IV	Group-V
0 day	67.83±2.79	73.00±4.73	70.33±4.80	72.00±6.48
15th day	67.83±2.79	255.83±11.14*	263.83±13.13*	245.17±8.16*

30th day	67.83±2.79	233.33±11.69*,#	233.50±7.58*,#	204.33±4.27*,#
60th day	67.83±2.79	171.33±5.35*,#,$	176.67±3.88*,#,$	164.83±5.15*,#,$
120th day	67.83±2.79	157.67±2.25*,#,$,‖	163.83±3.92*,#,$,‖	139.33±3.78*,#,$,‖

(*0 day compared with 15th, 30th, 60th and 120th day, #15th day compared with 0, 30th, 60th and 120th day, $30th day compared with 0, 15th, 60th and 120th day. ‖60th day compared with 0, 15th, 30th and 120th day. The designated symbols indicate significant comparison (p<0.05). Non significant comparisons are not showed in the table)

DISCUSSION

The present study conducted to determine the anti-diabetic activity of aqueous extract of *Syzygium cumini* seeds in streptozotocin induced diabetic male albino rats. The effects were compared with those of the standard oral hypoglycemic drug glibenclamide. *Syzygium cumini* seeds are widely used for the treatment of diabetes in ayurveda. Hence, we studied long-term administration of seed extract on glucose levels in rats. This study selected the streptozotocin induced diabetic rats as an experimental model because it is accepted and widely used model to screen new anti-diabetic agents. Streptozotocin administration caused development of diabetes within 72 hours. Animals have more than 200mg/dl selected and included in the study. All the groups except control administered standard and test drug for 120 days. In this study results showed decreased glucose levels in standard and high dose seed extract treated groups compared to other groups. Previous studies also showed same effect with plant. Our study results are consistent with those already reported by our previous publication. Seed extract 500mg/kg showed effect equal to the standard drug. The mechanism how seed extract reduced glucose levels not known. Phytochemical present in seed may be responsible for this. Over all study results showed aqueous extract of *Syzygium cumini* seed powder has anti-diabetic activity.

CONCLUSION

From this study results it can be concluded that *S.C* extract (500mg/kg) has significant hypoglycemic effect in STZ diabetic rats. *S.C* seed extract can be administered diabetic patients for better diabetic control. More preclinical, clinical and molecular studies required to find the active chemicals.

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