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RESEARCH ARTICLE

EVALUATION OF ANTI-DIABETIC POTENTIAL OF THE *SYZYGIUM CUMINII* (LINN) SKEELS BY REVERSE PHARMACOLOGICAL APPROACHES

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Oral administration of 50 and 100 mg/kg of the aqueous and methanol extracts of roots, leaves, seeds and barks of *Syzygium cuminii* in alloxan monohydrate (150 mg/kg *i.p.*) induced diabetic male Sprague Dawley (SD) rats, for 21 days resulted in a statistically significant (P variation 0.05 to 0.001) reduction in blood glucose level and biochemical parameters in dose dependent manner. They also prevented decrease in body weight. Oral administration of 100 and 200 mg/kg aqueous extract of leaves (AL) in the oral glucose tolerance test on streptozotocin (STZ 75 mg/kg *i.p.*) induced diabetes, in experimental diabetes induced by alloxan (150 mg/kg *i.p.*) and streptozotocin (70 mg/kg *i.p.*) resulted in a significant ($P < 0.001$) hypoglycemic activity. The safety profiles of extracts confirmed by acute toxicity study on mice and sub-chronic toxicity of AL extract on male rats. On the basis of these investigations, we may partially conclude that the *S. cuminii* leaves could be a potent antidiabetic agent.

Key words: *Syzygium cuminii*, Streptozotocin, Sub-chronic toxicity, Antidiabetic, Alloxan.

INTRODUCTION

Since decades, plants are well known to produce metabolites with diverse bioactivities (Jain *et al* 2011; Jenny *et al* 2012; Jain and Argal, 2013). Diabetes has emerged as a major healthcare problem in India. According to Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025. The countries with the largest number of diabetic people will be India, China and USA by 2030. It is estimated that every fifth person with diabetes will be an Indian. Due to this, economic burden due to diabetes in India is amongst the highest in the world. The real burden is however due to its associated complications which lead to increased morbidity and mortality (Gupta, 2008). A wide array of plants and active principles representing numerous chemical compounds like alkaloids, glycosides, galactomannan gum, polysaccharides, peptide glycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids etc. have demonstrated activity consistent with their possible use in the treatment of Non Insulin Dependent Diabetes Mellitus) (Grover *et al* 2002). With the advent of the western culture and the concurrent modern or allopathic

medicines, the rich Indian heritage of TSM (Ayurveda, Unani, Siddha and Tribal) and also the Homeopathic System were sidelined and neglected. Resultantly, the village-based wisdom of traditional system of treatment has not been seriously followed, not well documented and passed on properly over generations (Deb and Dutta, 2006). In this study, we have chosen a plant "*Syzygium cuminii* (Linn) skeel (Family: myrtaceae) or Jamun" which is very popular as a medicinal agent as mentioned in the ancient texts of the ethnic medicine. Earlier, extracts and fractions of seeds, roots, leaves and barks of the plants have been studied to establish the scientific uses as an anti-diabetic, antioxidant, anti-inflammatory, antibacterial, anticonvulsant, antihyperlipidaemic, anti-allergic. However, it is not reported that how antidiabetic activity distributed in the different parts of *Syzygium cuminii (*Linn) skeels. The leaves are rich in tannins, flavonoids and essential oils. Phenolic contents of leaves bark and fruits are correlated with antioxidant activity. *Syzygium cuminii* contains phytochemicals such as Glycosides, flavonoids, tannins and alkaloids. Their hypoglycemic activities have reported that saponins possess hypocholesterolemic and antidiabetic properties. The terpenoids have also been shown to decrease blood sugar level in animal studies (Pandey and Khan, 2002; Prince *et al* 1998; Panwala *et al* 2004; Nag Chaudhuri *et al* 1990; Chattopadhyay *et al* 1998; De Lima *et al* 1998; Krishnamoorthy *et al* 2006; Ruan *et al* 2008; Brito *et al* 2007; de Oliveira *et al* 2007; Saravanan and Leelavinothan, 2006; Nikhat *et al* 2008). However, the distribution of bioactive components responsible for anti-diabetic activity in different parts of the *S. cuminii* was unknown. With this background, we designed this study for exploring the anti-diabetic activity of different parts of *Syzygium cuminii* (Linn) skeels due to distribution of bioactive components.

MATERIALS AND METHODS

Collection of plant material

Syzygium cuminii (Linn) skeels (Myrtaceae) seeds, leaves, bark and roots were collected fresh from Mirza forest, South Tripura district, Tripura, India, in the month of November, 2004. The plant was identified and authenticated at Tripura forest department as well as in S.C.S. College of Pharmacy, Harapanahalli, Karnataka by botanist Mr. K. Prabhu. Herbarium (SCSCOP/ Pharmacognosy/HB/01/04) was prepared and submitted in the department of pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli, Karnataka, India. Seeds were thoroughly washed with distilled water three to four times (10 min each time).

The seeds were dried at room temperature. Leaves were collected fresh and dried at room temperature. Bark was collected and shade dried at room temperature. Fresh roots were collected and dried at room temperature. All those samples were brought to S.C.S. College of Pharmacy and powdered in a hand grinder to make a coarse powder (10# mesh).

Preparation of extracts

The powder was defatted first in soxhlet apparatus with petroleum ether for 18 h and the same marc was subjected to extraction with chloroform, methanol or distilled water respectively for 18 h in each time. The extracts were dried at 55°C in vacuum distillation till condensed, and then dried in hot air oven at 45°C till solid mass was obtained. These were stored in airtight container in refrigerator below 10°C. Aqueous and methanolic extract of each sample were selected for anti-diabetic investigation as those extracted comparatively in large quantity.

Experimental animals

Wister albino mice of either sex (18-25 g) used for acute toxicity test and male Sprague Dawley albino rats (200-320 g *b.w.*) were used throughout the experiments. The animals were procured from National Institute of Mental Health and Neuro Sciences (NIMHNS), Bangalore. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature (26±2°C), relative humidity (45-55%) and 12 h dark/light cycle were maintained in the quarantine. All the animals were fed with synthetic diet (gold Mohr, Lipton India Ltd., Bangalore) and water was allowed *ad libitum* under strict hygienic conditions. All the animal experiments were conducted according to the protocol approved by the Institutional Animal Ethics Committee.

Sample collection

Blood samples were collected by tail vein puncture method and blood glucose levels were estimated using an endpoint colorimetric method (glucose kit-span diagnostic).

Toxicity studies ALD_{50} (Fixed dose method) on mice according to OECD guidelines

The acute toxicity of methanolic and aqueous extracts of *Syzygium cuminii* (Linn) leaves, seeds, bark, roots was determined in albino mice of either sex (18-25 g, procured from Venkateshwara Enterprises, Bangalore). Acute oral toxicity 'acute toxic class method' (OECD Guideline no. 423, Annexure – 2d) adopted by CPCSEA, government of India was followed for acute toxicity studies. The mortality was observed after oral administration of test samples (Veerarghavan, 2001). In brief, three mice in a group initially given 2000 mg/kg body weight oral dose of each extract and mortality was recorded for a time period of 24 h from the time of administration of extracts. In case of 100% mortality with initial higher dose in any group, the respective test sample(s) were tested for next lower dose 300 mg/kg *b.w.* or 50 mg/kg *b.w.* or 5 mg/kg *b.w.* in similar manner, as per guideline. The ALD_{50} was identified from OECD chart (2 d) provided corresponding ALD_{50} values for each dose. Common side effects such as mild diarrhea, loss of weight and depression of treated groups of animals were recorded within the 7 days of observation (Veerarghavan, 2001).

Screening of antidiabetic potency of *Syzygium cuminii* (Linn) skeels (Myrtaceae) seeds, leaves, barks and roots extracts on alloxan-induced diabetic animals

Albino rats weighing 200-320 g of body weight, acclimatized and were segregated in 18 groups 6 animals/group) and were labeled as group I to XVIII respectively. Fasting blood sugar was determined after depriving food for 16 h, with free access to drinking water. From the following day, daily 6 group (II to VII) of animals were rendered diabetic by injecting alloxan monohydrate (Loba Chemic, Bombay) dissolved in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally upto completion of XVIIIth group (Ghosh and Suryawanshi, 2001). The rats were provided with 5% glucose solution for the next 24 h to prevent hypoglycemia. After 96 h, the animals from all the groups were subjected to blood glucose estimation using endpoint colorimetry method. The blood samples were collected from tail vein.

Animals in groups II to XVIII showing fasting blood sugar level more than 350 mg/dl were selected for the study and started treatment from same day except group I and II. In case of the group II the hyperglycemia condition was maintained for 7 days after which none of the animals survived. So on 7th day, all hematological and biochemical estimation were carried out. Taking this into consideration, an experiment was set up for the extracts' treatment for a period of 21 days.

During this period, animals in all groups had free access to standard diet and water. Body weights and blood glucose levels were estimated on 1st, 7th, 14th and 21st day of extract treatment. On the 21st day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for hematological and biochemical analysis.

Hematological and biochemical analysis

For the hematological and biochemical analysis, blood samples were centrifuged at 4000 rpm for 15 min, the plasma collected and fasting blood glucose level was determined by colorimetric assay according to the span diagnostic kit instruction manual. Same plasma used for estimations of plasma cholesterol, triglycerides, creatinine and urea by spectrophotometric assays according to the span diagnostic kit instruction manual.

Histopathological studies

The whole pancreas from all the animals were removed immediately and was kept in 10% formalin solution for histopathological work (Luna, 1968). Morphological parameter like body weight were also observed.

Sub-chronic toxicity studies of leaves aqueous extracts

Albino rats weighing 200-320 gm of body weight were divided into several groups of six animals. Each set of animals received either drinking water (35 ml/rat) or leaves aqueous extract of *Syzygium cuminii* (Linn) skeels daily. Three sets of control animals (Group - A) received drinking water only. One set of test animals (Group - B) received AL of *Syzygium cuminii* (Linn) skeels (100 mg/kg/day) whilst three sets of test animals (Group - C) received AL of *Syzygium cuminii* (Linn) skeels (500 mg/kg/day). All animals received treatment for 30 days. One set of six animals in each group was weighed on day 0 and then weekly until termination at day 30. For blood parameter studies, blood samples (1 ml each) of these six animals in each treatment group, were taken by tail bleeding, on days 0, 15 and 30 into separate Eppendorff tubes containing EDTA (1.5 mg) and heparin (0.125

mg) for haematological and biochemical analyses, respectively. Simultaneously, in same time interval (days 0, 15 and 30) urine of animals were also collected by using metabolic cage. For organ weight studies, the same animals were euthanized by cervical dislocation on last day of bleeding (termination) and their organs were excised and weighed.

Screening of antidiabetic potency of *Syzygium cuminii* (Linn) skeels (Myrtaceae) leaves aqueous extracts on alloxan-induced diabetic animals

Albino rats weighing 200-320 g of body weight, after acclimatization were segregated into several groups of 6 animals in each group. Group 1 served as control, group 2 as diabetic control and group 3 and 4 served as test group which were treated with aqueous extract (AL) of *Syzygium cuminii* leaves at the dose 200 mg/kg *b.w.* and 100 mg/kg *b.w.* respectively. Further, same procedure was followed as mentioned under screening of antidiabetic potency of *Syzygium cuminii* (Linn) skeels (Myrtaceae) seeds, leaves, barks and roots extracts on alloxan-induced diabetic animals.

Screening of antidiabetic potential of leaves aqueous extracts on streptozotocin induced diabetic model on rats

Albino SD rats weighing 200-320 g of body weight were divided into several groups of six each and were fed with standard diet (Ratan Brothers, Hyderabad) and water ad libitum. They were kept in clean and dry cages and maintained in well-ventilated animal houses with 12 h light-12 h dark cycle. Rats were rendered diabetic by injecting a freshly prepared streptozotocin (75 mg/kg, *i.p.*; dissolved in 0.1 M acetate buffer; pH 4.5) after a base-line blood glucose estimation was done.

After two weeks, animals with blood glucose levels above 450 mg/dl were selected for the study. For long term evaluation, groups of rats were given daily treatments for 21 days (Deb and Dutta, 2006). Animals in test groups were given orally, the aqueous extract of *Syzygium cuminii* (Linn) Skeels leaves at a dose of 200 and 100 mg/kg. One group served as control while one group received glibenclamide at a dose of 0.04 mg/kg. Blood samples were collected from the overnight fasted animals and fasting blood glucose levels were measured before and also at weekly intervals for 21 days. For urinary collection, rats were housed in metabolic cages at the start (pre-diabetic condition) and at the end (20th day) of the experiment. The 24 h urinary samples were collected from all the animals after they have been acclimatized in metabolic cages for 3 days. The measurements of urinary protein albumin and creatinine were done using commercial diagnostic kits (Span Diagnostic Limited, Mumbai) following manufacturer's instructions.

Oral glucose tolerance test (OGTT) on streptozotocin induced diabetic and normal rats

Albino rats weighing 200-320 g of body weight were divided into 8 groups of five each and were fed with standard diet (Ratan Brothers, Hyderabad) and water ad libitum. They were kept in clean and dry cages and maintained in well-ventilated animal house with 12 h light-12 h dark cycle. Rats were rendered diabetic by injecting a freshly prepared streptozotocin (75 mg/kg, *i.p.*; dissolved in 0.1 M acetate buffer; pH 4.5) after a base-line blood glucose estimation was done.

After two weeks, animals with blood glucose levels above 450 mg/dl were selected for the study prior to an oral glucose tolerance test (OGTT), rats were fasted for 16 h. Distilled water (control), leaves aqueous extract at the dose of 100 mg/kg, 200 mg/kg, 500 mg/kg, body weight and a reference drug, metformin at a dose of 500 mg/kg body weight were orally administered to groups of 5-6 rats each. Thirty minutes later, glucose (3 g/kg) was orally administered to each rat with a feeding syringe.

Blood samples were collected from the tail vein by tail milking at 30 min (just before the administration of distilled water fractions of leaves aqueous extract of *Syzygium cuminii* (Linn) skeels and metformin in respective group, 0 (just before the oral administration of glucose), 30, 60, 120, and 180 min after glucose load for the assay of glucose.

Statistical analysis

Data were expressed as mean ± Standard Error Mean (SEM). Differences were considered significant at ***P<0.001, or **P < 0.01 or *P<0.05 when compared test groups vs. diabetic control group. For numerical results, one-way analysis of variance (ANOVA) with Tukey-Kramer Multiple Comparisons post tests were performed using GraphPad InStat Version 3 (GraphPad Software) and all graphs were made by using GraphPad Prism software.

RESULTS

Acute toxicity study of the different extracts (except methanolic extract of seed), did not show mortality at the dose of 2000 mg/kg. Therefore 2000 mg/kg dose was considered as ALD_{50} cut off dose under Globally Harmonised Classification System (GHS) category 5 (safe dose), as per OECD guideline 423 and for methanolic seed extract ALD_{50} cut off was 200 mg/kg *b.w.* (GHS, category 3). Common side effects such as, mild diarrhea, loss of weight and depression in treated groups of animals were not recorded within the 7 days of observation. All extracts were administered as 1/10th the higher dose and 1/20th the lower dose of their respective LD_{50} values for *in vivo* experiments.

Aqueous extracts of roots (AR), barks (AB), leaves (AL), seeds (AS) and methanolic extracts of roots (MR), barks (MB), leaves (ML), seeds (MS) of *Syzygium cuminii* (Linn) skeels were subjected for antidiabetic activity in rats where alloxan monohydrate (150 mg/kg body weight) was used as the diabetogenic agent. A marked rise in blood glucose level was observed in diabetic control group of rats which also became hyperlipidemic, restless and irritable. Severe thirst and lack of appetite were observed. In these rats, AL, AR, ML, AS and MB treated groups of rats showed progressive reduction in blood glucose levels. All extracts produced a definite bioactivity on 14th and 21st day compared to 1st and 7th day of treatment (**Figure 1**).

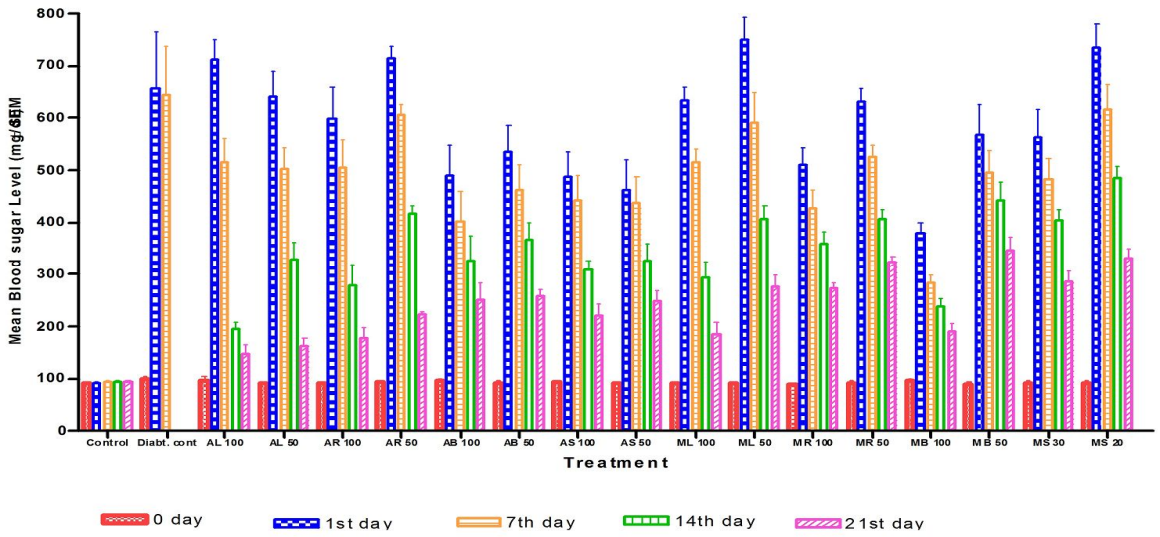


Fig. 1. Effect of *Syzygium cuminii* on fasting blood glucose levels in alloxan induced diabetic rats

An increase in body weight during treatment for diabetes is a positive indication for the effectiveness of treatment. Results shows less reduction in body weight in AL, AR, AS, MB and ML extract treated group of rats than other extracts treated group of rats in which the body weight was greatly reduced. Lower dose of the all extract showed less effect than higher doses of all respective extracts.. In histopathological studies, section of pancreas from normal control group showed normal architecture of islets of Langerhans and exocrine acini (plate no. I). Diabetic control group of rats showed degenerative and neurotic cells with sparse cellularity in the islets of Langerhans (plate no. II). Rats treated with aqueous extract of leaves at 100 mg/kg *b.w.* showed maintenance of normal architecture of islets of Langerhans with a few cells showing veculated cytoplasm (plate no. III). But rats treated with aqueous extract of leaves at 50 mg/kg *b.w.* showed maintenance of normal architecture of islets of Langerhans with cells having granular cytoplasm (plate no. IV). Rats treated with aqueous extract of roots at 100 mg/kg *b.w.* showed degenerating and neurotic cells with vacuolated and granular cytoplasm (plate no. V). Rats treated with aqueous extract of roots at 50 mg/kg b.w. showed sparse cellularity and cells with degenerating and neurotic cell of islet of Langerhans (plate no. VI). Group of rats treated with aqueous extract (100 mg/kg) of bark showed that cellularity has been restored although few β-cells revealed vacuolated appearance (plate no. VII) and aqueous extract (50 mg/kg *b.w.*) of bark showed extensive damage to islet cells with degenerative and lytic changes in the islet of Langerhans (plate no. VIII). Group of rats treated with

aqueous extract (100 mg/kg *b.w.*) of seed showed restoration of normal architecture of pancreatic islet. (plate no. IX) and aqueous extract (50 mg/kg) of seed treated group showed that cellularity has been restored although few *β*-cells revealed vacuolated appearance (plate no. X). Group of rats treated with methanolic extract of leaves at 100 mg/kg *b.w.* showed total restoration of Islet of Langerhans with hypercellularity (plate no. XI) and methanol extract at 50 mg/kg *b.w.* of leaves showed that cellularity has been restored although few *β*-cells revealed vacuolated appearance (plate no. XII). Group treated with methanol extract (100 mg/kg *b.w.*) of roots showed that few *β*-cells reveal vacuolated appearance (plate no. XIII) and methanol extract (50 mg/kg *b.w.*) of roots showed extensive damage to the islet cells with lytic changes in the islet of langerhans (plate no. XIV). Rats treated with methanol extract (100 mg/kg *b.w.*) of bark showed restoration of normal architecture of pancreatic islet (plate no. XV) while methanol extract (50 mg/kg *b.w.*) of bark showed extensive damage to the islet cells with degenerative and lytic changes in the islet of langerhans (plate no. XVI). Rats treated with methanol extract (30 mg/kg) of seed showed restoration of near to normal architecture of pancreatic islet (plate no. XVII) whereas seed methanol extract (20 mg/kg) treated group showed restoration of normal architecture of pancreatic islet (plate no. XVIII). Results of biochemical parameters are shown in **Figure 2A, 2B**. Plasma urea level increased 3.95 fold in diabetic control group, AL, AR, ML, MB and AS extracts treated groups showed a highly significant effect in controlling plasma urea levels in diabetic animals. Plasma creatinine increased 3.23 fold in diabetic control animals. AL, AR, ML, MB, and AS treated groups significantly reduced plasma creatinine levels but AB, MR, MS extract treated groups of rats produced less effect. Plasma triglycerides increased 2.39 fold in diabetic control group, AL, AR, MB, ML and AS extracts treated group of rats showed significantly reduced plasma triglyceride levels, but AB, MR, and MS extracts produced less effect. Aqueous extract of leaves of *Syzygium cuminii* (Linn) skeels showed positive anti diabetic activity on streptozotocin induced diabetic rats as well as in oral glucose tolerance test (OGTT) on streptozotocin induced diabetic and repeated study on alloxan induced diabetic animals in different dose. Subchronic toxicity studies of leaves aqueous extracts on male Wistar albino rats (6-8 week old) was not shown any significant changes in biochemical, hematological, urinary parameters and organ weight of animals. In case of organ, weight for liver, kidney, lung, pancreas, heart and spleen was 2.95 ± 0.06, 0.48 ± 0.01, 0.45 ± 0.04, 0.22 ± 0.01, 0.35 ± 0.01 and 0.23 ± 0.02 g% body weight respectively in normal animals (control group) whereas 3.14 ± 0.15, 0.51 ± 0.04, 0.44 ± 0.14, 0.23 ± 0.03, 0.36 ± 0.05, 0.24 ± 0.01 g% body weight was in leaves aqueous extract (100 mg/kg/day) treated groups and 3.05 ± 0.11, 0.54 ± 0.02, 0.50 ± 0.12, 0.27 ± 0.11, 0.35 ± 0.01, 0.23 ± 0.06 was in leaves aqueous extract (200 mg/kg/day) treated group of animals respectively. In case of hematological parameters at termination of treatment, heamoglobin, RBC, lymphocytes and platelets was recorded 15.0 ± 0.06 G/dL, 5.21 ± 0.01 × 10^{12}/L, 54.6 ± 5.8 % and 159 ± 15 × 10^{9}/L respectively in normal animals. Whereas, 15.7 ± 0.04 G/dL, 5.62 ± 0.31 × 10^{12}/L, 54.6 ± 3.7% and 149 ± 21 × 10^{9}/L was recorded in leaves aqueous extract (100 mg/kg/day) treated groups and 15.2 ± 0.03 G/dL, 5.74 ± 0.21 × 10^{12}/L, 53.6 ± 2.7%, 171 ± 09 × 10^{9}/L was recorded for leaves aqueous extract (200 mg/kg/day) treated group of animals respectively. In case of plasma biochemical parameters, urea, creatinine, albumin, ALP, cholesterol and triglyceride was recorded 7.97 ± 0.25 Mmol/L, 64.31 ± 4.01 μmol/L, 46.6 ± 2.8 g/L, 235 ± 15 U/L, 84.6 ± 1.3 mg/dl and 76.6 ± 2.3 mg/dl respectively in normal animals whereas 7.76 ± 0.11 Mmol/L, 64.21 ± 5.01 μmol/L, 46.8 ± 1.8 g/L, 231 ± 17 U/L, 86.6 ± 2.5 mg/dl, 73.7 ± 2.3 mg/dl L was recorded in leaves aqueous extract (100 mg/kg/day) treated groups and 7.69± 0.56 Mmol/L, 64.51 ± 3.01 μmol/L, 46.0 ± 3.1 g/L, 234 ± 12 U/L, 83.6 ± 1.7 mg/dl and 75.1 ± 1.2 mg/dl was recorded for leaves aqueous extract (200 mg/kg/day) treated group of animals respectively. In case of urine parameters, specific gravity and pH was recorded at termination of treatment as 1.03 and 6.4 respectively in case of all groups, but glucose, ketones, bilirubin, proteins and leucocytes were not recorded in any group of animals (**Table 1**).

DISCUSSION

Alloxan causes a massive reduction in insulin release by destruction of the beta cells of Islets of Langerhans and inducing hyperglycemia.

Table 1. Sub chronic toxicity studies of leaves aqueous extracts on male Wistar albino rats

Sl. No.	Parameters	Units	Treatment groups		
			Control	Leaves aqueous extract 100 mg/kg/day	Leaves aqueous extract 200 mg/kg/day
Organ weight (g % body weight)					
1	Liver	g % body wt	2.95 ± 0.06	3.14 ± 0.15	3.05 ± 0.11
2	Kidney	g % body wt	0.48 ± 0.01	0.51 ± 0.04	0.54 ± 0.02
3	Lung	g % body wt	0.45 ± 0.04	0.44 ± 0.14	0.50 ± 0.12
4	Pancreas	g % body wt	0.22 ± 0.01	0.23 ± 0.03	0.27 ± 0.11
5	Heart	g % body wt	0.35 ± 0.01	0.36 ± 0.05	0.35 ± 0.01
6	Spleen	g % body wt	0.23 ± 0.02	0.24 ± 0.01	0.23 ± 0.06
Hematological parameters at termination of treatment					
1	Hb	G/dL	15.0 ± 0.06	15.7 ± 0..04	15.2 ± 0.03
2	RBC	10^{12}	5.21 ± 0.01	5.62 ± 0.31	5.74 ± 0.21
3	Lymphocytes	(%)	54.6 ± 5.8	54.6 ± 3.7	53.6 ± 2.7
4	Platelets	10^9/L	159 ± 15	149 ± 21	171 ± 09
Plasma biochemical parameters at termination of treatment					
1	Urea	Mmol/L	7.97 ± 0.25	7.76 ± 0.11	7.69± 0.56
2	Creatinine	µmol/L	64.31 ± 4.01	64.21 ± 5.01	64.51 ± 3.01
3	Albumin	g/L	46.6 ± 2.8	46.8 ± 1.8	46.0 ± 3.1
4	ALP	U/L	235 ± 15	231 ± 17	234 ± 12
5	Cholesterol	(mg/dl)	84.6 ± 1.3	86.6 ± 2.5	83.6 ± 1.7
6	Triglyceride	(mg/dl)	76.6 ± 2.3	73.7 ± 2.3	75.1 ± 1.2
Urine parameters at termination of treatment					
1	Glucose	—	—	—	—
2	Ketones	—	—	—	—
3	Bilirubin	—	—	—	—
4	Specific gravity	—	1.03	1.03	1.03
5.	pH	—	6.4	6.4	6.4
6.	Proteins	—	—	—	—
7	Leucocytes	—	—	—	—

*Mean ± Standard error mean (SEM)

Jamun seed reverse this effect, (hypoglycemic action) may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from beta cells of Islets of Langerhans or its release from the bound form (Prince *et al* 1998). In our present study, we have observed a significant increase in the fasting blood glucose after 4 days of alloxan monohydrated treatment and AL (100 mg/kg), AR (100 mg/kg), ML (100 mg/kg), MB (100 mg/kg) and AS (100 mg/kg) extracts can significantly (***P < 0.001) reverse hyperglycemic activity (**Figure 1**) that again confirmed hypoglycemic potential of 'Jamun' seed and also established hypoglycemic activity of leaves, roots and bark of 'Jamun'. The anti-diabetic activity was assessed by monitoring fasting blood sugar levels and change in the body weight at 1st day, 7th day, 14th day and 21st day. Other supporting data on plasma cholesterol, plasma creatinine and plasma urea and plasma triglyceride was performed to substantiate the anti-diabetic action of the extracts. The loss of body weight was noted in alloxan induced diabetic rats and the recovery of body weight were observed in increasing order with the AS, AR, AL, MB and ML extracts. There is a gain in the body weight, effect of *Syzygium cuminii* on biochemical markers in alloxan induced diabetic rats and effect of *Syzygium cuminii* on plasma creatinine in alloxan induced diabetic rats are depicted in **Figure 2A, 2B**. No such significant effect was observed when other extracts were administered to respective groups.

In this anti-diabetic study, each extract was used in two different doses (lower and higher dose).

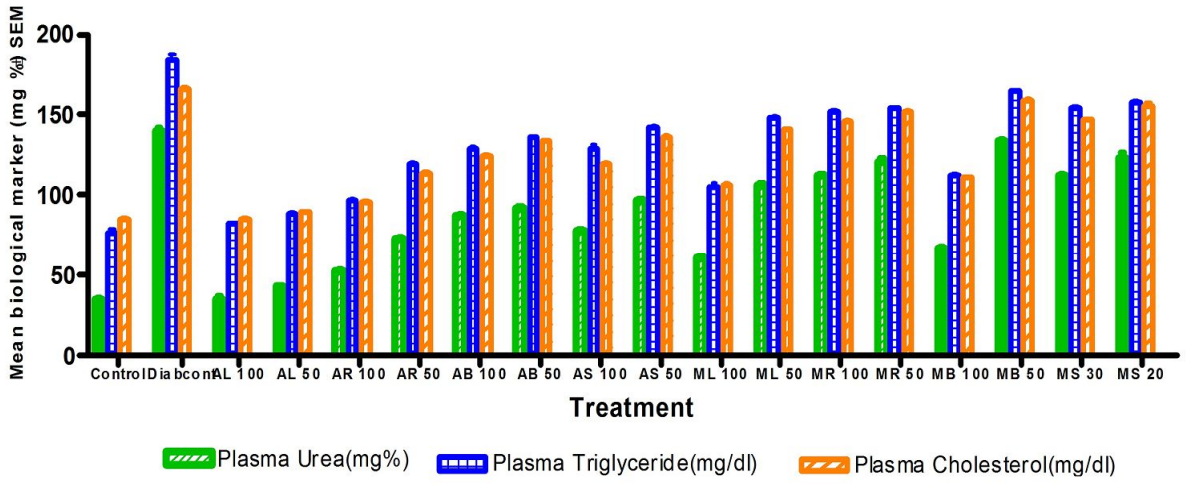


Fig. 2A. Effect of *Syzygium cuminii* on Biochemical markers in alloxan induced diabetic rats

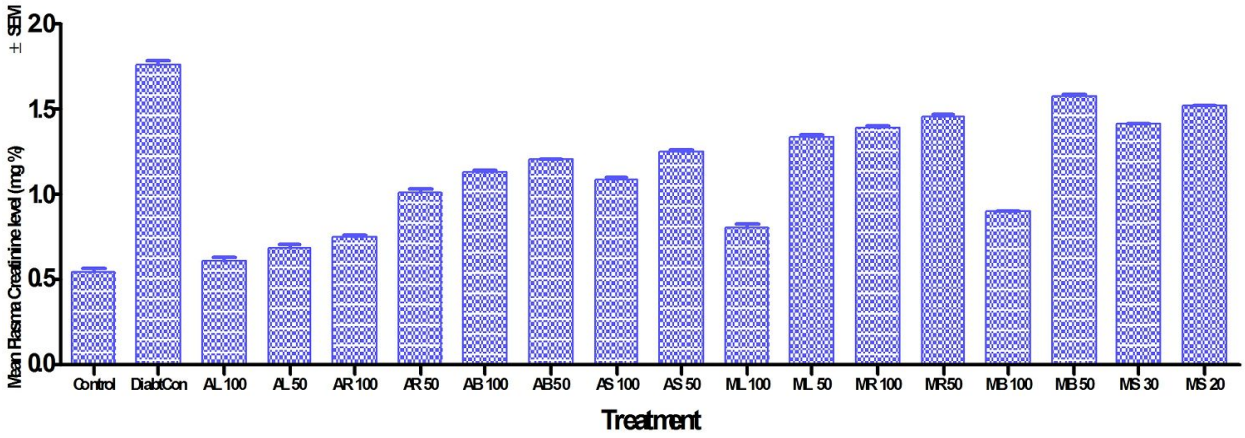


Fig. 2B. Effect of *Syzygium cuminii* on plasma creatinine in alloxan induced diabetic rats

Anti-diabetic activity for all the extracts were affected in dose dependant fashion *i.e.* higher doses of all extracts were more effective than that of lower doses of respective extracts. Hypoglycemic effect of *Syzygium cuminii* (Linn) Skeels seeds and leaves in albino rats was reported (Pandey and Khan, 2002; Prince *et al* 1998; de Oliveira *et al* 2007; Nikhat *et al* 2008; Teixeira *et al* 1997; Prince *et al* 2004). Taking a clue from this, the investigation of anti-diabetic activity on different parts of *Syzygium cuminii* (Jamun) was studied. It was observed that lipid profiles (plasma cholesterol and triglycerides) level was increased significantly and that showed a decreasing tendency in comparison with the diabetic control rats in extracts treated animals. In fact, these tendencies were advantageous to the diabetic conditions where these could put on Hallmark to the atherosclerosis and other delayed diabetic complications arising due to altered fat metabolism. The problem of hypercholesterol-emia is particularly significant in patients with diabetes mellitus.

This will increase the risk of premature coronary heart disease. The alteration in plasma lipoprotein level were common in diabetes which tend to exaggerate any pre-existing tendencies toward elevated lipid levels. Moreover, lipid peroxide-mediated tissue damage has been observed in the development of both type I and II diabetes. It has been observed that insulin secretion is closely associated with lipoxygenase derived peroxides (Prince *et al* 2004) and increased concentration of lipid peroxide in the liver can result in decreased activity of cytochrome P_{450} and cytochrome b_5 and this may affect the drug metabolism activity in chronic diabetes (Prince *et al* 1998; Teixeira *et al* 1997).

Any anti-diabetic drug having a favorable effect on elevated lipid levels would be advantageous for the patients. Diabetic nephropathy is a major cause of morbidity and mortality effecting 35% of (IDDM) and 3-17% of NIDDM patients. In addition diabetes is associated with altered kidney function. In our study, blood urea and creatinine levels also increased significantly in alloxan treated animals and showed a declining trend similar to that of control rats in extracts treated animals. It is fact that the kidney function is disturbed in diabetic conditions leading to elevated levels of urea and creatinine. Treatment with aqueous extracts of leave, root and seeds and methanolic extract leaves and barks may have almost normalized the kidney function as indicated by the reversal of blood plasma urea and creatinine.

Effect of *S. cuminii* leaves extract on fasting blood glucose level in alloxan induced diabetic rats is presented (**Figure 3**).

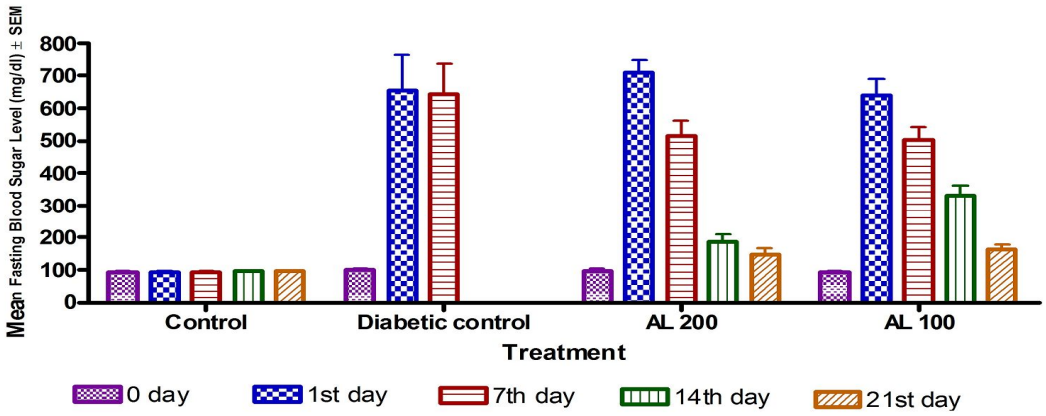


Fig. 3. Effect of *S. cuminii* leaves extract on fasting blood glucose level in alloxan induced diabetic rats

Histopathological studies also revealed that the groups treated with aqueous extract of leaves, roots, seed and methanolic extract of barks and leaves were able to restore or maintain normal architecture of Islet of Langerhens with hypercellularity compared to diabetic rats. This is indicating that there is recovery of beta cells of islet of langerhence in pancreas due to the treatment with various extracts. Thereby, insulin secretion may increase and reduce the blood glucose level by increasing transportation, utilizing glucose in body. Further, streptozotocin is well known for its selective pancreatic islet beta cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanism (Pari and Latha, 2002). In this study *Syzygium cuminii* (Linn) skeels leaves extract have shown maximum antidiabetic efficacy as compared to other extracts tested. In order to confirm the fact, streptozotocin induced diabetic model on rat, oral glucose tolerance test (OGTT) on streptozotocin induced diabetic and normal rats and further study on alloxan induced diabetic animals with different dose had been carried out (**Figure 4-6**) where leaves aqueous extract showed tremendous anti diabetic efficacy on all models used.

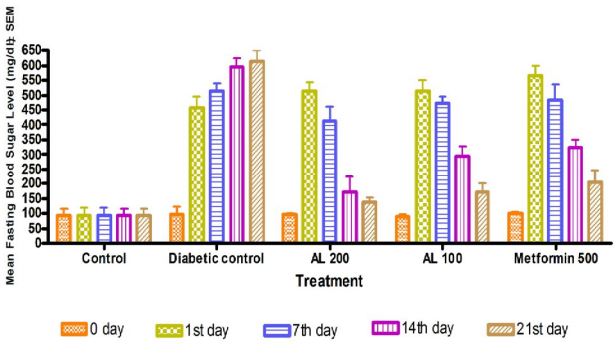


Fig. 4. Effect of *S. cuminii* extract on fasting blood glucose level in streptozotocin induced diabetic rats

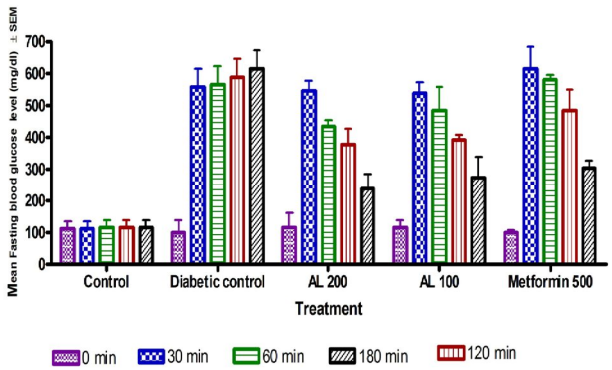
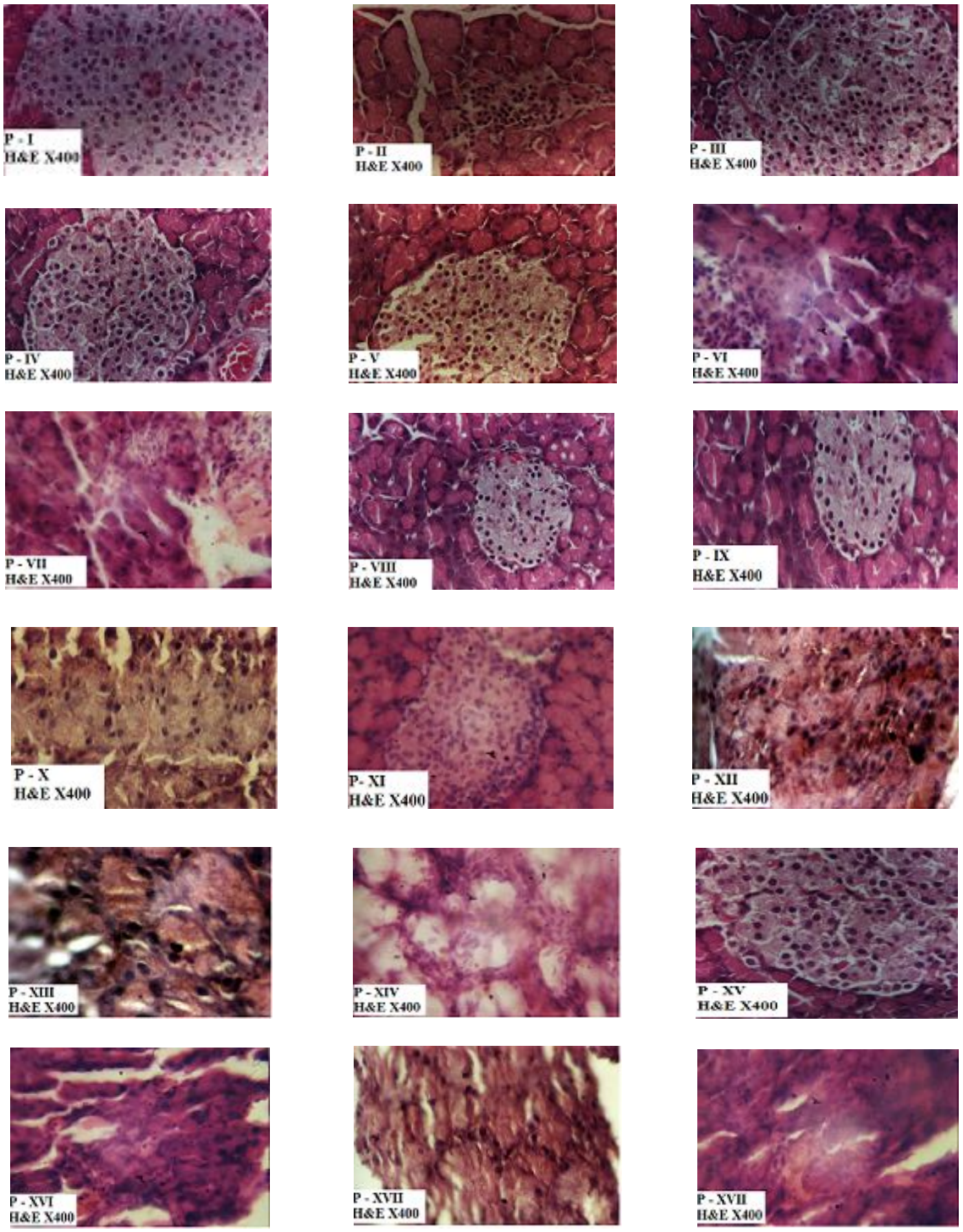


Fig. 5. Effect of *S. cuminii* leaves extract on OGTT in streptozotocin induced diabetic rats

Finally, subchronic toxicity studies of leaves aqueous extracts on male SD albino rats (6-8 week old) was performed to evaluate the safety profile of *Syzygium cuminii* (Linn) skeels leaves extract. Studies did not show any significant changes in biochemical, hematological, urinary parameters and organ weight of animals.



Normal control group. II. Diabetic control group. III. Aqueous leave extract (100 mg/kg *b.w.*) treated group. IV. Aqueous leave extract (50 mg/kg *b.w.*) treated group. V. Aqueous root extract (100 mg/kg *b.w.*) treated group. VI. Aqueous root extract (50 mg/kg *b.w.*) treated group.VII. Aqueous bark extract (100 mg/kg *b.w.*) treated group. VIII. Aqueous bark extract (50 mg/kg *b.w.*) treated group. IX. Aqueous seed extract (100 mg/kg *b.w.*) treated group. X. Aqueous seed extract (50 mg/kg *b.w.*) treated group. XI. Methanolic Leave Extract (100 mg/kg *b.w.*) treated group. XII. Methanolic Leave Extract (50 mg/kg *b.w.*) treated group.XIII. Methanolic root extract (100 mg/kg *b.w.*) treated group. XIV. Methanolic root extract (50 mg/kg *b.w.*) treated group. XV. Methanolic bark extract (100 mg/kg *b.w.*) treated group. XVI. Methanolic bark extract (50 mg/kg *b.w.*) treated group. XVII. Methanolic seed extract (100 mg/kg *b.w.*) treated group. XVIII. Methanolic seed extract (50 mg/kg *b.w.*) treated group.

Fig. 6. Transverse sections showing effect of *S. cuminii* on pancreas of alloxan induced diabetic rats

CONCLUSION

The diabetes mellitus is a disease that can be called as silent killer. To control such disorder with other than insulin could be possibly achieved through the plant sources. On the basis of this investigation, it can be concluded that the *S. Cuminii* (Linn) skeels leaves could be a potent anti-diabetic agent for next generation.

With such information, further studies on isolation and characterization of phytochemical constituents present in the aqueous leaves extracts are under progress in our laboratory; those are particularly responsible for such development to give protection to the mankind for treating such dreaded disease in future.

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