Active Hypoglycemic Fraction from *Syzygium cumini* L. Seed and its Safety Profile

Md. Al Amin Sikder[1], Md. Abul Kaisar[1], Mohammad S. Rahman[1], Maleeha Hussain[2] and Mohammad A. Rashid[1,*]

[1]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh
[2]National Institute of Cancer Research & Hospital, Mohakhali, Dhaka-1212, Bangladesh

Abstract

The seed of *Syzygium cumini* is well reputed as traditional medicine for treating diabetes but its active hypoglycemic constituent(s) is still unknown. With this aim, the methanol extract of the seed of *S. cumini* was fractionated to get petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble fractions and were provided to alloxan-induced diabetic rats to evaluate their antidiabetic efficacy. Among all, the petroleum ether soluble fraction of the methanol extract exhibited a rapid and significant ($P<0.001$) hypoglycemic action after a week of regular treatment (100 and 300 mg/kg body wt.). The safety profile was confirmed through liver function test. Besides, the histopathological study of the liver, heart, kidney and spleen tissues of the experimental rats demonstrated no untoward effect of this petroleum ether soluble fraction to the treated diabetic rats.

Keywords: *Syzygium cumini*, Alloxan, Antidiabetic, Hypoglycemic

Introduction

Diabetes is one of the most commonly occurred diseases in Bangladesh. It is a serious concern to medical practitioners for all ages of Bangladeshi population. At present, about 3.8 million or 4.8% of people living in Bangladesh are diabetics (International Diabetes Federation, 2006). As per WHO report, approximately 150 million people have diabetes mellitus worldwide and this number may be doubled by 2025 (Pattanayak *et al.*, 2009). For several centuries, the medicinal plants are used for treating diabetes (Rao *et al.*, 2001) but only a few have received advanced scientific scrutiny.

Syzygium cumini (Bengali name- Jam; Family- Myrtaceae) is a large evergreen tree attaining 30 m in height and found all over the Bangladesh (Rafiullah *et al.*, 2006). It is reported to possess antibacterial, anti-inflammatory and anti-HIV activities (Muruganandan *et al.*, 2001). The hypoglycemic efficacy of *S. cumini* seed is well known (Grover *et al.*, 2002) and in herbal preparation the seed powder is commonly used as antidiabetic drug in Bangladesh. But till now, no advancement is seen to identify its active hypoglycemic constituent(s). In this respect, this study was designed to isolate the hypoglycemic constituent(s) from the *S. cumini* seed as well as to study its safety profile by liver function test and histopathology for the first time.

Materials and Methods

Plant materials: The seeds of *S. cumini* were collected from a local market in Dhaka in March 2008. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. 32926).

Preparation of extract: The powdered plant sample (1500 g) was soaked in 3.0 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator and a portion (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993) into petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble fractions.

Collection of rats: Healthy Long Evan's rats 4-5 months of age, weighing between 170-220 g, were used for antidiabetic investigation. The rats were bought from the Animal Resources Branch of the International Centre

* Correspondence to: Mohammad A. Rashid; Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh E-mail: rashidma@univdhaka.edu

for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B). They were housed individually in cages and were kept at constant room temperature (25.0±3.0^{o}C), humidity 35-60% and 12 hours light and 12 hours dark cycle to get them adapted with the new environment of the laboratory, before being employed in any experiment (Hawk *et al.,* 1954).

Induction of diabetes: Diabetes was induced in rats by injecting a freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg, i.p.) (Rao et al., 1999) after a base line glucose estimation was done. After forty eight hours of alloxan administration, animals with blood glucose levels above 12 mmol / L were selected for the study.

Experimental design: A total of 40 experimental rats were grouped into five, each group consisting of 8 rats. Each of these groups was then sub-grouped into two, each sub-group containing 4 rats. On the other hand, a total of 18 rats were divided into 3 groups to be used as normal control, negative control and positive control. Each extractive under investigation was administered for 21 days at two different doses (100 and 300 mg/kg body weight) whereas standard antidiabetic drug glibenclamide (600 µg/kg) (Pari and Umamaheswari, 2000) was given to rats belonging to the positive control group.

Group 1 :	Normal untreated rats
Group 2 :	Alloxan treated diabetic rats (negative control)
Group 3 :	Alloxan treated diabetic rats given glibenclamide (600 µg/kg)
Group 4 :	Alloxan treated diabetic rats given petroleum ether soluble materials (PESM) (100 mg/kg and 300 mg/kg)
Group 5 :	Alloxan treated diabetic rats given carbon tetrachloride soluble materials (CTSM) (100 mg/kg and 300 mg/kg)
Group 6 :	Alloxan treated diabetic rats given dichloromethane soluble materials (DCMSM) (100 mg/kg and 300 mg/kg)
Group 7 :	Alloxan treated diabetic rats given aqueous soluble materials (AQSM) (100 mg/kg and 300 mg/kg)

In the study, the body weight was assessed weekly. Blood samples of normal and diabetic rats were drawn after an overnight fasting (12 hr) from rat's tail vein at different time intervals i.e. 1st, 7th, 14th and 21st days for determination of serum glucose level. The level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined after 21 days.

Preparation of sample suspension: The extractives (100 mg/kg and 300 mg/kg body weight) and Glibenclamide (600 µg/kg body weight) were prepared by suspending each in distilled water using 1% Tween-80 as a suspending agent.

Serum glucose and enzyme assessments: Serum glucose was estimated with the glucometer One Touch Basic Plus (Easy pain Supreme, Taiwan) and liver enzyme level was estimated by the method designed by Mansour *et al.,* 2002.

Histopathology of liver, heart, kidney and spleen of rats: Histopathological studies of the liver, kidney heart and spleen were performed to observe the change in cellular structure of the rats receiving various extractives of seeds of *S. cumini.* It was done by comparing the liver, kidney, heart and spleen tissues and cells of the experimental rats with that of the control rats. The sliced-tissues were immersed in 10% formalin for 3 days. The tissues were dehydrated with ethanol and embedded in paraffin. Blocks were made and sectioned with a rotating microtone at a thickness of 6 microns. Then they were deparaffinised with two changes of xylene (5 minutes in each) and hydrated in descending order of ethanol (2-3 minutes in each). The sections were washed in water, then kept in Erhlich's hematoxylin (2-3 minutes) and finally washed in running water. After this, they were counter stained in Eosin solution (2-3 minutes) and dehydrated in ascending order of ethanol (2-3 minutes in each). The sections were lastly cleared in two sets of xylene (5 minutes in each). Glass slides were wiped dry except for the area containing the tissue sections. A drop of canada balsam was put gently onto the sections. When the cover slips were pressed firmly onto the sections, the mounting medium was spreaded out to form a thin film between the coverslip and the microscopic glass slide and thus attached together. The prepared slides from liver, kidney, heart and

spleen of the control and experimental rats were examined under low to moderate magnification of the Olympus CX-41 microscope.

Data and statistical analysis: Data were expressed as means ± SEM. Statistical comparisons were performed using Student's t-test. $P<0.05$ was considered as significant.

Results and Discussion

The induction of diabetes in rats by alloxan produced a significant reduction of body weight in all hyperglycemic rats. Daily administration of various extractives of *S. cumini* for 21 days caused a statistically significant increase in body weight ($P<0.05$) when compared with diabetic control rats. On the other hand, no significant body weight anomaly was observed between glibenclamide treated and the plant extracts treated-groups (Table 1).

In the table 2, the blood sugar profile of all the experimental rats is illustrated. Among them, the petroleum ether soluble fraction of methanol extract showed a significant ($P<0.001$) and rapid blood glucose reduction on 7th day (71.68% at 100- and 72.53% at 300-mg/kg body weight), 14th day (72.51% at 100- and 73.73% at 300-mg/kg body weight) and 21st day (73.19% at 100- and 76.82% at 300-mg/kg body weight) (Table 2). No significant difference was observed between the sugar reducing potency of glibenclamide (at a dose 600 µg/kg body weight) and petroleum ether soluble fraction of seeds of *S. cumini* (100- and 300-mg/kg body weight) at the 7th, 14th and 21st days of treatment.

The safety of the extractives in animals was evaluated by observing the effects of the same on liver enzymes and histopathology of liver, kidney heart and spleen tissues. The levels of enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in plasma of normal and diabetic rats are depicted in table 3. The alloxan treatment increased these enzymes levels in the diabetic rats but the treatment with various fractions of the *S. cumini* seed showed no significant difference of the enzyme levels with normal control rats.

The experimental rats receiving various extracts at two different doses of 100 and 300 mg/kg body weight/day for consecutive 21 days were sacrificed at the end of drug treatment. Histopathological studies were made by comparing the liver, heart, kidney and spleen tissues of the experimental rats with those of the control (Gr-1). The results of histopathological studies have been summarized in table 4 which clearly demonstrated no abnormalities in the *S. cumini* treated rats when compared with untreated control rats.

The seed of *S. cumini* is very commonly used as antidiabetic agent. But till now, the active hypoglycemic fraction of the seed has not been identified. Further, the safety profile of the seed extractives has not also been yet reported. In this study, we used the seed of *S. cumini* growing in Bangladesh. Initially, we proceeded through partitioning of the methanol extract of the seed in order to get the petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble fractions. These fractions were provided to normal and alloxan-induced diabetic rats to evaluate their hypoglycemic efficacy. In this regard, body weight and blood sugar level were measured weekly to monitor the antidiabetic activity. In order to confirm the safety, the level of liver enzymes (AST, ALT and ALP) was checked. In addition, the histopathological studyies of liver, kidney, spleen and heart were also performed.

Our thorough and systematic investigation with the extractives revealed the fact that the petroleum ether soluble partitionate of methanol extract is the active fraction for controlling diabetes. It showed rapid onset of hypoglycemic action immediately after 7 days and continuation of the treatment gradually reduced the plasma sugar level to normal range. In parallel, the body weight of the petroleum ether treated diabetic rats was also improved in comparison with the untreated diabetic control rats (Table 1). Besides, the blood sugar reduction trend of petroleum ether and glibenclamide treated groups was also found similar. However, the other fractions of the seed did not show promising hypoglycemic effect.

Nowadays, the liver enzyme assay is a common tool for monitoring drug safety and toxicity. In this regard, the plasma level of AST, ALT and ALP were checked at the end of the study. In alloxan-induced diabetic rats, the levels of plasma AST, ALT and ALP were significantly ($P<0.001$) increased relative to their normal levels (Table 3).

Table 1. The changes in body weight of treated and untreated rats

Groups	Dose	Effects on body weight (g)			
		1st day	7th day	14th day	21st day
Group-1: Normal untreated	Vehicle only	172.5 ± 0.90	177.06 ± 0.81	181.88 ± 0.83	185.66 ± 0.69
Group-2: Alloxan treated (negative control)	Vehicle only	183.91 ± 1.74	178.73 ± 1.77	168.7 ± 1.6	157.25 ± 1.51
Group-3: Glibenclamide treated (positive control)	600 µg/kg	179.6 ± 1.38	175.08 ± 1.37	170.1±1.35	165.7 ± 1.26
Group-4: PESM treated	100 mg/kg	192.47 ± 2.53	186.9 ± 2.61	178.75 ± 2.30*	172.9 ± 2.01*
	300 mg/kg	188.62 ± 1.95	182.7 ± 1.96	176.61 ± 1.63*	170.75 ± 1.51*
Group-5: CTSM treated	100 mg/kg	184.5 ± 3.48	180.5 ± 3.48	174.6 ± 3.25	165.11 ± 2.43
	300 mg/kg	183.64 ± 1.72	178.83 ± 1.35	171.33 ± 1.08	162.33 ± 1.33
Group-6: DCMSM treated	100 mg/kg	182.0 ± 1.85	176.15 ± 1.46	174.1 ± 0.75	169.97 ± 0.53*
	300 mg/kg	180.82 ± 0.69	176.1 ± 0.66	175.52 ± 1.13	170.75 ± 0.64*
Group-7: AQSM treated	100 mg/kg	185.83 ± 2.79	179.0 ± 2.16	171.51 ± 1.45	164.52 ± 1.45
	300 mg/kg	184.8 ± 2.18	178.66 ± 2.14	172.0 ± 2.35	166.16 ± 2.42

Values are given in average body weight in gm ± SEM; Experimental groups 4-7 were compared with diabetic control group-2 on corresponding day; *P<0.05

Table 2. The effect of 3 weeks treatment of various extracts of *S. cumini* on blood sugar level of alloxan-induced diabetic rats.

Groups	Dose	mmol/L			
		1st day	7th day	14th day	21st day
Group-1: Normal untreated	Vehicle only	5.18 ± 0.034	5.18 ± 0.06	5.13 ± 0.053	5.21 ± 0.045
Group-2: Alloxan treated (negative control)	Vehicle only	23.86 ± 0.60**	22.31 ± 0.45**	27.16 ± 0.41**	28.98 ± 0.54**
Group-3: Glibenclamide treated (positive control)	600 µg/kg	20.55 ± 0.60	4.98 ± 0.041**	6.36 ± 0.36**	5.63 ± 0.058**
Group-4: PESM treated	100 mg/kg	21.9 ± 0.43	6.2 ± 0.25**	6.02 ± 0.197**	5.25 ± 0.077**
	300 mg/kg	22.65 ± 0.37	6.22 ± 0.22**	5.95 ± 0.126**	5.2 ± 0.120**
Group-5: CTSM treated	100 mg/kg	22.32 ± 3.82	20.27 ± 1.50	21.23 ± 0.80	19.75 ± 0.88
	300 mg/kg	21.45 ± 2.01	17.47 ± 1.45	18.07 ± 0.97	17.42 ± 0.58
Group-6: DCMSM treated	100 mg/kg	27.62 ± 1.58	15.52 ± 1.89	10.04 ± 2.08	12.42 ± 0.59
	300 mg/kg	19.5 ± 1.67	12.65 ± 1.59	11.8 ± 1.44	11.15 ± 0.89
Group-7: AQSM treated	100 mg/kg	27.07 ± 0.0.31	22.27 ± 0.50	19.97 ± 0.77	21.65 ± 0.202
	300 mg/kg	26.52 ± 2.19	24.07 ± 1.29	27.47 ± 1.65	22.75 ± 3.21

Values are given as mean ± SEM; Diabetic control group-2 was compared with normal group-1 on corresponding day. Experimental groups 4-7 were compared with diabetic control group 2 on corresponding day; *P<0.05, **P<0.001

Table 3. The effect of 3 weeks treatment of various extracts of *S. cumini* on AST, ALT and ALP of alloxan induced diabetic rats.

Groups	Dose	U/L		
		ALT	AST	ASP
Group-1: Normal untreated	Vehicle only	5.1±0.33	12.58±0.22	271.16±2.93
Group-2: Alloxan treated (negative control)	Vehicle only	39.82±0.79**	26.03±0.72**	335.47±4.78*
Group-3: Glibenclamide treated (positive control)	600 µg/kg	15.96±0.32*	16.12±0.29	279.31±2.36
Group-4: PESM treated	100 mg/kg	9.0±0.55	17.88±1.66	266.42±15.41
	300 mg/kg	8.2±0.68	16.31±0.55	234.72±6.85
Group-5: CTSM treated	100 mg/kg	17.07±0.74**	30.27±1.75**	345.7±17.71*
	300 mg/kg	16.06±0.39**	25.14±2.36*	299.33±11.12
Group-6: DCMSM treated	100 mg/kg	8.8±0.32	13.95±0.54	375.58±24.81**
	300 mg/kg	8.22±0.10	15.68±0.72	317.76±17.11*
Group-7: AQSM treated	100 mg/kg	13.63±0.65*	33.82±2.01**	341.86±6.80*
	300 mg/kg	13.22±1.01*	33.23±1.72**	317.86±10.04*

Values are given as mean ± SEM; Groups 2-7 were compared with normal control group 1 on corresponding day; *P<0.05, **P<0.001

Table 4. Histopathological status of liver, kidney, heart and spleen after administration of *S. cumini* extractives for 3 weeks.

Test group	Histopathological status			
	Liver	Kidney	Heart	Spleen
Group-1: Normal untreated	No change	No change	No change	No change
Group-2: Alloxan treated (negative control)	No change	No change	No change	No change
Group-3: Glibenclamide treated (positive control)	Mild fatty change	No change	No change	No change
Group-4: PESM treated	No change	Mild fatty change	No change	No change
Group-5: CTSM treated	No change	No change	No change	No change
Group-6: DCMSM treated	No change	Focal infiltration of lymphocytes	No change	No change
Group-7: AQSM treated	No change	No change	No change	No change

However, the petroleum ether fraction treated group showed an optimum and significant decrease ($P<0.05$) of these enzymes in comparison to those of diabetic control rats. The histopathological studies of liver, kidney heart and spleen tissues of all the groups were also performed (Table 4). It demonstrated no major abnormalities in the viscera of *S. cumini* extractives (petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble fractions) treated rats when compared with normal rats.

From this animal model trial, it is clearly evident that the petroleum ether soluble fraction of the seed of *S. cumini* is highly effective in controlling hyperglycemia with no untoward effects on the liver, heart, kidney and spleen tissues. Further phytochemical study is underway to identify the active antidiabetic principle(s) from this less polar extractive of the seed.

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