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Full Length Research Paper

Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats

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Syzygium cumini **(Myrtaceae) is widely used traditional system of medicine to treat diabetes in India. The present study was carried out to isolate and identify the putative antidiabetic compound from the *S. cumini* [SC] seed. A compound, mycaminose was isolated from SC seed extract. The isolated compound mycaminose (50 mg/kg) and ethyl acetate [EA] and methanol [ME] extracted compounds of *S. cumini* seed (200 and 400 mg/kg) was undertaken to evaluate the anti-diabetic activity against streptozotocin (STZ)-induced diabetic rates. The compound 'Mycaminose' and ethyl acetate and methanol extracted produced significant ($p<0.05$) reduction in blood glucose level. The standard drug, glibenclamide (1.25 mg/kg) also produced significant ($p<0.05$) reduction in blood glucose level against STZ-induced diabetic rats. The results of this experimental study indicate that isolated compound 'Mycaminose', ethyl acetate and methanol extracts possess anti-diabetic effects against STZ-induced diabetic rats.**

Key words: *Syzygium cumini*, ethyl acetate, methanol, mycaminose, anti-diabetic.

INTRODUCTION

Diabetes mellitus [DM] is a metabolic disorder of the endocrine system. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. Diabetes is becoming the third 'killer' of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality (Li et al., 2004) (Li et al., 2004). Approximately 4% popula-tion worldwide and is expected to increase by 5.4% in 2025 (Kim et al., 2006). The number of adults suffering from diabetes in India is expected to increase threefold, from 19.4 million in 1995 and 57.2 million in 2025. Recent studies on geographical and ethical influences have shown that people of Indian origin are highly prone to diabetes. Diabetes is characterized by hyperglycemia due to an absolute or relative deficiency of insulin (WHO, 1994).

DM is a metabolic disorder affecting carbohydrate, fat and protein metabolism. The worldwide survey reported that the DM is affecting nearly 10% of the population (Siddharth, 2001). The treatment of DM is based on oral hypoglycaemic agents and insulin. However, DM is also treated in Indian traditional medicine using anti-diabetic medicinal plants (Chattopadhyay et al., 1993; Ponnachan and Panikkhar, 1993; Subramonium et al., 1996). The oral hypoglycaemic agents currently used in clinical practice have characteristic profiles of serious side effects (Prout, 1974; Holman and Tuener, 1991). Hence, there is a need to search for newer anti-diabetic agents that retain therapeutic efficacy and are devoid of side effects that could be important sources of such agents.

The *Syzygium cumini* (or *Eugenia jambolana*) tree belongs to the Myrtaceae family. This is also called as Jamun, Jambul and Jambol in India and Malaya. The barks, leaves and seeds extracts of SC have been reported to possess anti-inflammatory (Chandhuri et al., 1990), antibacterial (Bhuiyan et al., 1996) and anti-diarraheal effects (Indira and Mohan, 1992). The present study was designed to evaluate the anti-diabetic activity of isolated compound mycaminose, EA and ME extracts of the SC seeds against STZ-induced diabetic rats. The effect of SC extracts was

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compared to glibenclamide, which is often used as a standard drug.

MATERIALS AND METHODS

Plant material

The fully mature SC seeds were collected in June-July 2006 from Kattuppalayam Village in Erode District of Tamil Nadu, India from a single tree. The seed was identified and authenticated by Dr. S. Amerjothy, Head of the Department of Plant Biology and Plant Biotechnology, Presidency College, Chennai and voucher specimen (No.1586) was deposited in the Herbarium of the same department.

Preparation of plant extract

The SC fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with ethyl acetate and methanol using cold percolation method. The percentage yields were 1.81% in ethyl acetate and 10.36% in methanol.

Preliminary phytochemical screening

The phytochemical screening of SC seed contains alkaloids, amino Acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids.

Isolation and identification of the active compound

Five grams of the pure SC seed methanol extract was admixed with 10 g of silica gel (60 - 120 mesh), dried for uniform mixing and the admixture was loaded in a column (5 cm diameter X 50 cm height) packed with silica gel (150 g) using hexane as the solvent. The column was eluted with increasing order of polarity gradually from 100% hexane, 100% chloroform and methanol in ethyl acetate (0 - 100%). The fraction eluted at 100% methanol, yield of 350 mg obtained. The compound was obtained as pale brown semi solid. The fraction was characterized by spectroscopy techniques like ^{1}H NMR, ^{13}C NMR and Mass Spectrum.

Animals

Wistar rats (160 - 180 g) were purchased from King Institute, Chennai for experimental study. They were acclimated to animal house conditions fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal).

Acute toxicity studies

Acute oral toxicity (Ecobichon, 1997) study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n = 6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extracts (ethyl acetate and methanol) were administered orally at the dose level of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2 - 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300 and 2000 mg/kg body weight.

Anti-diabetic evaluation

Experimental induction of diabetes

Induction of diabetic mellitus: After fasting for 18 h, 60 rats were injected by 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 and water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock. The development of diabetes was confirmed after 48 h of the streptozotocin injection. The rats having fasting blood glucose level more than 200 mg/dL were selected for experimentation. From, the out of 60 animals, 6 animals were died before grouping and 5 animals were omitted from the study, because mild hyperglycemia (below 150 mg/dL). From the 49 diabetic animals, they were divided into seven groups each having 7 animals (Brosky and Logothelopocelos, 1969).

Collection of blood samples and glucose determination

Blood samples were collected by end tail vein cutting method and blood glucose level was determined by using one touch electronic glucometer. Using glucose strips (Lifescan, Johnson and Johnson Ltd.) (Kumar et al., 2005).

Experimental protocol

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 1% SCMC 10 ml/kg per orally for 15 days; Group II–STZ induced diabetic animals received 1% SCMC 10 ml/kg, p.o. for 15 days; Group III and IV– STZ induced diabetic animals received ethyl acetate extract at the dose of 200 and 400 mg/kg p.o. daily for 15 days; Group V and VI– STZ induced diabetic animals received methanolic extract at the dose of 200 and 400 mg/kg daily p.o. for 15 days; Group VII – STZ induced diabetic animals received mycaminose 50 mg/kg daily p.o. for 15 Days; Group VIII–STZ induced diabetic animals received standard drug, glibanclamide 1.25 mg/kg daily p.o. for 15 days.

All the group of animals received the treatment by the above schedule for 15 days. Blood samples were collected one hour after drug administration on the day 1, 5, 10 and 15^{th} day to determine the blood glucose level by electronic glucometer (Babu et al 2002).

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ± SD. Differences between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnet's *t*–test. p value < 0.05 were considered as significant.(Dixon and Jennrich, 1990).

RESULT

Identification of the compound

On isolation of the methanol extract, a pale brown semi solid was obtained. Structural determination of the compound was done using spectroscopy techniques and

Table 1. Anti-diabetic activity of *Syzygium cumini* seed extracts and isolated compound against Streptozotocin –induced diabetic rats.

Group (15 days)	Blood sugar level in mg/dL (mean ± SD)				
	Initial	Day 1	Day 5	Day 10	Day 15
Group – I (n=6)	70.78 ± 7.03	65.05 ± 9.33	66.70 ± 9.85	67.00 ± 7.41	65.48 ± 5.88
Group – II (n=5)	249.76 ± 8.85	262.28 ± 14.75	285.85 ± 4.78	309.20 ± 8.09	313.28 ± 4.73
Group – III (n=6)	250.85 ± 8.40	252.49 ± 5.57a^{NS}	239.23 ± 8.42a^{NS}b*	204.38 ± 5.84a*b*	192.03 ±5.80a*b*
Group – IV (n=7)	249.04 ± 3.89	249.65 ± 7.85a^{NS}	221.24 ± 5.41a*b*	189.10 ± 8.22a*b*	178.14 ± 9.30a*b*
Group – V (n=6)	248.70 ± 8.85	256.08 ± 4.98a^{NS}	239.88 ± 8.84a^{NS}b*	214.23 ± 3.33a*b*	182.85 ± 4.58a*b*
Group – VI (n=7)	251.84 ± 4.90	256.57 ± 5.57a^{NS}	233.45 ± 6.30a*b*	192.77 ± 4.89a*b*	154.85 ± 10.24a*b*
Group – VII (n=7)	248.38 ± 3.50	251.17 ± 8.14a^{NS}	217.97 ± 4.52a*b*	190.10 ± 7.91a*b*	180.21 ± 8.68a*b*
Group – VIII (n=7)	249.62 ± 8.53	247.51 ± 8.11a^{NS}	190.07 ± 11.04a*b*	167.84 ± 9.37a*b*	123.93 ± 5.89a*b*

Values are mean ± SD of respective groups, NS –Non Significant, *p<0.05 Comparison were made a – Initial Vs day 1, day 5, day 10 and day 15 of respective groups b – Group II Vs group III, IV, V, VI, VII and VIII

it was confirmed as mycaminose. The yield of mycaminose was 0.73 % in *S. cumini* seed power. The compound was identified based on the following evidence: Molecular weight 191; ^{1}H NMR (200 MHz): 1.01, 3.19, 3.26, 3.56 – 3.60 and 3.81; ^{13}C NMR (200 MHz): 12.30, 49.65, 70.14, 71.99, 76.56, 82.00 and 98.82. The molecular formula of mycaminose is $C_8H_{17}NO_4$.

Acute toxicity studies

This study showed no mortality up to the dose of 2,000 mg/kg body weight. So, the extracts safe for long term administration.

Anti-diabetic activity

The blood sugar levels measured in normal and experimental rats in initial and at the 1, 5, 10 and 15 days of treatment are given in Table 1. Streptozotocin-induced diabetic rats showed significant increase in the levels on blood sugar as compared to normal rats. Oral administration of ethyl acetate and methanol extracts (200 and 400 mg/kg) showed significant decrease ($p<0.05$) in blood sugar level. The isolated compound, mycaminose at a dose level of 50 mg/kg also showed significant decrease ($p<0.05$) in blood sugar level. The standard drug, glibenclamide decreased blood sugar level in 15 days treatment.

DISCUSSION

The aim of the present study was to evaluate the anti-diabetic effect of ethyl acetate and methanolic extracts of SC seed and isolated compound mycaminose, against streptozotocin-induced diabetic rats. The continuous treatment of the extracts of SC for a period of 15 days produced a significant decrease in the blood sugar levels of diabetic rats. These results confirmed the use of SC seed of traditional practice as an anti-diabetic (Anonymous, 1985). The standard drug, Glibenclamide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic -cells (Tian et al., 1998). It may be suggested that the mechanism of action of mycaminose is similar to glibenclamide, this is may be the first report that demonstrates antidiabetic properties for mycaminose.

The possible mechanism by which seed brings about a decrease in blood sugar level may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from -cells of the islets of Langerhans or its release from the bound form. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effects (Twaij and Al-Badr, 1988; Gupta, 1994).

Pepato et al. (2005) reported that the therapeutic potential of Eugenia jambolana is related to the geographic region in which the plant was grown and to the part of the plant used. The leaves of Brazilian Eugenia jambolana have no effect on diabetes (Teixeira et al., 1990, 1997, 2000; Pepato et al., 2001). But, the SC seed which was collected from Kattuppalayam Village in Erode District of Tamil Nadu, India have anti-diabetic properties.

These results confirmed the use of *S. cumini* seed 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 SC seed.

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