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EVALUATION OF ANTI DIABETIC ACTIVITY OF LEAVES OF *SYZYGIUM CUMINI* MONO HERBAL FORMULATION IN WISTAR RATS

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ABSTRACT

Syzygium cumini (Myrtaceae) is widely used traditional system of medicine to treat diabetes in India. Thepresent study was carried out to isolate and identify the putative antidiabetic compound from the *S. Cumini* leaves. A compound, mycaminose was isolated from SC leaf extract. The isolated compound mycaminose (50 mg/kg) and ethyl acetate and ethanol extracted compounds of *S. cumini* leaves (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) alsoproduced significant ($p<0.05$) reduction in blood glucose level against STZ-induced wister rats. The results of this experimental study indicate that isolated compound 'Mycaminose', ethyl acetate and ethanol extracts possess anti-diabetic effects against STZ-induced wister rats.

KEYWORDS: *Syzygium cumini*, ethyl acetate, ethanol, mycaminose, anti-diabetic.

INTRODUCTION

Diabetes mellitus 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 Approximately 4% population worldwide and is expected to increase by 5.4% in 2025. 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. DM is a metabolic disorder affecting carbohydrate, fat and protein metabolism. The worldwide survey reportedthat the DM is affecting nearly 10% of the population. The treatment of DM is based on oralhypoglycaemic agents and insulin. However, DM is alsotreated in Indian traditional medicine using anti-diabeticmedicinal plants. The oral hypoglycaemic agents currently used in clinicalpractice have characteristic profiles of serious sideeffects (Prout, 1974; Holman and Tuener, 1991). Hence, there is a need to search for newer anti-diabetic agentsthat retain therapeutic efficacy and are devoid of sideeffects that could be important sources of such agents. The *Syzygium cumini* (or *Eugenia jambolana*) treebelongs to the Myrtaceae family. This is also called asJamun, Jambul and Jambol in India and Malaya. Thebarks, leaves and seeds extracts of SC have been reported to possess anti-inflammatory antibacterial and antidiarraheal effects. The present study was designed to evaluate the anti-diabetic activity of isolated compound mycaminose, ME extracts of the SC seeds against STZ-induced wistar rats.

MATERIALS AND METHODS

Plant material

The fully mature SC leaves were collected in June-July 2016 from Kattuppalayam Village in Erode District of Tamil Nadu, India from asingle 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 leaves were first washed well and pulp was removed from the leaves. Leaves were washed several times with distilled water to remove the traces from the leaves. The leaves 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 ethanol.

Preliminary phytochemical screening

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

Isolation and identification of the active compound

Five grams of the pure SC leaves ethanol extract was admixed with10 g of silica gel (60 - 120 mesh), dried for uniform mixing and the mixture 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 from100% 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 1HNMR, 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 study was performed as perOECD-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 intra gastric 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 49diabetic animals, they were divided into seven groups each having7 animals.

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 JohnsonLtd.) (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/kgper 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 ethanolic 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 15th day to determine the blood glucose level by electronic glucometer.

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.

RESULTS AND DISCUSSION

Identification of the compound

On isolation of the methanol extract, a pale brown semisolid was obtained. Structural determination of the compound was done using spectroscopy techniques and it was confirmed as mycaminose. The yield of mycaminose was 0.73 % in *S. cumini* leave power. The compoundwas identified based on the following evidence:Molecular weight 191; 1H NMR (200 MHz): 1.01, 3.19,3.26, 3.56 – 3.60 and 3.81; 13C NMR (200 MHz): 12.30,49.65, 70.14, 71.99, 76.56, 82.00 and 98.82.

Acute toxicity studies

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

Anti-diabetic activity

The blood sugar levels measured in normal and experimental rats in initial and at the 11, 16, and 21 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 ethanol extracts (200 and400 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 21 days treatment.

Table 1: Effect of administration of *Syzigium cumini* leaf extract + Glibenclamide for 21 days on blood glucose levels in diabetic rats.

Group	Treatment	Glucose level at 11th Day	Glucose level at 16th day	Glucose level at 21st day
Group -1	Control (Normal control)	105 ± 1.61	97.86 ± 1.56	94.58 ± 1.32
Group -2	Diabetic control	278.53 ± 5.51	264.93 ± 9.31	262.22 ± 9.16
Group -3	*Syzigium cumini* leaf extract (100mg/kg, p.o)	207.13 ± 15.41	189.73 ± 36.50	140.06 ± 4.48
Group -4	*Syzigium cumini* leaf extract (200mg/kg, p.o)	269.14 ± 1.16	186.37 ± 33.76	147.65 ± 3.38
Group-5	*Syzigium cumini* leaf extract (5 micro g/kg, p.o) (standard)	269.43 ± 5.48	189.65 ± 34.65	165.58 ± 5.49

Values are expressed in mean ± SEM, n=6. Experimental groups statistically compared with control P ≤ 0.001.

The aim of the present study was to evaluate the antidiabetic 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 21 daysproduced a significant decrease in the blood sugar levels of diabetic rats. These results confirmed the use of SC leaf of traditional practice as an anti-diabetic. The standard drug, Glibenclamide has been used for many years to treat diabetes, to stimulateinsulin secretion from pancreatic-cells. 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 hypoglycaemic activity through insulin release-stimulatory effects. Pepato et al reported that the therapeutic potentialof Eugenia jambolana is related to the geographic region in which the plant was grown and to the part of theplant used. The leaves of Brazilian Eugenia jambolana have no effect on diabetes 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 intraditional 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 leaf.

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