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Experimental evaluation of antidiabetic activity of *Syzygium aromaticum* extract in streptozotocin induced diabetic rats

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ABSTRACT

Diabetes mellitus is a globally prevalent chronic debilitating illness. The goals in management of diabetes are to alleviate symptoms and signs of hyperglycaemia, and to prevent or reduce the acute and chronic complications of diabetes. There are a range of oral hypoglycaemic agents available for the treatment of diabetes but they are costly and have many adverse effects which warrant the continued research for newer drugs. In the present study, the flower buds of *Syzygium aromaticum* was studied for its antidiabetic activity against Streptozotocin (55mg/kg, i.p) induced diabetes in rats. 50% of ethanol was used as solvent to extract the constituents of *Syzygium aromaticum* buds. Three dose levels of *Syzygium aromaticum* (100, 300 and 500mg/kg) were used in the study. The test drugs were administered once daily for 15 days and the mean body weight, blood sugar were measured on 0, 5, 10 and 15th day using Glucometer and percentage reduction in blood sugar levels were also determined. All the doses of ethanolic bud extract of *Syzygium aromaticum* exhibited antidiabetic effect, but 500mg/kg showed significant antidiabetic effect as compared to other doses. From the result it was concluded that, *Syzygium aromaticum* exhibited antidiabetic effect against Streptozotocin induced diabetes in rats. Further studies are required to establish its anti-diabetic activity and to find out the exact mechanism for its anti-diabetic effect.

Keywords: *Syzygium aromaticum*, Antidiabetic, Glibenclamide and Streptozotocin .

INTRODUCTION

Diabetes mellitus is the world's largest endocrine disease resulting in disordered metabolism, usually due to a combination of hereditary and environmental

causes, resulting in abnormally high blood sugar levels due to defects in either insulin secretion or insulin action in the body or both [1]. The worldwide prevalence of diabetes has risen dramatically over the

past two decades, from an estimated 30 million cases in 1985 to 366 million in 2011. Based on current trends, it is estimated that more than 522 million people will be suffering with the disease by year 2030[2]. The most dramatic increases have been recorded in developing countries such as India, and diabetes accounts for a major portion of healthcare expenditure, further emphasizing the need for newer and cheaper modalities of treatment.

Although many drugs and interventions are available to manage diabetes, these are expensive for the large diabetic population of developing countries, apart from their inherent adverse effects. So it is necessary to look for new cheap alternatives to manage this major health problem [3]. Different indigenous drugs had been used in the Indian subcontinent for several centuries for treatment of Diabetes mellitus with conflicting report of their efficacy because of lack of scientific investigation in a laboratory setting. One such plant, *Syzygium aromaticum* (clove) whose dried flower buds has long been used traditionally in the treatment of Diabetes mellitus in South Asian countries is selected for the study.

Syzygium aromaticum belonging to family "Myrtaceae" is a spice which is well known for its medicinal properties. It is commonly known as clove. The clove tree is an evergreen that grows to a height ranging from 8–12 m, having large square leaves and 25 sanguine flowers in numerous groups of terminal clusters. The dried flower buds (cloves) of *Syzygium aromaticum* is extensively used in Ayurvedha, Siddha and Chinese medicinal systems. Cloves are used as an anodyne for dental pain, as a carminative, and as an anthelmintic [4]. Clove oil is used as an antiseptic and analgesic [5]. Other studies have shown that cloves have significant antioxidant property [6]. As a spice, cloves are commonly used for culinary purposes [7]. In this study, the antidiabetic potential of the ethanolic extract of dried flower buds of *Syzygium aromaticum* will be screened on laboratory animal model.

METHODOLOGY

Plant material and extraction

Dried clove buds (*Syzygium aromaticum*) were purchased from local market (Karimnagar, Andhra Pradesh, India) and identified by the Department of Botany, Govt. Junior College of Sciences,

Karimnagar. The voucher specimen was deposited in the herbarium for future reference.

The buds of *Syzygium aromaticum* were cleaned and dried. The buds were ground to coarse particles and extraction was carried out with Soxhlet apparatus using 50% ethanol as solvent. Excess solvents were evaporated to dryness using rotary evaporator and the concentrated extract was stored in desiccators.

Animals

The study was carried out in Post Graduate research laboratory of the department of Pharmacology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar. Laboratory bred Wistar rats of either sex weighing between 150 – 250 gms were obtained from M/S Sainath Agencies, Hyderabad and placed in individual cages in central animal house of the institute. On arrival, all the animals were randomly allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of $24\pm 2^{\circ}\text{C}$ and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chow (M/s. Hindustan Lever Ltd, Mumbai). Ethical clearance was obtained from the Institutional Animal Ethics Committee prior to the commencement of the experiment.

INDUCTION OF DIABETES

Streptozotocin was stored at $4 - 8^{\circ}\text{C}$. It was dissolved in sterile normal saline. It was always prepared freshly for immediate use. All rats were fasted overnight before diabetes was induced. STZ was given in the dose of 55 mg/kg body weight, single intraperitoneal injection. The animals were observed to be diabetic from the 3rd day onwards. The animals showing a blood glucose level of 250 mg/dl and above were considered diabetic and were included for the study.

EXPERIMENTAL DESIGN

- **Group I:** Consisted of 6 rats which served as normal control group and were given distilled water orally every day.
- **Group II:** Consisted of 6 STZ induced diabetic rats which served as diabetic control group and were given distilled water orally daily.

- **Group III:** Consisted of 6 STZ induced diabetic rats which served as standard group and were given Glibenclamide at a dose of 0.5 mg/kg body weight orally daily.
- **Group IV:** Consisted of 6 STZ induced diabetic rats and were given *Syzygium aromaticum* extract at a dose of 100 mg/kg dose orally daily.
- **Group V:** Consisted of 6 STZ induced diabetic rats and were given *Syzygium aromaticum* extract at a dose of 300 mg/kg dose orally daily.
- **Group VI:** Consisted of 6 STZ induced diabetic rats and were given *Syzygium aromaticum* extract at a dose of 500 mg/kg dose orally daily.

All the test drugs were administered orally using oral feeding tube once daily for 15 days. Blood was withdrawn from tail vein on 0, 5th, 10th and 15th day of test drug administration and glucose levels were measured using glucometer (One Touch - Horizon glucometer). Body weights of each animal were also observed on 0, 5th, 10th and 15th day of test drug administration.

STATISTICAL ANALYSIS

All the values were expressed as Mean \pm SEM and percentage reduction were calculated. The test of significance was done by using student 't'-test.

RESULT AND DISCUSSION

Table 1. Effect of *Syzygium aromaticum* on blood sugar levels of Streptozotocin induced diabetes in rats.

Drug Treatment	Mean Blood Sugar Level (mg/dl)			
	0 day	5 th Day	10 th day	15 th Day
Group - I	102.33 \pm	105.17 \pm	101.83 \pm	98.00 \pm
Normal Control	5.26	4.85	5.35	3.86
Group - II	334.17 \pm	380.50 \pm	390.67 \pm	379.17 \pm
Diabetic Control	17.06	19.33	24.67	13.65
Group - III	270.83 \pm	221.17 \pm	137.33 \pm	105.00 \pm
Diabetic Standard Glibenclamide (0.5mg/kg)	8.28	11.28**	8.71**	12.72**
Group - IV	322.50 \pm	291.83 \pm	245.50 \pm	194.67 \pm
(<i>Syzygium aromaticum</i>) 100mg/kg	15.41	14.50*	12.89**	10.29**
Group - V	319.17 \pm	278.17 \pm	202.83 \pm	119.83 \pm
(<i>Syzygium aromaticum</i>) 300mg/kg	8.36	8.74*	11.27**	7.26**
Group - VI	311.17 \pm	261.17 \pm	185.00 \pm	109.17 \pm
(<i>Syzygium aromaticum</i>) 500mg/kg	6.43	11.46**	8.04**	3.74**

Values are in mean \pm SEM (n=6), *P<0.001, **P<0.0001 Vs Control

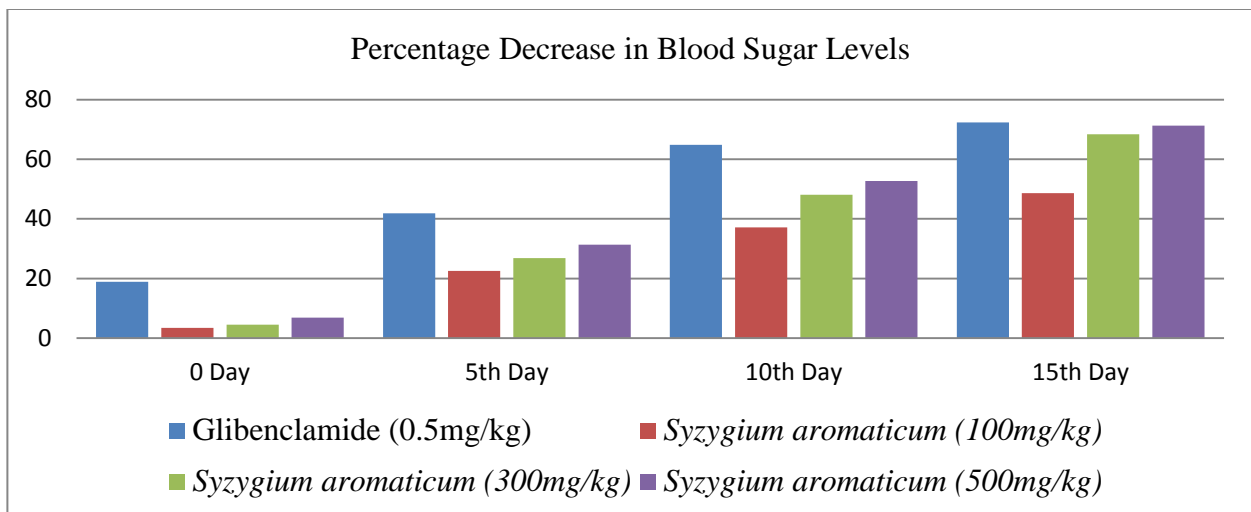


Figure 1. Effect of *Syzygium aromaticum* on percentage decrease in blood sugar levels against Streptozotocin induced diabetes in rats.

Table 2. Effect of *Syzygium aromaticum* on body weight of Streptozotocin induced diabetes in rats.

Drug Treatment	Mean Body Weight (gm)			
	0 day	5 th day	10 th day	15 th day
Group - I	211.67±	213.33±	211.33±8.	214.33±7.
Normal Control	8.22	7.50	31	11
Group - II	208.00±	187.33±	166.67±3.	145.33±3.
Diabetic Control	4.71	4.34	57	50
Group - III	210.33±	191.33±	184.33±7.	181.00±7.
Diabetic Standard	6.50	7.02	42*	51*
Group - IV	213.33±	199.00±	182.67±4.	166.00±3.
(<i>Syzygium aromaticum</i>)	3.53	2.91	09*	43*
100mg/kg				
Group - V	211.00±	194.00±	183.00±6.	172.00±3.
(<i>Syzygium aromaticum</i>)	8.87	6.75	04*	65*
300mg/kg				
Group - VI	209.67±	194.33±	186.67±5.	174.67±4.
(<i>Syzygium aromaticum</i>)	6.90	6.01	10*	02*
500mg/kg				

Values are in mean ± SEM (n=6), *P<0.001, **P<0.0001 Vs Control

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental factors. The factors contributing to hyperglycaemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The complications associated with diabetes involve multiple organ systems and impose a tremendous burden on the individual with diabetes and on the health care

system. Plants have played a major role in the development of new therapeutic agents. But there is still an extensive demand for new oral anti-diabetic drugs which are cheaper and have lesser side effects. Different indigenous drugs are used in the traditional systems of medicine for the treatment of diabetes mellitus but they lack scientific investigations for their efficacy in a laboratory setting.

One such plant, *Syzygium aromaticum* (clove) whose dried flower buds has long been used traditionally for culinary purposes in South Asian and

African countries, is selected for the study. Oil from clove has been shown to possess many beneficial therapeutic properties like analgesic, anti-inflammatory, antimicrobial, antioxidant properties. The present study was carried out to evaluate the anti-diabetic effect of extract of dried flower buds of *Syzygium aromaticum* in streptozotocin induced diabetic rats.

The effect of *Syzygium aromaticum* on blood sugar and body weight were shown in table 1,2 and figure 1. The present study, 50% ethanolic extract of dried flower buds of *Syzygium aromaticum* was given to different groups of animals at a dose of 100, 300 and 500 mg/kg body weight (groups IV, V and VI) and the blood glucose lowering effect of these groups were compared with the diabetic control group.

Statistical analysis of the test groups with the control group showed significant reduction in blood glucose levels, especially from the 5th day onwards. When compared to the diabetic standard group, the following observations were made. The diabetic test group -IV which were treated with 100 mg/kg extract showed antidiabetic effect but it was not as

significant as the diabetic standard group treated with Glibenclamide. The diabetic test groups V and VI (300 and 500 mg/kg respectively) both showed statistically comparable anti-diabetic effect as to the diabetic standard group and percentage reduction was also as effective as Glibenclamide. There was no significant difference between the glucose lowering effects of diabetic test group -V (300 mg/kg) and diabetic test group – VI (500 mg/kg) which implies 300 mg/kg to be the ideal dose below which the effect is not significant and further increment in dose doesn't increase its effect. These results suggest that ethanolic extract of *Syzygium aromaticum* buds exhibits anti-diabetic property against STZ induced diabetes in rats.

CONCLUSION

The present study concludes that the extract of dried flower buds of *Syzygium aromaticum* has anti-diabetic property and further studies are needed to evaluate its antidiabetic effect in different species and to find out the most probable mechanism of action.

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