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Research Article

HYPOGLYCAEMIC EFFECT OF *SYZYGIUM CARYOPHYLLATUM* (L.) ALSTON ON ALLOXAN INDUCED DIABETIC ALBINO MICE

C. SAVITHA RABEQUE[1]*, S. PADMAVATHY[2]

[1] Research Scholar, R&D Centre, Bharathiar University, Coimbatore [2]Department of Botany, Nirmala College For Women, Coimbatore
Email: savitha.rabeque@gmail.com

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ABSTRACT

Objective: The primary objective of this study was to investigate the hypoglycaemic activity of *S. caryophyllatum* in alloxan induced diabetic mice.
Methods: Alloxan was administered as a single dose (60mg/kg, b. wt) to induce diabetes. Methanol extracts from *S. caryophyllatum* (250mg/kg body weight/day) was administered for 14 days to alloxan induced diabetic mice and the body weight changes and fasting blood sugar levels were monitored on 0, 7th and 14th day. Glycosylated haemoglobin levels also estimated.
Result: The results suggest that the administration of *S. caryophyllatum* have hypoglycaemic activity in alloxan induced diabetic mice and their effect was equivalent to that of reference drug Glibenclamide. Conclusions: Methanol extract of S. *caryophyllatum* possesses anti-diabetic effect on Alloxan induced diabetic mice due to the presence of secondary metabolites.

Keywords: Alloxan monohydrate, Glibenclamide, Glycosylated haemoglobin,

INTRODUCTION

Diabetes Mellitus is a universal metabolic disorder, with an increasing global trend and of particular concern to India; it has significant impact on the health, quality of life and life expectancy of patients as well as healthcare expenditure. The prevalence of diabetes is about 366 million people worldwide and it is likely to increase to 552 million and more by the year 2030 and its occurrence was found to be high in India, China, and USA (Whiting *et al*., 2011). India now has the world's largest diabetic population, encompassing an estimated 35 million people out of an overall population of 1 billion. In just over 13 years (i.e. 2025) the country will have almost 200 million people (approximately 15% of the population) affected by diabetes (Geetanjali *et al*., 2010). With increasing incidence and mortality from diabetic complications, prompt and adequate glycaemic control is paramount if the management can meaningfully improve the quality of life and increase life expectancy.

The World Health Organization (WHO) has also recommended the evaluation of traditional plant treatments for diabetes, as they are effective, non-toxic, with less or no side effects and is considered to be an excellent candidate for oral therapy (Shokeen *et al*., 2008). Many valuable sources of knowledge are available in different cultures dealing with medication for various diseases and disorders from ancient time particularly for diabetes. Many of these recommendations make use of natural herbs with no side effects to the individuals. Since the information is too old, there arises a need to revive these recommendations carefully by making use of modern scientific techniques. These recommendations may lead to the development of "alternative systems" of medicine. Very few plants have been so far studied further, in depth, for investigating their site and mechanism of action for possible development of anti-diabetic drugs. Plants like *Allium cepa* (Onion, piyaj), *Allium sativum* (garlic, lasun), *Syzygium cumini* (Syn. *Eugenia jambolana*; *Momordica charantia* (bitter gourd; karela) *Gymema sylvestre* (Gurmar), *Pterocarpus marsupium* etc. have attracted more attention to the scientists as well as laymen in the field of antidiabetic activity in recent years (Sheela *et al*., 1995; Sitasawad *et al*., 2000 and Soni *et al*., 2011).

Many species of Myrtaceae family has been widely used to treat diabetes by the traditional practioners over many centuries (Nadkarni, 1954). Among them, root, leaf, bark, fruit and seed extract of *S. cumini* have shown very good antihyperglycemic activity by many workers (Shrotri *et al*., 1963; Bansal *et al*., 1981; Achrekar *et al*., 1991; Grover *et al*., 2000; Sharma *et al*., 2003). On the other hand, one could expect that the antihyperglycemic activity may also be present among the very close related species of Myrtaceae family such as *S. caryophyllatum. Syzygium caryophyllatum* is one of the species that has been categorized as endangered tree species under the international nature for conservation of nature (IUCN) red list of threatened species. It is known as Wild black plum. In Tamil it is known as Kattu naval. It was used in the folklore medicine for various ailements, but there are only very few report available regarding the antihyperglycemic activity. Hence, the present investigation was aimed to prove the antihyperglycemic activity of leaf extract of *S. caryophyllatum* on alloxan induced diabetic animal model.

MATERIALS AND METHODS

Collection of the plant material

Syzygium caryophyllatum (L.) Alston (Myrtaceae) is collected during the month of October 2009 from Palani Hills, Tamil Nadu, India. It is identified and authenticated by Dr. S. Padmavathy, Associate Professor, Department of Botany, Nirmala College for Women (Autonomous), Coimbatore, Tamil Nadu and also the voucher specimen (T_1) was deposited.

Preparation of the extract

Freshly collected sample of *S. caryophyllatum* leaves, were washed 2-3 times with water followed by distilled water and shade dried. All the dried parts were pulverized by mechanical grinder (Willy mill) to get the powder through 100-mess sieve and then stored in a refrigerator. The shade dried powdered plant material (250g) was extracted with methanol using a soxhlet apparatus. Then the extract was concentrated in a rotary evaporator.

Animals

Three months old Swiss albino mice (24-25g) were obtained from the animal- breeding center of Kerala Agricultural University, Trissur, Kerala. All animals were kept in an environmentally controlled room with a 12h light/12h dark cycle. All the experiments were performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA).

Induction of diabetes

The mice were injected alloxan dissolved in sterile normal saline at a dose of 60mg/kg body weight, intraperitoneally. After a fortnight, mice with marked hyperglycaemia were selected and used for the study

Experimental design

The animals were randomly divided into four groups with 5 rats in each group and treated as follows

Group I: Normal control (Saline) (by using an intragastric catheter tube (IGC).

Group II: Diabetic control

Group III: Diabetic rats received Glibenclamide (2mg/kg b.wt.) for 14 days. for 14 days by

IGC

Group IV: Diabetic rats received *S. caryophyllatum* Methanol extract (250 mg/kg b.wt) for 14 days by IGC.

The change in body weight and fasting plasma glucose (FPG) levels of all the rats were recorded at regular intervals during the experimental period. No sign of toxicity was noticed on the behaviour and general health of the animals when exposed to the extract. Animals described as fasted were deprived of food for at least 12 h but allowed free access to drinking water. Blood samples were drawn at the end of study. Blood glucose estimation (Sasaki *et al.,* 1972) and glycosylated haemoglobin (Kynoch and Lehmann, 1977) were done on 14th day of the study.

RESULT

Body weight

Diabetes is characterized by weight loss and it was also seen in this study. Normal control animals were found to be stable in their body weight but diabetic mice showed statistically significant reduction in body weight on day 7 and 14. Alloxan caused body weight reduction, which was reversed by the treatment with methanol extracts of *S. caryophyllatum* after 7 and 14 days of treatment. However, the treatment with *S. caryophyllatum* weight increase is low when compared to the glibenclamide treated groups (Table -1).

Table 1: Effect of methanol leaf extract of *S. caryophyllatum* leaf extracts on body weight changes in normal and alloxan induced diabetic mice

Treatment	Day 0(g)	Day 7(g)	Day 14(g)
Group - I	24.40 ± 0.26	25.38 ± 0.5	25.12 ± 0.26
Group - II	24.72 ± 0.27	19.56 ± 0.19**	18.62± 0.12**
Group - III	24.64 ± 0.02	25.55 ± 0.21a	26.69 ± 0.22aa
Group - IV	24.66 ± 0.23	25.20 ± 0.12a	26.06 ± 0.21aa

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01;*** P<0.001 Compared normal control vs -Diabetic mice.

a -P < 0.05; aa - P<0.01 Compared -Diabetic mice vs drug treated

Group I: Mice received normal saline were served as a normal control. (By using an intragastric catheter tube (IGC).

Group II: Diabetic mice received normal saline 14 days by IGC and served as diabetic control.

Group III: Diabetic mice received glibenclamide at the dose of 2mg/ Kg body weight, daily, orally for 14 days by IGC.

Group IV: Diabetic mice received *S. caryophyllatum* extract at the dose of 250 mg/kg b.wt for 14 days by IGC.

Changes in serum glucose concentration

The daily administration of *S. caryophyllatum* extract (250 mg/kg) on alloxan induced diabetic mice caused a significant reduction in blood glucose level when compared with the vehicle-treated (P<0.05) group and day zero value (P<0.05). Similarly, repeated administration of glibenclamide (2mg/kg) twice a day for 7 and 14 days caused a significant reduction (P<0.01) in the blood glucose level in alloxan induced diabetic mice when compared to vehicle and day zero values. *S. caryophyllatum* treated group have shown good hypoglycaemic activity (Table-2).

Table 2: Effect of methanol leaf extract of *S. caryophyllatum* on fasting plasma glucose level in normal and alloxan induced diabetic mice

Treatment	Day 0(g)	Day 7(g)	Day 14(g)
Group - I	104.30 ± 10.99	109.20 ± 10.47	110.56 ± 9.84
Group - II	220.34 ± 8.19	267.15 ± 11.15*	292.40 ± 10.16*
Group - III	216.40 ± 3.97	165.40 ± 17.57	106.00 ±9.49**
Group - IV	223.26 ± 3.26	174.40 ± 18.13*	112.16 ± 9.26*

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01;*** P<0.001 Compared normal control vs -Diabetic mice.

a -P < 0.05; aa - P<0.01 Compared -Diabetic mice vs drug treated

Group I: Mice received normal saline were served as a normal control. (By using an intragastric catheter tube (IGC).

Group II: Diabetic mice received normal saline 14 days by IGC and served as diabetic control.

Group III: Diabetic mice received glibenclamide at the dose of 2mg/ Kg body weight, daily, orally for 14 days by IGC.

Group IV: Diabetic mice received *S. caryophyllatum* extract at the dose of 250 mg/kg b.wt for 14 days by IGC.

Gycosylated haemoglobin

Glycosylated haemoglobin levels were significantly increased in diabetic group when compared with normal group of animals. Administration of *S. caryophyllatum* extract and glibenclamide to diabetic mice significantly reversed all the changes to near normal levels. As expected, the Hb A_1 C level of *S. caryophyllatum* extract and glibenclamide treated groups has shown significant reduction when compared to the diabetic untreated groups. This effect was bringing down to normal in *S. caryophyllatum* extract and standard drug treated groups (Table-3).

Table 3: Effect of methanol leaf extract of *S. caryophyllatum* on Glycosylated haemoglobin level in normal and alloxan induced diabetic mice

Treatment	Hb A_1c (%)
Group - I	3.43±0.23
Group - II	9.78±0.34**
Group - III	3.89±0.11 aa
Group - IV	3.97±0.15 aa

Each Value is SEM ± 5 individual observations * P < 0.05; ** P<0.01;*** P<0.001 Compared normal control vs -Diabetic mice.

a -P < 0.05; aa - P<0.01 Compared -Diabetic mice vs drug treated

Group I: Mice received normal saline were served as a normal control. (By using an intragastric catheter tube (IGC).

Group II: Diabetic mice received normal saline 14 days by IGC and served as diabetic control.

Group III: Diabetic mice received glibenclamide at the dose of 2mg/ Kg body weight, daily, orally for 14 days by IGC.

Group IV: Diabetic mice received *S. caryophyllatum* extract at the dose of 250 mg/kg b.wt for 14 days by IGC.

DISCUSSION

Experimental diabetes mellitus was induced by injecting alloxan, a beta cytotoxin induces chemical diabetes (Alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves way for the decreased utilization of glucose by the tissues (Gurusamy *et al*., 2008). Many workers have shown that extracts of *S. cumini* have shown very good antihyperglycemic

activity (Shrotri *et al*., 1963; Bansal *et al*., 1981; Achrekar *et al*., 1991; Grover *et al*., 2000; Sharma *et al.*, 2003). In the present study, the effect of the leaf extracts of *S. caryophyllatum* reflected in body weight. The body weight was slightly increased in the normal control mice compared to initial body weight, whereas in the diabetic control mice there was a significant decrease in the body weight. Our results were supported by Esharat (2002), who reported that diabetic induced animals showed loss in body weight. The hypoglycaemic activity was compared with glibenclamide a sulphonylurea, stimulate insulin secretion from pancreatic ß cell (Tiedge and Lenzen, 1995). The significant body weight gain after treatment with the plant extracts revealed the previous report of *E. jambolana* in diabetic albino rats (Srivastava *et al*., 2012). The hypoglycemic activity of methanolic leaf extract of *S. caryophyllatum* was evaluated in alloxan induced diabetic mice. The results showed that fasting plasma glucose (FBG) level remains practically same before and after treatment with vehicle (saline only) in case of normal control mice. Whereas, in diabetic control mice the fasting plasma glucose levels rises significantly in 2 weeks (14 days) after treatment with vehicle. Methanolic leaf extracts of *S. caryophyllatum* caused significant ($p<0.01$) decrease in the fasting glucose in group IV. However, diabetic mice treated with glibenclamide a standard drug has exhibited maximum reduction in fasting blood sugar when compared to normal groups because the standard drug glibenclamide stimulate insulin secretion from pancreatic β-cells and it may be suggested that the mechanism of action of methanolic extracts of *S. caryophyllatum* was similar to glibenclamide. The possible mechanism by which the plant extract decreases the blood sugar level may be by potentiation of insulin effect either by increasing the pancreatic secretion of insulin from β-cells of islets of langerhans or by increasing the peripheral glucose uptake (Aybar *et al*., 2001).

Glycosylated haemoglobin determinations are self-monitoring of blood glucose therefore, it play an important complementary role for the management of diabetes mellitus (Thai *et al*., 1983). In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including haemoglobin. Glycosylated haemoglobin level is increased in patients with diabetes mellitus to approximately 16% and the amount of increase was found directly proportional to the fasting blood glucose level. During diabetes, the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in alloxan diabetic rats (Pari and Amarnath, 2004). Administration of the standard drug and methanolic extracts of *S. caryophyllatum* for 14 days prevented a significant elevation of glycosylated haemoglobin thereby, increasing the level of total haemoglobin in diabetic mice. This could be due to the result of improved glycemic control produced by the plant extracts. These results were in accordance with the studies of Jamshid *et al.* (2008).

CONCLUSION

The present study suggested that the methanolic extract of *S. caryophyllatum* leaves possess a potent hypoglycaemic activity as it significantly reduced the fasting blood glucose levels and glycosylated haemoglobin levels in alloxan induced mice as compared to the diabetic control. And it may be due to the presence of secondart metabolites in the extract.

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