International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 24-12-2021, Accepted: 08-01-2022, Published: 25-01-2022
Volume 7, Issue 1, 2022, Page No. 479-483

Serum lipid profile of diabetic Albino mice treated with leaf methanolic extract of *Syzygium caryophyllatum* (L.) Alston and *Syzygium densiflorum* Wall

Savitha Rabeque C[1], S Padmavathy[2]
[1] Department of Botany, Mercy College, Palakkad, Kerala, India
[2] Department of Botany, Nirmala College for Women, Coimbatore, Tamil Nadu, India

Abstract
Diabetes Mellitus is a major degenerative disorder that is characterized by hyperglycemia and hyperlipidemia which has been rated as a major contributing factors underlying the development of several metabolic diseases nowadays. The present study was carried out to assess the lipid profile of alloxan induced diabetic albino mice treated with leaf methanolic extract of *Syzygium caryophyllatum* and *Syzygium densiflorum* and standard glibenclamide, The serum concentration of cholesterol, HDL, LDL and triglycerides as well as body weight of all the animals in each group were determined after the 14th day of treatment. There was significant ($P<0.001$) reduction in body weight, serum cholesterol, LDL and Triglycerides when compared with the diabetic control mice. These reductions were dose dependent and compared well with values obtained in the standard drug treated control group. However, HDL-C level was lowered in the diabetic control and restored after the administration of the extracts at the dose of 250mg/kg b.w. and also in Group III treated with glibenclamide. The results showed that the leaf methanolic extract of *Syzygium caryophyllatum* is more potent than *Syzygium densiflorum.*

Keywords: alloxan, hyperlipidemic, LDL, HDL, *Syzygium caryophyllatum*, *Syzygium densiflorum*

Introduction
Diabetes mellitus is a metabolic heterogeneous disorder, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. It also causes significant morbidity and mortality due to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications. Diabetes Mellitus is a major degenerative disorder that characterized by hyperglycemia and hyperlipidemia which has been rated as a major contributing factors underlying the development of several metabolic diseases nowadays. It is becoming the third major threat to the health of humans of both developed and developing countries. According to the Diabetes Atlas, the global prevalence of diabetes is estimated to be 4.6%, representing 151 million people and is expected to go up to 333 million people by 2025 (Wild *et al*., 2004) [29]. Recent reports have estimated an increase in these figures, with the global prevalence was 6.6%, representing 285 million people in 2010 but it will rise up globally in 2030 to 7.8% (438 million people). According to WHO, India had 31.7 million diabetic subjects in the year 2000, and this number would increase up to 79.4 million by the year 2030 (Wild *et al*., 2004) [29]. Currently, India has the highest number of diabetic patients, and is being called the diabetic capital of the world (Mohan *et al*., 2007) [15]. At present the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas and D- phenylalanine derivatives, alpha glucoside inhibitors in addition to insulin. However, due to untoward side effects the efficacy of these compounds are debatable and there is a demand for new plant based compounds for the treatment of diabetes (Jackson and Bressler, 1981 and Thirunavukkarasu *et al*., 2003) [26]. According to the World Health Organization (WHO), up to 90% of the population in developing countries uses plants and its products as traditional medicine for primary health care (WHO, 2002) [31]. It is reported that about 21,000 plants are having medicinal properties and of which, about 800 plants have been reported to show antidiabetic potential (Patel *et al*., 2011) [19].
The family Myrtaceae comprises trees and shrubs with simple leaves, these mostly entire, usually evergreen, commonly opposite, or more rarely alternate (Long and Lakela, 1971 and Mabberley, 1997) [11, 13]. Plants in this family are noted for their spicy, aromatic odour caused by essential oils and for the presence of numerous stamens in the flowers (Gentry, 1993) [6].
Syzygium is the most important genera and are traditionally used for various purposes. In Asia and South America, the development and use of cheap and easily accessible phytomedicines for diabetes is obtained from the genus *Syzygium* (Teixeira, 1997) [25].
Oral feeding of *E. jambolana* (Bansal *et al*., 1981) [3] and fruit of *S. cumini* (Acherekar *et al*., 1991) [1] the effect of *S. aromaticum* flower bud showed reduction in glucose levels in diabetic induced albino mice (Kim *et al*., 1999) [9] bud extract *Eugenia uniflora* to mice has been shown to retain the plasma glucose levels during OGTT

and plasma triglyceride level in oral corn oil tolerance test (Arai *et al.*, 1999). Patel *et al.* (2008) have investigated the antihyperglycemic antihyperlipidemic and antioxidant activities of Dihar a poly-herbal formulation including *S. cumini* in streptozotocin induced type 1 diabetic mice. Hence the present study also aimed to evaluate the lipid profile of leaf methanolic extract of *Syzygium caryophyllatum* and *Syzygium densiflorum* in alloxan induced albino mice.

Materials and Methods

Collection of plant material

Syzygium caryophyllatum (L.) Alston and *Syzygium densiflorum* Wall. (Myrtaceae) were collected during the month of October 2009 from Palani Hills, Tamil Nadu, India. The plants were identified and authenticated by Dr.S. Padmavathy, Associate Professor, Department of Botany, Nirmala College for Women (Autonomous), Coimbatore.

Preparation of extract

250 g of freshly collected sample of *Syzygium caryophyllatum* (L.)Alston and *Syzygium densiflorum* Wall. 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 was extracted with methanol using a soxhlet apparatus. Then the extract was concentrated in a rotary evaporator to yield 5 gm of a syrupy residue. The residues were used for the analysis of following parameters.

Analysis of Hypoglycaemic activity of *S. caryophyllatum* and *S. densiflorum* extracts on experimental animals

Experimental induction of diabetes in mice

When administrating a foreign chemical substance to a biological system, various types of interactions could occur and a series of dose-dependent results may be occurred. These responses are desired and useful however, a number of other effects may be disadvantageous. These effects on the biological systems are harmful or beneficial. The types of toxicity studies that are carried out by several pharmaceutical industries for a new drug are acute, sub-acute and chronic toxicity.

Acute toxicity involves LD50, the dose that has proved to be lethal to 50% to the tested group of animals. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds.

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.

The animals had free access to water and standard rat diet. 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

In the experiment, a total of 25 mice (20 diabetic surviving mice, 5 normal mice) were used. The mice were divided into five groups, each group comprised of 5 mice.

Group I: Control.

Group II: Alloxan induced diabetic mice.

Group III: Diabetic mice treated with glibenclamide (2mg/ kg b.wt).

Group IV: Diabetic mice treated with methanolic leaf extract of *S. caryophyllatum* at the dose of 250mg/kg b.wt. daily using an intragastric gavage.

Group V: Diabetic mice treated with methanolic leaf extract of *S.densiflorum* at the dose of 250 mg/kg b.wt. daily using an intragastric gavage.

All these experiment were performed according to ethical guidelines for the investigation of experimental pain in conscious animals (659/02/a/CPCSEA). The animals were carefully monitored and weighed on 7^{th} and 14^{th} day. No sign of toxicity was noticed on the behaviour and general health of the animals when exposed to 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 was collected from overnight fasted mice, allowed to clot and centrifuged at 3000 rpm for 15 minutes. Serum samples were separated and used for biochemical analysis. The samples were stored at 4º C, till further use.

Biochemical analysis: Serum total cholesterol was estimated by Parekh and Jung, (1970) serum triglycerides was estimated according to the method described by Rice (1970). HDL- Cholesterol (Warnick *et al.*, 1985) and LDL-Cholesterol was estimated according to the method described by Friedbald *et al.*, (1972) formula.

LDL cholesterol = Total cholesterol – (VLDL+HDL Cholesterol)

All the values of blood glucose level and other biochemical estimations were expressed as Mean±SD of six determinations. Statistical analysis was done by Analysis of Variance (ANOVA) between the groups were considered significance at $p \leq 0.05$ level.

Results and Discussion

Hyperlipidemia is always associated with the body weight changes in the alloxan induced albino mice. Normal control animals were found to be stable in their body weight but diabetic mice showed significant reduction in body weight on day 7 and 14. Alloxan caused body weight reduction, which is reversed by methanol extracts of *S. caryophyllatum and S. densiflorum* after 7 and 14 days of treatment. The same trend was noted in glibenclamide treated groups (Table 1.)

In the present study also reveals the **c**hanges in the serum lipid profile in the non-diabetic control, alloxan induced diabetic control and different drug treated diabetic mice is shown in the Table 2. A significant elevation in the concentration of serum total cholesterol ($P<0.01$), triglyceride ($P<0.05$), LDL-C ($P<0.01$), VLDL-C ($P< 0.05$) and phospholipid ($P<0.01$) except HDL-C ($p<0.01$) were noted in the alloxan induced diabetic control animal, when compared to the normal non-diabetic control group. Except *S. densiflorum* leaf extract treated groups, all other two drug treated groups (*S. caryophyllatum* and standard drug) the lipid profile was significantly reduced to normal when compared to the non-diabetic control except HDL-C, which is elevated in all the drug treated groups. No significant changes were noted in the cardiac risk factor (TC/HDL) ratio.

The resultant body weight loss could be due to reduction in nutrient intake caused by high cholesterol content of the diet which might have impaired the absorption of protein and other nutrients (Matos *et al*., 2005; Woo and Henry, 1996) [30]. This reversal of the effect of leaf methanolic extract of *S. caryophyllatum and S. densiflorum* agrees with other investigators who noticed an increase in body weight gain upon the improvement of hyperlipidemia status (Lamiaa, 2011) [10]. The mechanism of action is unknown but it may be due to improvement in the nutritional status of the animals (Prasad, 2010) [21].

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridaemia (Pushparaj *et al*., 2007; Okoli *et al*., 2010) [22, 17] and also insulin deficiency is associated with hypercholesterolaemia. Insulin deficiency may be responsible for dyslipidaemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. The mechanisms responsible for the development of hypertriglyceridemia and hypercholesterolemia in uncontrolled diabetes in humans are due to a number of metabolic abnormalities that occur sequentially (Murali *et al*., 2002) [16]. Bruan and Severson (1992) [4] and Lopes-Virella *et al.* (1983) have reported that treatment of diabetes with insulin served to lower plasma triglyceride levels by returning lipoprotein lipase levels to normal. In the current study, the increased level of serum total cholesterol, total triglyceride, LDL-C, VLDL and PL except HDL-C in diabetic mice showed hypercholesterolaemia and hypertriglyceridaemia and the treatment with plant extracts significantly decreased both total cholesterol (TC) and total triglyceride (TG) levels and improvement in HDL-C. This implies that methanolic extract of *S. caryophyllatum* and *S. densiflorum* could prevent or be helpful in reducing the complications of lipid profile seen in some diabetics. In this study *S. caryophyllatum* once again showed better result than the *S. densiflorum.* These findings also support the hypothesis that the activity of plant extracts may be directly attributed to improvements in insulin levels upon treatment (Kameswara *et al*., 2003; Sharma *et al*., 2003) [8, 24].

Table 1: Effect of *S. caryophyllatum* and *S. densiflorum* leaf extracts on body weight in Alloxan induced diabetic mice

Treatment	Day 0(g)	Day 7(g)	Day 14(g)
Group - I	24.00 ± 0.26	25.38 ± 0.5 29	25.12 ± 0.26
Group - II	24.72 ± 0.27	19.56 ± 0.19**	18.62± 0.12**
Group - III	25.04 ± 0.02	25.55 ± 0.21a	26.09 ± 0.22a
Group - IV	23.50 ± 0.23	24.10 ± 0.12a	24.86 ± 0.21aa
Group - V	24.68 ± 0.22	25.32 ± 0.26a	26.56 ± 0.26a

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 mice induced 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 SCLextract at the dose of 250 mg/kg b.wt for 14 days by IGC.

Group –V: Diabetic mice received SDL extract at the dose of 250 mg/kg b.wt for 14 days by IGC.

Table 1: Effects of Methanol extract of *S. caryophyllatum* and *S. densiflorum* on serum lipid profiles in normal and Alloxan induced diabetic mice

Treatment	TC (mg/dl)	TG (mg/dl)	HDL – C (mg/dl)	LDL – C (mg/dl)	VLDL –C (mg/dl)	PL (mg/dl)	TC/HDL
Group - I	73.17±1.26	62.23±1.27	31.32±1.47	29.40±0.31	12.45±0.84	133.12±3.54	1.69:1
Group - II	112.25±2.14**	86.28±1.66*	27.22±1.08**	42.78±0.95**	17.25±1.15*	167.90 ±3.32**	2.14:1
Group - III	82.12±1.84a	54.37±1.64a	36.28±1.31	34.97±1.07a	10.87±0.64a	132.18 ±3.78a	2.26:1
Group - IV	76.27±1.26a	59.45±1.39a	35.67±1.83	28.71±0.32aa	11.89±0.83a	135.88 ±3.05a	2.13:1
Group - V	74.56±1.32	66.53±1.85	32.26±1.91a	32.01±1.33	13.30 ±0.36	145.03 ±2.91ns	2.68:1

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 mice induced 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 SCLextract at the dose of 250 mg/kg b.wt for 14 days by IGC.
Group-V: Diabetic mice received SDL extract at the dose of 250 mg/kg b.wt for 14 days by IGC.

Conclusion

In the present study, the administration of methanolic leaf extract of *S. caryophyllatum* and *S. densiflorum* shows a significant hypolipidemic activity in alloxan induced albino mice.
This implies that methanolic extract of *S. caryophyllatum* and *S. densiflorum* could prevent or be helpful in reducing the complications of lipid profile seen in some diabetics. In this study *S. caryophyllatum* once again showed better result than the *S. densiflorum.* However Further studies are needed to isolate the active constituents of *S. caryophyllatum* and *S. densiflorum***.**

References

1. Acherekar S, Kaklij GS, Kelkar SM. Hypoglycemic activity of *Eugenia jambolana* and *Ficus bengalensis*: mechanism of action. *In vivo*,1991:5:143-147.
2. Arai I, Amagaya S, Komatzu Y, Okada M, Hayashi T, Kasai M, Arisawa M et al. Improving effects of the extracts from *Eugenia uniflora* on hyperglycemia and hypertriglyceridemia in mice. *J. Ethnopharmacol*.,1999:68:307-314.
3. Bansal R, Ahmad N, Kidwai JR. Effect of oral administration of *Eugenia jambolana* seeds and chlorpropamide on blood glucose level and pancreatic cathepsin B in rats. *Indian J. Biochem. Biophys*.,1981:18:377-381.
4. Bruan JEA, Severson DL. Lipoprotein lipase released from myocytes is increased by decavandate but not insulin. *Am. J. Physiol*.,1992:262(E):663-670.
5. Friedbald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plama, without use of the preparative ultracentrifuge. *Clin. Chem*.,1972:18(6):499-502.
6. Gentry AH. A field guide to the families and genera of woody plants of northwest South America (Columbia, Ecuador, Peru). Conservation International. Washington, D.C, 1993, 291.
7. Jackson JE, Bressler R. Clinical pharmacology of sulphonylurea hypoglycemic agents: Part 1. *Drugs,1981:*22:211-245.
8. Kameswara RB, Kesavalu MM, Apparao C. Evaluation of antidiabetic effect of *Momordic cymbalaria* fruit in alloxan-diabetic rats. *Fitoterapia*,2003:74:7-13.
9. Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH. *et al*., 1999. Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. *J. Ethnopharmacol*.,1999:60:125-131.
10. Lamiaa AA Barakat. Hypolipidemic and Antiatherogenic Effects of Dietary Chitosan and Wheatbran in High Fat- High Cholesterol Fed Rats. Aust. J. Basic Appl. Sci,2011:5(10):30-37.
11. Long RW, Lakela O. A flora of tropical Florida. Banyan Books, Miami, 1971, 962.
12. Lopes Virella MF. Comparison of phosphotungstate and dextran sulfate- Mg2+ precipitation procedures for determination of high-density lipoprotein cholesterol. *Clin. Chem*.,1983:23:166-169.
13. Mabberley DJ. The plant book. 2nd ed. Cambridge Univ. Press, New York, 1997.
14. Matos SL, Paula H, Pedrosa ML, Santos RC, Oliveira EL, ChiancaJr DA, et al. Dietary models for inducing hypercholesterolemia in rats. Braz. Arch. Biol. Technol,2005:48(2):203209.
15. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. *Indian. J. Med. Res*.,2007:125:217-230.
16. Murali B, Upadhyaya UM, Goyal RK. Effect of chronic treatment with *Enicostemma littorale* in non-insulin dependent diabetic (NIDDM) rats. *J. Ethnopharmacol*.,2002:81:199-204.
17. Okoli CO, Ibiam AF, Ezike AC, Akah PA, Okoye TC. Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats, *Afr. J. Biotechnol*.,2010:9(2):248-259.

18. Parekh AC, Jung. Cholesterol determination with ferric acetate, uranium acetate and sulphuric acid, ferrous sulphate reagent. *Anal. Chem*.,1970:112:1423-1427.
19. Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and *in vitro* antioxidant potential of *Hybanthus enneaspermus* (Linn) F. Muell in streptozotocin-induced diabetic rats. *Asian Pac. J. Trop. Biomed*.,2011:1(4):316-322.
20. Patil, R. Patil, B Ahirwar, Ahirwar D. Current status of Indian medical plants with antidiabetic potential; a review. Asian Pacific Journal of Tropical Biomedicine,2011:2:S291-S298.
21. Prasad K. Effects of vitamin E on serum enzymes and electrolytes in hypercholesterolemia. Mol. Cell. Biochem,2010:335:67-74.
22. Pushparaj PN, Low HK, Manikandan J, Tan BKH. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. *J. Ethnopharmacol*.,2007:111:430-434.
23. Rice EW. Triglycerides in serum in standard methods in clinical chemistry, Roderick MP (Ed), Academic press, New York,1970:6:215-222.
24. Sharma S, Nasir A, Prabhu KM, Murthy PS, Dev G. Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan- induced diabetic rabbits. *J. Ethnopharmacol*.,2003:85:201-206.
25. Teixeira CC, Pinto LP, Kessler FHP, Knijnik J, Pinto CP, Gastaldo G, et al. The effect of *Syzygium cumini* (L) skeels on postprantial blood glucose levels in non-diabetic rats and rats with streptozotocin-induced diabetes mellitus. *J. Ethnopharmacol*.,1997:56:209-213.
26. Thirunavukkarasu V, Anuradha CV, Viawanathan P. Protective effect of fenugreek (*Trigonella foenumgraecum*) seeds in experimental ethanol toxicity. *Phytother. Res.*,2003:17:737-743.
27. Warnick GR, Nguyan T, Albers AA. Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol, *Clin. Chem*.,1985:31:217.
28. WHO, 1999
29. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*,2004:27:1047-53.
30. Woo J, Henry JB. Metabolic intermediates and inorganic ions in clinical diagnosis and management by laboratory animals. Philadelphia: W.B Saunders Company, 1996, 162-193.
31. World Health Organization. Traditional medicine – growing needs and potential, WHO policy perspective on medicines,20002:2:1-6.