International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 31-12-2021, Accepted: 15-01-2022, Published: 31-01-2022
Volume 7, Issue 1, 2022, Page No. 667-671

Assesment of serum protein and hepatic marker enzyme profiles of diabetic Albino mice treated with glibenclamide and leaf methanolic extract of *Syzygium caryophyllatum* (L.) Alston

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, once considered a disease of minor significance to world health, is now a major threat to human health in the 21st century. Several hypoglycemic agents have been reported to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea. So the present study was aimed to investigate the effect of leaf methanolic extract of *Syzygium caryophyllatum* (L.) on liver and kidney function on alloxan induced diabetic mice. The result indicated that a significant reduction in serum protein, albumin and globulin and in the elevation of SGPT and SGOT levels in alloxan induced diabetic group (Group II), when compared to control (Group I) and glibenclamide treated mice (Group III). On administration of methanol leaf extracts of *S caryophyllatum* to the diabetic group restored the level of protein, albumin and globulin and liver marker enzymes levels.

Keywords: diabetes mellitus, glibenclamide, SGPT, SGOT, globulin

Introduction
Globally, medicinal plants have been used in virtually all cultures as a source of medicine It is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles (Elujoba *et al*., 2005 and Ignacimuthu *et al*., 2006) [17, 2]. In India, indigenous plants have been used for the treatment of diabetes mellitus since the time of Charaka and Sushruta (Grover *et al*., 2002) [4]. The World Health Organization (WHO) has also recommended the evaluation of traditional plants for treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy (Shokeen *et al*., 2008) [21]. Indian Council of Medical Research (ICMR) reported recently as diabetes is one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated. (Verma *et al*., 2010) [24].
Researchers conducted studies over last several decades and illustrated the plant and plant based therapies to control and treat diabetes, this may be due to the presence of glycosides, flavonoids, alkaloids, terpenoids, carotenoids etc., (Bailey and Day, 1989 and Malviya *et al*., 2010) [1]. Today, these phytochemicals in medicinal plants have received a great deal of attention mainly on their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen (O_2), superoxide anion (O_2-) and hydroxyl (.OH) radical and hydrogen peroxide (H_2O_2). These reactive species exert oxidative damaging effects by reacting with nearly every molecule found in living cells including DNA (Sharma *et al.*, 2001). They play an important role in aging and its related disorders such as cancer, diabetes hypertension, atherogenesis, Alzheimers disease, Parkinsons disease (Modak *et al*., 2007) [14] or impaired antioxidant defences (Saxena *et al*., 1993) [19].
The genus *Syzygium* is the part of myrtle family (Myrtaceae). In general, the genus *Syzygium* are evergreen trees with a simple arrangement of leaves appearing opposite each other. The leaves are generally smooth and hairless. They are mostly found in tropical and subtropical areas and different species are used for food, wood, hedging and general ornamental use etc. Traditionally, *Syzygium cumini* seeds, fruits, leaves, flowers and bark have been used in folk medicine for various diseases. Charaka prescribed seeds, leaves and fruits as decoctions for diarrhoea and the bark as an astringent. Sushruta prescribed the fruit for obesity, vaginal discharges and menstrual disorders and cold infusion in intrinsic haemorrhage (Kumar *et al*., 2008) [9]. Among the *Syzygium* genus, *Syzygium caryophyllatum* is a very important species and is enlisted in the Red List of Threatened Species (IUCN, 2006). Although a lot literatures are available in the therapeutic effect of *Syzygium* sps. in the family Myrtaceae for the treatment of Diabetes, no detailed hypoglcaemic studies were carried out in *Syzygium caryophyllatum*. Hence the present study is aimed to investigate the serum protein and hepatic marker enzyme levels in diabetic Albino mice treated with glibenclamide and leaf methanolic extract of *Syzygium caryophyllatum* (L.) Alston.

Materials and Methods

Collection of the plant material

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

Description of the plant

Syzygium caryophyllatum (L.) Alston in Trimen, Handb. Fl. Ceylon 6 (Suppl):116-1931; RHFC 2:450. 1981; FTN 1:155. 1983; IFPH: t. 318. 1986; FPH: 495. 1999 .*Myrtus caryophyllata* L., Sp. Pl.: 472. 1753. *Eugenia caryophyllaea Wight*, Icon. Pl. Ind. Orient. 2(3): 3. t. 540. 1842; FBI 2: 490. 1878; RBSI 9 (1): 80. 1921. *Syzygium caryophyllaeum* sensu Gamble, Fl. Madras: 480 (339). 1919, non Gaerth. 1788.

Tree, Ca 12 m tall. Leaves obovate, obovate oblong or elliptic to obovate – elliptic, acute or cuneate entire, obtuse or shortly acuminate, 3.2-7.7 x 1.8 -3.2 cm, coriaceous, dark above, reddish brown and persistent calyx, purple. Habitat- wet evergreen Forests, ca 1500 m

FL and Fr: Jan – June.

Vernacular name

Tamil: Malai naval
Malayalam: Kattunjaval
English: Wild black plum

Preparation of the extract

250 g of freshly collected leaves of *Syzygium caryophyllatum* (L.) Alston were washed 2-3 times with water followed by distilled water and shade dried. All the dried leaves 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 further analysis.

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.

All these experiments 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 7th and 14th 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 studies

Biochemical analysis such as Protein estimation (Lowry *et al*., 1951), albumin and globulin (Wolfson *et al.*, 1948), aspartate transaminase (SGOT) and alanine transaminase (SGPT) (Reitman and Frankel, 1957) and alkaline phosphatase (King and Armstrong, 1934) were analysed.

Statistical analysis was done by Analysis of Variance (ANOVA) between the groups were considered significance at $p \leq 0.05$ level.

Results and Discussion

Scientific interest in 'alternative' or 'traditional system' in diabetes mellitus have concentrated, mainly on the screening of plant drugs (from all possible sources) for their blood-sugar lowering effect. Very few plants have been studied further, in depth, for investigating their site, bioactive compounds and mechanism of action for possible development of anti-diabetic drugs.

In the present study, experimental diabetes mellitus was induced by injecting alloxan, a beta cytotoxin which 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). The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells (Szkudelski, 2001). Generally, the hyperglycemia is characterized by alterations in the metabolisms of carbohydrate, proteins and lipids. Hyperglycemia induces the over production of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation. Among the parameters of protein metabolism, the present study showed a slight decline in total protein, sharp fall in serum albumin and globulin in diabetic rats. After treatment with *S. caryophyllatum* leaf extract, glibenclamide, total protein, albumin, globulins were brought back to near normal levels (Table 1.). The protein oxidation in insulin dependent diabetic mellitus was increased with decreasing the plasma levels of total protein, albumin, globulin and to non-diabetic subjects. This is in agreement with hypoalbuminia observed in diabetes (Porte *et al.,* 1981) [16]. On the other hand, the extract treated diabetic mice protein metabolism never deviated from normal range. Hypoalbuminemia is a common problem in diabetic animals and generally attributed in the presence of nephropathy. An overall reduction in serum total protein in diabetic animal and consequence in albumin were observed in the present study. This corroborates earlier reports (Soon and Tan, 2002) [21]. The reversal of these changes by *S.caryophyllatum* extracts therapy proved that insulin deficiency had been grassly corrected.

Table 1 also summarizes the hepatic marker enzymes in the serum of diabetic and drug treated albino mice. In alloxan induced diabetic control, significant elevation level of SGPT and SGOT and alkaline phosphatase levels were noted. But after treatment with *S. caryophyllatum* leaf methanolic extract and glibenclamide, liver marker enzymes were brought back to near normal. These results were in consistant with the results of *Wattakaka volubilis* leaf in diabetic rats (Maruthupandian *et al*., 2000) [12]. The increased level of serum protein, albumin and globulin in alloxan induced diabetic mice were presumed to be due to the increased protein catabolism and gluconeogenesis during diabetes (Palanivel *et al*., 2001) [15]. In the present study, the levels of SGPT and SGOT in alloxan induced diabetic mice were elevated. It may be due to the leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan (Stanely *et al*., 1999) [22]. The increased levels of transaminases, which are active in the absence of insulin because of increased availability of amino acids in diabetes, are responsible for the increased glucogenesis and ketogenesis observed in diabetes (Feling *et al*., 1970) [3]. This type of elevation of liver marker enzymes in diabetic mice indicates the hepatic damage. Aspartate amino transaminases and Alanine transaminase were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats (Hwang *et al*., 2005) [6]. In this study, the methanol extract of *S.caryophyllatum* regulated the activity of SGPT and SGOT in liver of mice intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study (Preethi and Kuttan, 2009) [17]. The restorations of SGPT and SGOT to their respective normal levels after treatment with both glibenclamide and methanol extract of *S.caryophyllatum*, further strengthen the antidiabegenic effect of this extract. Moreover, SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. Since the alloxan can also affect the liver by free radical mechanism. In addition to the assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants. In the present study, serum ALP increased in alloxan induced diabetic rats. Elevated level of this enzyme in diabetes may be due to extensive damage to liver in the experimental animal by alloxan. Treatment with methanol extract of *S.caryophyllatum* in alloxan induced diabetic group produces a decline in ALP level.These results were in consistent with the studies of *E. floccose* on diabetic rats (Mary *et al*., 2012)

Table 1: Effect of methanol extract of *S. caryophyllatum* on serum protein profiles and hepatic marker enzymes levels in normal and alloxan induced diabetic mice

Treatments	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group - I	7.21±0.45	4.03±0.18	3.18±0.25	15.37±0.34	11.62±0.44	90.16±2.41
Group - II	5.18±0.72*	3.12±0.23	2.06±0.13	31.61±0.26	24.13±0.51	136.50±3.2*
Group - III	7.84±0.44	4.18±.02	3.77±0.11	14.21±0.11a	12.12±0.73	90±1.69aa
Group - IV	7.77±0.67	4.16±0.27	3.30±0.15	19.18±0.36	16.23±0.24	93.33±1.54

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.

Conclusion
Alloxan, a β-cytotoxin, causes a massive destruction of β-cells of the islets of Langherhans, resulting in reduced synthesis and release of insulin. Generally, the hyperglycemia is characterized by alterations in the metabolisms of carbohydrate, proteins and lipids. Hyperglycemia induces the over production of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation. But the reversal of serum proteins and hepatic marker enzymes were noted after treatment with Glibenclamide and Syzygium caryophyllatum. This may be due to the bioactive constituents present in the extract. The present investigation has also opened avenues for further research, especially with reference to the different dose studies and development of potent formulation for diabetes mellitus from *Syzygium caryophyllatum* leaves.

References

1. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. *Diabetes Care*,1989:12:553-564.
2. Elujoba AA, Odeleye OM, Ogunyemi CM. Traditional medical development for medical and dental primary health care delivery system in Africa. *Afr. J. Trad. CAM*.,2005:2(1):46-61.
3. Feling P, Marliss E, Ohman J, Cahill JR. Plasma amino acids level in diabetic ketoacidosis. *Diabetes*,1970:19:727-730.
4. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol*.,2002:81(1):81-100.
5. Gurusamy K, Kokilavani R, Sowmya C, Arumugasamy K. Non enzymatic antioxidant properties of ethanolic extract of polyherbal formulation (PHF) against CCL4 induced rats. *Res. J. Biotechnol*.,2008:3:51-54.
6. Hwang HJ, Kim SW, Lim JM *et al*. Hypoglycemic effect of crude epoxypolysaccharides produced by a medicinal mushroom *Phellinus baumii* in streptozotocin induced diabetic rats. *Life Sci*.,2005:76:3069-3080.
7. Ignacimuthu S, Ayyanar M, Sivaraman SK. Ethnobotanical investigations among tribes in Madurai district of Tamil Nadu (India). *J. Ethnobiol. Ethnomedicine*,2006:2:1-7.
8. King EJ, Armstrong AR. Convenient method for determining serum and bile phosphatase activity. *Can. Med. Assoc. J*.,1934:31:376.
9. Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N, et al. Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. *J. Med.Plants Res*.,*2008:*2(9):246-249.
10. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin's phenol reagent. *J. Biol. Chem*.,1951:193:265-275.
11. Malviya N, Jain S, Malviya S. Antidiabetic potential of medicinal potential plants. *Acta. Pol. Pharm*.,2010:67(2):113-118.
12. Maruthupandian A, Mohan VR, Sampathraj R. Antidiabetic, antihyperlipidaemic and antioxidant activity of *Wattakaka volubilis* (L.f) Stap leaves in alloxan induced diabetic rats. *Int. J. Pharm. Sci. Res*.,2000:1:83-90.
13. Mary JKS, Tresina PS, Mohan VR. Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia Floccosa* Bedd. leaves in alloxan induced diabetic rats, *J. Basic. Clin. Pharma.*,2012:3(1):235-240.
14. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. *J. Clin. Biochem. Nutr*.,2007:40:163-73.
15. Palanivel R, Thangavel M, Selvendran K, Sakthisekaran D. Insulino mimetric effect of ammonium paratungstate on protein metabolism in streptozotocin induced diabetic rats. *Biomed*.,2001:21:23-30.
16. Porte DJ, Halter JB. In: Williams R. H. (ed.) *Textbook of Endocrinology.* W.B. Saunders Co., *Philadelphia*, 1981, 715.
17. Preethi KC, Kuttan R. Hepato and Reno protective action o *Calendula officinalis* L. flower extract. *Ind. J. Exp. Biol*.,2009:47:163-168.
18. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Path*.,1957:28:56-63.
19. Saxena AK, Srivastava P, Kale RK, Baquer NZ. Impaired antioxidant status in diabetic rat liver. Effect of vanadate. *Biochem. Pharmacol*.,*1993:*45(3):539-542.
20. Shokeen P, Anand P, Murali YK, Tandon V. Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions. *Food Chem. Toxicol*.,2008:46(11):3458-3466.
21. Soon YY, Tan BKH. Evaluation of the hypoglycemic and antioxidant activities of *Morinda officinalis* in streptozotocin- induced diabetic rats. *Singapore Med. J.*,*2002:*43:77-85.

22. Stanely P, Prince M, Menon V. Hypoglycemic and other related actions of *Tinospora cordifolia* roots in alloxan induced diabetic rats. *J. Ethnopharmacol*.,1999:70:9-15.
23. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*.,2001:50:537-46.
24. Verma L, Singour PK, Chaurasiya PK, Rajak H, Pawar RS, Patil UK. Effect of ethanolic extract of *Cassia occidentalis* Linn. for the management of alloxan-induced diabetic rats. *Pharmac. Res*.,*2010:*2(3):132-137.
25. Wolfson WQ, Cohn C, Calvary E, Ichiba F. A rapid procedure for the estimation of total protein, true albumin, globulin, globulin and globulin in 1 ml. of serum. *AM. J. clin. Pathol*.,1948:18:723-730.