



Research Article

ANTI-HYPERGLYCEMIC EFFECTS OF ETHANOLIC EXTRACT OF *SYZYGium ALTERNIFOLIUM* (WIGHT) WALP. IN ALLOXAN INDUCED DIABETES RAT MODELS

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ABSTRACT

The present study carried out as a sequel to our previous report on the rare botanical *Syzygium alternifolium* (Wight) Walp evaluates medicinal use of the species in the treatment of diabetes. Antidiabetic activity of ethanolic extract of *Syzygium alternifolium* (EESA) evaluated for hypoglycemic function in Alloxan monohydrate induced diabetic animal model presented data convincing enough to deal with this vexing health issue. In an experiment designed in accordance to the standard procedure with the clearance of animal ethics committee, different pharma clinical parameters were screened to determine the blood glucose levels as per approval norms. Animal groups treated with EESA have a significant influence in keeping the blood glucose level under control comparable to standard drug control group. Anti-glycemic function in the treatment of the tested two doses of extracts were comparable to the standard drug glipizide. Blood glucose level tested by glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method show that *Syzygium alternifolium* (EESA) prevented the diabetic condition in direct quantitative terms. The concentration of the higher dosage of the fruits fared well prompting a chance to tap ingredients which might help making new drug providing for the search for novel anti-diabetic agents.

Keywords: *Syzygium alternifolium*, Anti diabetic activity, Blood glucose, Glucose kit, Alloxan monohydrate, Induced diabetes.

INTRODUCTION

Diabetes mellitus is an ailment where the affected individual, be it human or the sick animal, fails to produce or effectively use insulin for the regulation of blood sugar level. This causes alterations in the metabolic prolife and the complications are manifested in the form of associated ailments and health complications. Diabetes mellitus is a condition in diabetes evinced as a profound alteration in the concentration and composition of lipid. The global figure of people with diabetes set to rise from the present estimate of 150-220 million about 300 million in 2025¹. The body has to maintain the blood glucose levels at very narrow range are done by mediation of insulin and glucagons are not an issue normally in children and young adults. Insulin is a hormone produced by special cells (called beta cells) in pancreas. The pancreas is located deep in the upper part of the abdomen, behind the stomach attached to the duodenum. Though the pancreatic secretion and regulation of insulin is part of routine in natural healthy individual, individuals of malfunction and growing disabilities is now turning common episodes res of health disorder owing to change in feeding pattern and altered health styles hit commonly by pressure, hypertension, stress and trauma. It s on this context that the recommendations made by WHO on diabetes mellitus calls for speeding up of investigations on hypoglycemic agents, especially from medicinal plants²⁻⁴.

Several useful reviews on the use Indian medicinal plants claim that botanical can be trusted with blood sugar lowering potentials. India is well known for its herbal wealth. Despite the frequent and robust studies that have been made in the understanding and management of diabetes the disease, diabetic related

complications remain increasing unabated. Despite of the availability of the many diabetic drug in market the search for plant-based remedies are still in demand for the reason the botanicals are spared from side effects and unintended fallouts owing their bio-digestibility. Quite interesting some common species namely *Andrographis paniculata*, *Azadirachta indica*, *Ocimum sanctum*, *Trigonella foenum-graecum*, *Swertia chirayita*, *Pterocarpus marsupium*, *Aegle marmelos*, *Heliotropium zeylanicum*, *Opuntia ficus*, *Caralluma attenuata*, *Salacia reticulata*, *Raphanus sativus*, *Amaranthus spinosus* have been studied for the treatment of diabetes. However, detailed study on the efficacy, mechanism of action and safety of many of these plant extracts still remain ill- explored⁵⁻⁷.

Many traditional plants treatments for diabetes are used throughout the world and there is an increasing demand by patients to use the natural products with anti-diabetic activity⁸. Several common and uncommon species of *Syzygium* have been taken up for detailed investigation⁹. Though researchers studying *Syzygium alternifolium*¹⁰ and natives¹¹ are presumed to tab this taxon for the blood sugar regulation, detailed investigation particularly on the utility of ripe and unripe whole fruits have not been attempted well so far. Recognizing that *Syzygium alternifolium* fruits are sold in local market based common notion and full belief but without any substantiated claim, this study is taken up to fill the void. It aims at evaluating the effect of Ethanolic Extract of *Syzygium alternifolium* (EESA) on changes in Body weight, Plasma glucose, Hemoglobin and glycosylated hemoglobin and lipid profile.

MATERIALS AND METHODS

Experimental models

For the study on hyperglycemic control, the experimental animal model is selected in such a way that it would satisfy the following, namely,

- The animal should be amenable to entrained hyperglycemia rapidly,
- Pathological changes in the site of induction should result from pancreatitis or damage of β -cells and
- Symptoms should be ameliorated or prevented by a drug treatment with plausible simulation to be effective in human beings.

Materials

- Animals: Male albino wistar rats (180-220gm)
- Drugs: Ethanolic Extract of *Syzygium alternifolium*
- Chemical: Alloxan monohydrate (S. D Fine. Chem. Ltd, Mumbai)

Selection and acclimatization of animals

Wistar strains of male albino rats weighing between 180-220 gram each are used as test animals for this study. The animals groomed well in large cages were fed with commercial feed as pellets and access to water ad libitum throughout the period of study. As the animals were adequately acclimatized to the standard environmental condition of temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) and humidity ($55 \pm 5\%$) and 12 h light dark cycles this investigation on the bio-efficacy for the *S. alternifolium* as therapeutic plant extract is undertaken.

Induction of Diabetes mellitus

Hyperglycemia is induced in Wistar rats by single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (150 mg/kg BW) in physiological saline after an overnight fasting of animals for 12 hrs¹². Alloxan is commonly used in similar such studies is known to cause diabetes mellitus in experimental animals for detrimental action on the β -cells and production of reactive oxygen species such as H_2O_2 , free radicals of oxygen and $\text{HO}^{\cdot}$. The development of hyperglycemia in rats is confirmed by plasma glucose estimation 72 hours after the Alloxan injection. Rats with fasting plasma glucose level of 160-220 mg/dl were used as test animals for this experiment.

Experimental Design

In this present exercise a total of 30 rats, inclusive of 24 diabetic surviving treated rats evaluated against 6 normal rats were used. Diabetes in each case was induced in rats 3 days before starting the experiment. The rats were divided into 5 groups after the intra-peritoneal administration of Alloxan. All the experiments are conducted in accordance with the ethical guidelines of the CPCSEA (661/02/C/IAEC/KMCP). The experimental protocol was approved by the Institutional Animal Ethical Committee of K.M. College of Pharmacy, Madurai District, Tamil Nadu, India.

Treatment protocol

Test Group - I: (Normal control) consist of normal rats given with 10 ml/Kg of normal saline, orally.

Test Group - II: (Toxic control) Diabetic animals receiving 150 mg/Kg of Alloxan monohydrate through I.P.

Test Group III: (Diabetic control) rats treated with glipizide at a dose of (10 mg/Kg orally) for 28 days.

Test Group IV: (Treatment Group-a): Diabetic control rats treated with *Syzygium alternifolium* extracts at a dose of 200 mg/Kg orally) for 28 days.

Test Group V: (Treatment Group-b): Diabetic control rats treated with *Syzygium alternifolium* extracts at a dose of 400 mg/Kg orally) for 28 days.

Methodology

Parameters studied

Body Weight, Blood Glucose, Haemoglobin, Glycosylated Haemoglobin, Plasma Insulin, Total Cholesterol, Triglycerides, HDL-Cholesterol and Phospholipids were determined. Blood was collected from the eyes (venous pool) by sino-ocular puncture in EDTA coating plasma tubes for the estimation of blood parameters¹³.

Biochemical analysis

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method¹⁴ while Plasma insulin was determined by ELISA method using a Boehringer-Mannheim kit¹⁵ with an ES300 Boehringer analyzer (Mannheim, Germany). Estimation of total hemoglobin and glycosylated hemoglobin: Total hemoglobin was determined by the method of Drabkin and Austin (1932)¹⁶ and glycosylated hemoglobin was estimated by the method of Sudhakar Nayak and Pattabiraman (1981)¹⁷. Plasma lipids were determined by auto analyzer according to the method of Parkeh and Jung (1970)¹⁸ analysis and the data of the cited biochemical parameters was analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keul's multiple range test (NKMRT).

RESULTS

Differences in the levels of initial and final blood glucose and change in body weight, in normal rat and treatment control animals were assessed in each group. The mean body weight of diabetic rats (TG II) is seen significantly to decrease as compared to normal control rats and the extent to which the standard treatment and the tow extracts treatments offered a relief in TG III, IV and V was measured as per standard procedures. It is found that in comparison the body weight of diabetic control rats, those treated with Ethanolic Extract of *Syzygium alternifolium* at a dose of 200 mg/kg and 400 mg/kg clearly offered to a positive and favorable result.

It can be inferred form Table 2 that the levels of total hemoglobin, glycosylated hemoglobin and plasma insulin in normal rat and treatment control animals improved both qualitatively and quantitatively. As the levels of total hemoglobin and plasma insulin levels were decreased significantly whereas glycosylated hemoglobin levels increased significantly as compared to normal control rats. Changes in the level of total hemoglobin, glycosylated hemoglobin and plasma insulin were recorded with a clear positive connation.

Table 1: Effects of Ethanolic Extract of *Syzygium alternifolium* on initial and final body weight and blood glucose in normal and treated animals

Group	Body weight (g)		Blood glucose (mg / 100 ml)	Blood glucose (mg / 100 ml)
	Initial	Final	Initial	Final
G1	235 ± 7.32	238 ± 7.35	84.60 ± 3.30	87.70 ± 3.85
G2	224 ± 6.68	168 ± 4.40** ^(a)	84.75 ± 3.42	213.45 ± 6.80** ^(a)
G3	230 ± 7.28	234 ± 7.32	86.65 ± 4.22	120.40 ± 4.40** ^(b)
G4	228 ± 7.25	238 ± 7.34	85.80 ± 3.70	135.40 ± 5.30** ^(b)
G5	230 ± 7.38	235 ± 7.42	86.45 ± 3.80	124.50 ± 4.45** ^(b)

Values are expressed as mean ± SEM.

Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

** (a) Values are significantly different from normal control G1 at P < 0.001.

** (b) Values are significantly different from Diabetic control G2 at P < 0.01.

Table 2: Effects of Ethanolic Extract of *Syzygium alternifolium* on plasma insulin, Hemoglobin and Glycosylated hemoglobin in normal and treated animals

Groups	Hemoglobin (gm/100 ml)	Glycosylated hemoglobin HbA _{1c} (%)	Plasma Insulin (μU/ml)
G1	11.85 ± 1.65	0.38 ± 0.05	34.50 ± 2.98
G2	6.20 ± 0.70** ^(a)	0.96 ± 0.15** ^(a)	12.72 ± 1.78** ^(a)
G3	11.18 ± 1.35** ^(b)	0.40 ± 0.06** ^(b)	28.40 ± 2.50** ^(b)
G4	10.40 ± 0.95** ^(b)	0.46 ± 0.09** ^(b)	24.68 ± 2.38** ^(b)
G5	10.96 ± 1.20** ^(b)	0.42 ± 0.05** ^(b)	27.92 ± 2.65** ^(b)

Values are expressed as mean ± SEM.

Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

** (a) Values are significantly different from normal control G1 at P < 0.001.

** (b) Values are significantly different from Diabetic control G2 at P < 0.01.

Table 3: Serum lipids of Normal and experimental groups

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	Phospholipids (mg/dl)	LDL (mg/dl)
G1	84.95 ± 2.65	88.65 ± 2.60	53.40 ± 1.86	123.70 ± 2.55	15.44 ± 1.36
G2	224.35 ± 6.78** ^(a)	155.62 ± 4.60** ^(a)	30.70 ± 1.35** ^(a)	212.45 ± 6.35** ^(a)	38.66 ± 2.44** ^(a)
G3	114.88 ± 3.35** ^(b)	94.95 ± 2.65** ^(b)	42.92 ± 1.45	149.46 ± 3.94	22.36 ± 1.88** ^(b)
G4	125.55 ± 3.60** ^(b)	115.85 ± 2.92** ^(b)	38.45 ± 1.40** ^(b)	157.58 ± 4.08** ^(b)	28.30 ± 1.90** ^(b)
G5	118.45 ± 3.38** ^(b)	98.50 ± 2.65** ^(b)	41.50 ± 1.60** ^(b)	150.42 ± 3.94** ^(b)	24.36 ± 1.75** ^(b)

Values are expressed as mean ± SEM.

Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.

** (a) Values are significantly different from normal control G1 at P < 0.001.

** (b) Values are significantly different from Diabetic control G2 at P < 0.01.

Table 3 reflects changes in the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Low density lipoprotein (LDL) and phospholipids between normal untreated control and the experimental animals in each group. Total cholesterol, triglycerides, high density lipoprotein differed from group to group. Low density lipoprotein (LDL) and phospholipids levels were increased quite significantly as the HDL-C level were found lowered in the alloxan induced diabetic rats. Marked improvement in responses was seen in extract treated groups. When compared to normal rats, those animals fed with glipizide and doses of 200 mg/kg and 400 mg/kg (EEAS) for 28 days evinced effective regulation of the total cholesterol, triglycerides, Low density lipoprotein (LDL) and phospholipids levels. An increase in HDL-C levels as compared to Alloxan induced diabetic can be inferred to be a consequence of Alloxan induced diabetes.

DISCUSSION

That the said drug caused a massive reduction in insulin release through the destruction of β-cells of the islets of Langerhans explains the mechanism of alloxan action which eventually results in uncontrolled or poorly controlled diabetes. An increased glycosylation in a number of proteins including hemoglobin and α-crystalline protein depicts the severity of alloxan triggered diabetes in rats^{19,20}. Administration of Ethanolic Extract of

Syzygium alternifolium at a dose of 200 mg/kg and 400 mg/kg (EEAS) helped increases in the body weight of alloxan induced diabetic rats. The sharp variations between healthy individual and induced with diabetes offered scope to ascertain the extract of diabetic control those Ethanolic Extract of *Syzygium alternifolium* at a dose of 200 mg/kg and 400 mg/kg (EEAS) exerted significant positive influence that potentiation of the insulin effect of plasma could be inferred from this treatment. Plant extracts and drugs have been reported to increase the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin²¹.

Alberti and Press (1982) showed that in uncontrolled or poorly controlled diabetes there will be an increased glycosylation in a number of proteins including hemoglobin and α-crystalline of lens²². Glycosylated hemoglobin (HbA_{1c}) had been found to increase in patients with diabetes mellitus to approximately 16%²³ and the amount of increase is directly proportional to the fasting blood glucose level²⁴. It has pointed out that in diabetes excess glucose present in blood reacts with hemoglobin. Therefore, the total hemoglobin level is decreased in alloxan induced diabetic rats with a concomitant increase in glycosylated hemoglobin²⁵. Administration of Ethanolic Extract of *Syzygium alternifolium* at a dose of 200 mg/kg and 400 mg/kg (EEAS) for 28 days in this study had prevented a significant elevation in glycosylated hemoglobin and consequently the increase the level

of total hemoglobin in diabetic rats can be construed as a clear anti-diabetic action.

The body weight was seen decreased in alloxan diabetic rats. Administration of Ethanolic Extract of *Syzygium alternifolium* at a dose of 200 mg/kg and 400 mg/kg (EEAS) increases the body weight in alloxan induced diabetic rats. The ability of Ethanolic fruit Extract at a dose of 200 mg/kg and 400 mg/kg (EEAS) to protect massive body weight loss seems to reflect its ability to check hyperglycemia. The level of serum lipids usually gets elevated in diabetes mellitus implying the risk of coronary heart disease (CHD)²⁶. Lowering of serum lipids concentration through diet or drug therapy can spare the risk of vascular disease. The abnormally high concentration of serum lipids in the diabetic subjects is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase²⁷.

A perusal of literature shows that glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidaemia that characterized the diabetic state may therefore be regarded as a consequence of the uninhibited action of lipolytic hormones on the fat depots. In the alloxan-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides and Low-density lipoprotein (LDL) levels brought down to near normal level by the treatment controls indicate that the Ethanolic Extract of *Syzygium alternifolium* (at a dose of 200 mg/kg and 400 mg/kg EEAS) can exert a positive and favorable influence in alloxan induced diabetic rats.

The quantitative increase in the effects of Ethanolic Extract of *Syzygium alternifolium* at a dose of 200 mg/kg and 400 mg/kg (EEAS) on diabetic hypertriglyceridemia emerging through the control of hyperglycaemia suggests that (i) the level of glycaemic control is the major determinant of total and very low density lipoprotein (VLDL), triglyceride, concentrations²⁸ and (ii) improved glycemic control following sulfonylurea therapy decreases the levels of serum VLDL and total triglycerides²⁹. Consistency of results noticed in the study demonstrates that *Syzygium alternifolium* extracts can be certainly anti-glycemic in its effects.

Anti-atherogenic lipoprotein (HDL) reported to be involved in the transport of cholesterol from peripheral tissues into liver³⁰ corroborates with the idea that the extracts can find a clear use as protective factor against coronary heart disease (CHD)³¹. The level of HDL-cholesterol was decreased in diabetic rats when compared with the standard drug glipizide and ethanolic fruit extracts in comparable and justifiable terms connate the popular notion and indigenous belief that as species of the genus *Syzygium*, *S. alternifolium* (Wight) Walp. can well be hired as a bio remedy in dealing with the growing and vexing problem of treating hyperglycemic conditions.

CONCLUSION

The present study revealed that the rare botanical *Syzygium alternifolium* (Wight) Walp evaluated for hypoglycemic function in Alloxan monohydrate induced diabetic animal model. Particularly this experiment designed in accordance to the standard procedure, different pharma clinical parameters were screened. Animal groups treated with EESA have a significant influence in keeping the blood glucose level under control comparable to standard drug control group. Antiglycemic function in the treatment of the tested two different doses of extracts was comparable to the standard drug glipizide. The concentration of the higher dosage of the fruits fared well

prompting a chance to tap ingredients which might help making new drug providing for the search for novel anti-diabetic agents.

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