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Evaluation of the hypoglycemic, hypolipidemic and hepatic glycogen raising effects of *Syzygium malaccense* upon streptozotocin induced diabetic rats

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Abstract

Objective: To study the effect of *Syzygium malaccense* on serum glucose, lipid profile and liver glycogen content in both normal and hyperglycemic rats. Methods: The aqueous and alcoholic extracts were compared with glibenclamide for their influence on fasting blood sugar, lipid profile and liver glycogen in both normoglycemic and to streptozotocin induced (50mg/kg ip) hyperglycemic rats. Results: In normoglycemic rats the aqueous and alcoholic extracts produced hypoglycemia but did not affect the lipid profile and liver glycogen content even on chronic treatment. In the hyperglycemic rats on chronic treatment both the extracts caused reduction in FBS and significantly reversed the diabetes induced hyperlipidemia and liver glycogen depletion. The alcoholic extract was found to be more active than aqueous and equivalent to that of glibenclamide. Conclusion: The extracts of *Syzygium malaccense* with their beneficial effects on blood sugar and hyperlipidemia associated with diabetes could serve as good adjuvant to other oral hypoglycemic agents.

Key words: *Syzygium malaccense,* hypoglycemic activity, lipid profile.

1. Introduction

The ancient Indian literature has prescribed various herbs and metals for cure of diabetes mellitus, like Amalaki (*Phyllanthuas emblica),* Karela (*Momordica charantia),* Amrita *(Tinospora cordifolia),* Tulsi *(Ocimum sanctum),* Agarvadhah *(Cassia fistula)* etc [1]. Picking up this lead, extracts of various plant materials capable of decreasing blood sugar have been tested in experimental animal models and their effects confirmed.The astringent bark of Malay apple i.e. *Syzygium malaccense,* an indigenous plant is recommended as a local remedy for a variety of disorders like cough, constipation, headache, diabetes, antibacterial activity, diuretic, abortifacient etc. It has been reported that the aqueous extract of the plant produced 15-25% fall in fasting blood sugar in four to five hours after giving a single dose

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orally [2]. The use of this plant is reported in treatment of diabetes mellitus in folklore medicine but no substantial scientific studies have been reported. The present study was to investigate the hypoglycemic activity of *Syzygium malaccense* in normal and streptozotocin induced diabetic rats and compare the anti diabetic and hypolipidemic effects with glibenclamide.

2. Materials and method

2.1 Plant material

Bark of *Syzygium malaccense* was obtained fresh from the nearby forest area around Manipal after authentication by Dr. Gopalkrishna Bhat of the department of Botany, Poorna Prajna College,Udupi.

2.2 Preparation of extracts

Aqueous extract - The dried bark was crushed into moderately coarse powder. It was immersed in distilled water in a flask and allowed to stand for seven days. The solid residue obtained by straining the liquid was pressed and filtered. The filtrate was concentrated on water bath to get a viscous paste. It was finally dried in a desiccator.

Alcoholic extract - The coarse powder was packed in a thimble made of whatman filter paper no.1 and extracted in soxhlet extraction column with 95% alcohol obtained by double distillation. Each batch was extracted for 30 cycles and then concentrated by distilling. Final residue was air dried and later stored in a dessicator. The yield of aqueous extract was 15% and of alcoholic extract was 20%.

2.3 Phytochemical test

Phytochemical analysis of the extract was performed using standard methods. Tests for glycosides, flavonoides, triterpenoides and tannins were carried out.

2.4 Animals

Wistar albino rats of either sex (150-200g) were used. All were locally bred and housed in polypropylene cages in well-ventilated rooms under hygienic conditions at room temperature. Animals were given water *ad libitum,* and were fed with rat pellet feed (Hindustan lever Ltd.).

2.5 Drugs

The extracts and glibenclamide were suspended in 2% gum acacia solution and administered in the volume of 5 ml/kg p.o. Dose selected for the extract was 1/10 of safe dose (300 mg/kg) found in acute toxicity studies conducted by up and down stair case method [3]. Glibenclamide was used as a reference drug at a dose of 0.25 mg/kg, which was calculated by computing the minimum human dose to rats as per the method of Paget and Barnes [4].

2.6 Methodology

The effect of extracts was studied in the normoglycemic and hyperglycemic rats. Diabetes was induced by administering streptozotocin 50 mg/kg intraperitonially to overnight fasted male rats [5]. The normal rats and the diabetic rats were divided into four groups of 7-8 rats each. Group 1-received vehicle (2% gum acacia), group 2-received aqueous extract, group 3-received alcoholic extract, and group 4-received Glibenclamide. The effects of these orally administered drugs was studied following 30 days of treatment in normoglycemic rats and 15 days of treatment in diabetic rats.

Hypoglycemic studies: Blood samples were drawn from the retro-orbital venous plexus in the overnight fasted animals on day 0, 10, 20, and 30, in normal rats and on day 0, 5, 10, and 15 in diabetic rats following 3 h of treatment with vehicle/extract/glibenclamide and before initiation of treatment on day 0. Fasting blood glucose levels were estimated using Glucose oxidase peroxidase method, Ranbaxy, Glucose estimation kit.

Lipid profile studies: It was done with 2 ml of blood withdrawn after vehicle/drug

administration on 30th day in diabetic rats and on 15th day in normal rats. Serum was obtained by centrifuging the blood samples at 3000 rpm for 10 min. Total cholesterol and HDL cholesterol were estimated [6].

Liver glycogen estimation: All the groups were sacrificed by cervical dislocation, liver was excised and liver glycogen was quantitatively estimated using Anthrone method [7].

2.7 Statistical analysis

The total variation present in a data was calculated by one-way analysis of variance (ANOVA). Differences among the means were analyzed by Scheffe's test. For this, a Window based SPSS Computer package was used. For comparing the blood sugar values, before and after drug administration (acute effect) paired Student's *t* - test was employed. P<0.05 was taken as the level of significance.

3. Results

Both the extracts i.e. aqueous and alcoholic showed the tests for presence of flavonoids, triterpenoids, tannins and glycosides. In normal rats with single dose administration study on day 0 both the alcoholic as well as aqueous extracts significantly reduced FBS levels, similar to reference drug glibenclamide (Table 1). Upon chronic administration also both the extracts showed significant hypoglycemic effect as compared to control. Their hypoglycemic effect was similar to that of the standard drug glibenclamide on all the days the FBS was tested (Table 2).

The diabetic rats did not show a significant decrease in FBS levels as compared to the levels

Table 1.
Effect of single dose administration of extracts of *Syzygium malaccense* in normal rats

Treatment group (mg/kg)	Fasting blood sugar (mg/dl)	
	Before administration of drug / vehicle	3 h after administration of drug / vehicle
Control (5ml of 2% gum acacia)	71.3±1.11	72.2±1.13
Aqueous extract (300)	74.4±1.23	67.3±2.86*
Alcoholic extract (300)	70.9±1.36	63.5±1.70*
Glibenclamide (0.25)	70.7±1.21	59.7±2.18*

n=6; values expressed as Mean±S.E; *P<0.05 before administration of drug. (Paired student's *t* - test).

Table 2.
Hypoglycemic effects on chronic administration of extracts of *Syzygium malaccence* in normal rats.

Treatment group (mg/kg)	Fasting blood sugar (mg/dl)			
	Day 0	Day 10	Day 20	Day 30
Control (5ml of 2% gum acacia)	71.3±1.11	70.9±1.60	70.0±2.70	69.5±2.27
Aqueous extract (300)	74.4±1.23	69.9±2.88	64.7±3.56	52.23± 3.25*
Alcoholic extract (300)	70.9±1.36	69.0±1.82	64.1±2.30	55.23±2.42*
Glibenclamide (0.25)	70.7±1.21	65.3±0.84	56.58±1.33*	49.5±2.62*
Allowance value by Scheffe's test.		6.90	9.30	9.54

n=6; values expressed as Mean±S.E; *P<0.05 *vs* normal control; df-3, 20. (Anova)

Table 3.
Hypoglycemic effects on chronic administration of extracts of *Syzygium malaccense* in diabetic rats.

Treatment group (mg/kg)	Fasting blood sugar (mg/dl) Day 0	Day 5	Day 10	Day 15
Normal Control (5ml of 2% gum acacia)	71.3±1.11	70.0±1.60	70.9±1.60	70.0±2.70
Diabetic Control(5ml of 2% gum acacia)	265.48±10.01*	260.36±7.83*	259.58±7.33*	254.75±7.72*
Aqueous extract (300)	251.65±9.34*	237.21±5.97*	221.9±3.66*,**	212.19± 7.33*,**
Alcoholic extract (300)	253.03±10.49*	234.23±10.43*	209.56±8.09*,**	186.96±12.55*,**
Glibenclamide (0.25)	242.33±6.64*	224.56±9.91*,**	192.51±9.94*,**	180.13±9.23*,**
Allowance value by Scheffe's test.		30.56	26.96	33.1

n=6; values expressed as Mean±S.E; *P<0.05 vs normal control; **P<0.05 *vs* diabetic control; df-4, 25. (Anova)

before administration of extract/drug after the first dose administration on day 0. In the diabetic control group of animals FBS levels remained constant on all the days tested (250 mg/dl). The aqueous and the alcoholic extracts administration did significantly alter these values after 10 days of treatment. Glibenclamide also showed significant reduction in FBS levels on day 5, 10, 15 in post drug administration period (Table 3).

Lipid profile and Liver glycogen

Neither the standard drug nor the extracts did significantly affect the lipid profile and liver glycogen in normal rats (Table 4). In the diabetic rats both the extracts affected the lipid profile significantly ($p<0.05$) compared to the standard drug. A significant reduction of the total cholesterol level and increase in HDL cholesterol compared to control group was observed (Table 5). The diabetic rats treated with standard drug and extracts showed a significant ($p<0.05$) increase in liver glycogen compared to normal and diabetic control groups (Table 5).

4. Discussion

Diabetes mellitus (DM) is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic beta cells (Type 1 Diabetes mellitus/IDDM) or because of inadequate release of insulin from the pancreatic beta cells or insensitivity of target tissues to insulin (Type 2 Diabetes mellitus/ NIDDM) [8]. NIDDM is the most common type of DM. Streptozotocin (STZ) is a valuable agent for the experimental production of DM [9].

The use of lower dose of STZ (50 mg/kg) produced an incomplete destruction of pancreatic beta cells even though rats became permanently diabetic[10]. In a number of studies sulphonylureas have shown hypoglycemic effects in streptozotocin-induced diabetes [11,12].

The fact that Glibenclamide produced hypoglycemia in STZ induced diabetic rats; in the present study we consider it as NIDDM. Single dose administration of extracts of *S.malaccense* showed hypoglycemia in

Table 4.
Effect of various extracts of *Syzygium malaccense* on lipid profile and liver glycogen in normal rats

Treatment group (mg/kg)	Total cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Liver Glycogen (mg/gm)
Normal Control (5ml of 2% gum acacia)	70.96±1.72	50.65±1.79	11.46±0.43
Aqueous extract (300)	74.6±2.79	50.6±2.34	11.56±0.50
Alcoholic extract (300)	71.7±2.69	51.4±2.32	12.19±0.68
Glibenclamide (0.25)	70.7±2.81	53.1±2.15	12.57±0.70
Allowance value by Scheffe's test.	9.12	7.75	2.12

n=6; values expressed as Mean±S.E; *P<0.05 *vs* normal control; df-4,25.(Anova)

Table 5.
Effect of various extracts o *Syzygium malaccense* on lipid profile and liver glycogen in diabetic rats

Treatment group (mg/kg)	Total cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Liver Glycogen (mg/gm)
Normal Control (5ml of 2% gum acacia)	70.96±1.72	50.65±1.79	11.46±0.43
Diabetic Control (5ml of 2% gum acacia)	95.08±2.17*	42.45±1.04*	7.69±0.18*
Aqueous extract (300)	80.51±3.70*.**	51.58±1.65**	9.49±0.73*.**
Alcoholic extract (300)	57.23±1.99*.**	66.90±1.67*.**	13.51±0.31*.**
Glibenclamide (0.25)	63.06±1.68**	54.08±1.54**	15.09±0.34*.**
Allowance value by Scheffe's test.	9.27	6.11	1.76

n=6; values expressed as Mean±S.E; *P<0.05 *vs* normal control; **P<0.05 vs diabetic control; df-4, 25. (Anova)

normoglycemic rats. Reports are available to show that medicinal plants with hypoglycemic property may affect circulating insulin level [13].

It is well established that sulphonylureas cause hypoglycemia by stimulating insulin release from pancreatic beta cells [14]. The comparable effect of the extracts with glibenclamide suggests the possibility of a similar mode of action. The triterpenoidal glycosides could be the active principles eliciting these effects. These observations are in accordance with the previous reports on the hypoglycemic potentials of *triterpenoids in Panax ginseng, Cornus officinalis, Momordica cochinchinesis* [15]. However, further studies are necessary to identify the exact mechanism of action.

Non-insulin dependant diabetes mellitus is a multi-factorial disease, which is characterized by hyperglycemia and lipoprotein abnormal-ities [16]. In the present study we observed significant increase in the total cholesterol of rats treated with STZ in accordance with earlier reports [17].

Following treatment with the active extracts of *S.malaccense* and glibenclamide, there was a significant reduction in cholesterol level. Significant lowering of total cholesterol and rise in HDL cholesterol is a very desirable biochemical state for the prevention of atherosclerosis and ischemic conditions [18]. The observed decrease in the ratio of total cholesterol/HDL cholesterol (atherogenic index) lessens the risk of heart disease.

The decrease in hepatic glycogen content in diabetic rats has been observed earlier by others [19]. In this study administration of glibenclamide and extracts significantly improved the hepatic glycogen level in diabetic rats. The prevention of depletion of glycogen in the liver tissue is possibly due to stimulation of insulin release from beta cells that activate the glycogen synthase system [20].

In conclusion, it could be said that the claims on *S.malaccense,* for its anti-diabetic activity is vindicated. The active extracts of *S.malaccense* could merit its role as an adjuvant in the management of diabetes mellitus.

References

1. Shah VK. (1995) *Diabetes in Indian medicine,* Chankhabia Orientalia: Varanasi; 1-11
2. Shroti DS Kelkar, Deshmukh M, Aiman R. (1963) *Indian J. Med.Research,* 51:464.
3. Ghosh MN. (1984) *Fundamentals of experimental pharmacology* Scientific book agency: Calcutta;153-158
4. Paget GE, Barnes JM. (1964) In: Laurence DR. Bacharach AL. (eds) *Evaluation of drug activities, pharmacometrics,* vol I, Academic press: New York;135-166
5. Clare A, Williams RC. (1994) *J. Pharm. Pharmacol.* 46: 321.
6. Harold Varley. (1976) *Estimation of total serum cholesterol.* In practical clinical biochemistry, fourth ed. Arnold Heinemann Publishers: India; 313
7. Caroll NV, Langelly RW, Row RH. (1956) *J.Biol.Chem.* 26:583.
8. Persaud SJ, Al-Majed H, Raman A, Jones PM. (1999) *J.Endocrinol.* 163: 207-212.
9. Zhang XF, Tan BK, (2000) *Singapore Med J.* 41: 9-13.
10. Aybar MJ, Sanchej Riera AN, Grau A, Sanchez SS. (2001) *J. Ethnopharmacol.* 27: 243-275.
11. Bopanna KN, Bhagyalakshmi N, Rathod SP, Balaraman R, Kannan J. (1997) *Indian J Pharmacol.* 29; 105-109.
12. Ignacimuthu S, Amalraj T. (1998) *Indian J Pharmacol.* 30(2): 107-108.
13. Lamela M, Cadavid I, Gato A, (1985) *J. Ethnopharmacol.* 41: 83-91.
14. Stephen N. Davis, Daryl K. Granner. (2001) Goodman & Gillman *"The pharmaco-logical basis of therapeutics"* 10 thed,Mc-Graw Hil comp Medical Publishing division: New Delhi; 1679-1714
15. Ivorra MD, Paya.M, Villar A. (1989) *J. Ethnopharmacol.* 27: 243-275.
16. Scoppola A, Montecchi FR, Mezinger G, Lala A. (2001) *Atherosclerosis.* 156: 357-361.
17. Archana Sachdeva LD, Khemani. (2003) *J. Ethnopharmacol.* 89: 61-66.
18. Luc G, Fruchart JC. (1991) *American J Clinical Nutrition,* 53: 2065-2095.
19. Grover JK, Vats V, Yadav S. (2002) *Molecular Cellular Biochemistry* 241: 53-59.
20. Kamalakkanan N, Rajaurai M, Stanley P. (2003) *J. Medicinal Food* 6: 93-98.