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Research Article

POTENTIAL HYPOGLYCEMIC PROPERTY OF *ALBIZIA MYRIOPHYLLA* AND VIRGIN COCONUT OIL IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Objectives: The claim made by a small segment of the Malay population in Pasir Mas, Kelantan Malaysia that the bark of *Albizia myriophylla* added with the use virgin coconut oil is able to reduce the sugar levels in diabetes was investigated by assessment of the anti diabetic properties. Methods: The hypoglycaemic activity of the aqueous bark extract of *Albizia myriophylla* (ABZ) and virgin coconut oil was evaluated after its oral administration of streptozotocin-induced diabetic rats and normal rats. Rats were divided into 8 groups. 4 groups were normal rats and another 4 groups were induced with diabetes. Diabetes was induced by injection of 60 mg/kg body weight intraperitoneally and fasting blood glucose level and body weight were monitored from day 0, 15, 30 and 45.

Results: The administration of aqueous bark extract of *Albizia myriophylla* and virgin coconut oil simultaneously produces a significant fall in blood glucose levels to almost normal levels. This combination of treatment also brought about an increase in the body weight of diabetic rats.

Conclusions: The aqueous bark extract of *Albizia myriophylla* and virgin coconut oil has anti diabetic activity as it lowers serum glucose levels in diabetic rats. The treatment also increases the body weight of diabetic rats.

Keywords: *Albizia myriophylla*, Virgin coconut oil, Streptozotocin induced diabetes, Anti diabetic effect.

INTRODUCTION

For the most part of this century, health concerns in the field of human nutrition which has been centered around deficiency disorders of macro and micronutrients with emphasis on the role of essential nutrients in health and disease has become increasingly important. In recent years, various dietary constituents have been found to provide protections against any disease. Any significant role by dietary intervention is encouraging and emerging as an acceptable approach for controlling the diabetes mellitus incidence worldwide [1]. Currently there are over 150 million diabetics worldwide and this number is likely to increase to 300 million or more by the year 2025 due to increase in sedentary lifestyle, consumption of energy rich diet, and obesity [2]. Prevalence of diabetes mellitus among Malaysians was 10.5% in 1996 and is dangerously increasing to 15% in 2003 [3].

Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, fat and protein metabolism and by complications like retinopathy, microangiopathy and nephropathy. Currently, medicinal plants have been proven to be of beneficial use in the treatment of diabetes mellitus [4]. Many plant extracts and plant products have been shown to have significant hypoglycaemic properties [5,6,7]. In order to decrease the number of diabetes complication and to postpone their development, the use of biologic active components in natural plant source is recommended.

In recent years, numerous traditional medicinal plants were tested for their anti diabetic potential in experimental animals. In the present study, the stem of *Albizia myriophylla* (ABZ) and the virgin coconut oil are to be tested for their hypoglycaemic activity.

ABZ is a common plant in Asia, from Mimosaceae family profoundly used in traditional medicine for the usage of mouth wash in Thailand [8] and for rice beer production [9]. Its hypoglycaemic effect has never been proven scientifically *in vivo* though there is one study showing that it has α-glucosidase inhibitory activity [10] done through *in vitro* investigations.

Besides ABZ, virgin coconut oil (VCO) has been used commonly as herbal medicine. Approximately 50% of the fatty acids in coconut fat are lauric acid. Lauric acid is a medium chain fatty acid, which has the additional beneficial function of being formed into monolaurin in the human or animal body. Monolaurin is the antiviral, antibacterial and antiprotozoal monoglyceride used by the human or animal to destroy lipid coated viruses such as HIV, herpes, cytomegalovirus, influenza, various pathogenic bacteria including *Listeria monocytogenes, Helicobacter pylori* and protozoa such as *Giardia lamblia.*

Coconut oil puts less of a demand on the enzyme production of the pancreas. This lessens the stress on the pancreas during mealtime when insulin is produced most heavily, thus allowing the organ to function more efficiently. Coconut oil also helps to supply energy to cells because it is easily absorbed without the need of enzymes or insulin. It has been shown to improve insulin secretion and utilization of blood glucose [11]. Coconut oil in the diet enhances insulin action and improves binding affinity compared to other oils [12,13].

Thus, it has been proven to have antidiabetic properties but no studies have been done to assess the degree of hyperglycemic control in the management of diabetes.

MATERIAL AND METHODS

Collection of plant material

The freshly picked parts of the plants were air-dried at room temperature for 2 weeks, with no direct sunlight. The bark or stem of the ABZ were collected in from Pasir Mas, Kelantan and authenticated by Dr. Samsul Kamis, a Botanist at the Institute of Bioscience, Universiti Putra Malaysia with a given voucher specimen number of ACPO122. The coconuts were collected from Taman Pertanian UPM.

Preparation of aqueous bark extract of ABZ

The chips of dried bark of ABZ were pulverized to a coarse powder in a mechanical grinder. Then 300 grams of dried bark powder of ABZ is then mixed into 1.5 liter of boiling hot distilled water for 2 minutes. The aqueous extract was then filtered, concentrated and lastly freeze dried. The residue was then stored in a refrigerator at 2 - 8° C for use in subsequent experiments.

Preparation of virgin coconut oil

The solid endosperm of mature coconut (West coast tall variety) was crushed, made into a viscous slurry andsqueezed through cheese cloth to obtain coconut milk whichwas refrigerated for 48 h. After 48 h, the milk was subjected to mild heating (50°C) in a thermostat oven. The obtained virgin coconut oil was filtered through cheese cloth and was used for the present study [14].

Animals

Healthy adult male *Sprague-dawley* (SD) rats between 6 – 8 weeks of age and weighing 200 - 300 g were used for the study. The rats were obtained from The Laboratory Animal Center, Fakulti Perubatan dan Sains Kemanusiaan, University Putra Malaysia. Cages were arranged in such a way that possible effects due to cage placements are minimized. The animals were identified uniquely (via ear punch) and acclimatized for at least 7 days in their cages prior to the start of the study.The rats were maintained on standard laboratory rat pellets with water *ad libitum*.The study was permitted by the Animal Care and Used Committee of Universiti Putra Malaysia (UPM/FPSK/PADS/BR-UUH/00347).

Collection of blood and determination of blood glucose levels

Blood samples were collected from the tail vein and glucose levels were estimated by using a glucose oxidase-peroxidase reactive strips and a glucometer (Accu-Chek, Roche diagnostics, USA).

Induction of diabetes mellitus

Diabetes will be induced [15] in overnight fasted *Sprague dawley* male rats weighing 200 – 300 g by a single intraperitoneal injection of 65 mg/kg streptozocin (Sigma Aldrich, Germany). Streptozocin (STZ) will be dissolved in 0.9% normal saline. Hyperglycemia will then be confirmed by the elevated glucose levels in plasma, determined at 48 h. The rats found with permanent hyperglycemia (>13.8 mmol/L) will be used for the anti diabetic study.

Animal preparation for anti diabetic study

In this study, a total of 48 rats were used. Food and water were given *ad libitum* through out 45 days of the experiment.

The rats were divided into 9 groups (n=6) in the following manner

Group I: normal control rats administered drinking water

Group II: normal rats given VCO (2 mg/kg)

Group III: normal rats given ABZ (5 mg/kg)

Group IV: normal rats given ABZ (5 mg/kg) and VCO (2mg/kg)

Group V: diabetic control rats administered drinking water

Group VI: diabetic rats given VCO (2 mg/kg)

Group VII: diabetic rats given ABZ (5mg/kg)

Group VIII: diabetic rats given ABZ (5mg/kg) and VCO (2 mg/kg)

Group IX: diabetic rats given glibenclamide (5mg/kg)

The fasting blood glucose levels and body weight of the experimental animals were determined every 15 days. The weight of the rats was also monitored daily and the means were calculated for every 15 days.

Biochemical Analysis

At the end of the experiment, after a period of 28 days, the rats were anaesthetized under diethyl ether following a 12 hours fast. 3 ml of blood samples were withdrawn via cardiac puncture into plain tubes. For the serum analysis, blood were collected in separate tubes and centrifuged at 2000 rpm for 10 minutes.Total cholesterol and triglyceride level were examined by Hitachi machine at Hematology and Clinical Biochemistry Laboratory in Universiti Putra Malaysia. C-peptide and Insulin level were analyzed by using the ELISA Enzyme-Linked Immunosorbant Assay) method using Mercodia Rat Insulin ELISA (Mercodia AB, Uppsala, Sweden).

Statistical Analysis

Data were statistically evaluated using one-way analysis of variance (ANOVA), followed by *post hoc* Tukey's test using SPSS, version 17. The values were considered significant when p value < 0.05.

RESULTS

Effect of ABZ and VCO on Body Weight of Rats

Diabetic rats showed a marked decrease in body weight during the experimental period when streptozotocin in a dose of 60 mg/kg b.wt. was given by intraperitoneal injection in the diabetic control rats. This effect was successfully antagonized by the extract. The increase in weight of the rats is mostly seen when treated with virgin coconut oil, followed by diabetic rats treated with ABZ in combination with VCO and least seen in the diabetic rats treated with ABZ alone.

Table 1: Effect of ABZ and VCO on Body Weight

Group	Body Weight			
	0 day	15 day	30 day	45 day
Normal Control	243.17±27.61	247.67±28.99	244.33±34.09	249.17±35.72
Normal + VCO(2mg/kg)	251.67±31.83	268.00±21.45	272.00±36.94	315.17±19.78
Normal + ABZ (5mg/kg)	256.50±23.14	268.33±20.89	281.33±18.29	303.00±20.12
Normal + ABZ +VCO	240.50±33.11	263.50±33.67	284.83±30.81	309.00±29.42
Diabetic control	242.50±24.40[a]	196.67±21.89[a]	170.67±22.15[a]	146.33±15.73[a]
Diabetic + VCO (2mg/kg)	267.00±29.93	270.67±27.43[b]	259.17±67.93[b]	277.00±86.03[b]
Diabetic + ABZ (5 mg/kg)	265.83±15.69	219.83±42.30	225.00±52.61[b]	230.67±60.57[b]
Diabetic + ABZ + VCO	233.00±31.23	176.00±29.36	187.50±46.91	209.17±45.22[b]
Diabetic + Glibenclamide (5mg/kg)	248.33±36.94	234.67±30.98	205.5±32.24[b]	196.83±37.54[b]

Values are expressed as mean ± S.D (n = 6). [a]represents statistical significance versus normal control ($p<0.05$). [b]represents statistical significance versus diabetic control ($p<0.05$).

Table 2: Effect of aqueous bark extract of ABZ and VCO on fasting blood glucose level (mmol/L) in normal and diabetic rats

Group	Fasting Blood Sugar			
	Day 0	Day 15	Day 30	Day 45
Normal control (NC)	5.67±0.38	5.15±0.40	4.60±0.18	5.95±0.57
Normal+VCO(2mg/kg)(NVCO)	6.10±0.46	5.10±0.43	4.55±0.54	5.9±0.33
Normal+ABZ(5mg/kg)(NABZ)	6.10±0.37	5.08±0.55	4.62±0.23	5.65±0.75
Normal+ABZ+VCO(NABZVCO)	6.65±0.18	5.08±0.35	4.42±0.33	5.98±0.62
Diabetic control (DC)	18.90±4.16[a]	24.02±5.38[a]	25.35±3.45[a]	28.52±2.12[a]
Diabetic+VCO(2mg/kg)(DVCO)	16.85±6.05[a]	10.87±3.58[b]	7.40±2.62[b]	6.95±0.38[b]
Diabetic+ABZ(5mg/kg)(DABZ)	22.07±7.23[a]	15.08±3.55[b]	11.57±5.29[b]	7.63±3.77[b]
Diabetic+ABZ+VCO(DABZVCO)	26.83±3.35[a]	13.02±5.09[b]	6.83±1.60[b]	5.58±0.75[b]
Diabetic+Glibenclamide(5mg/kg)(DG)	20.72±3.25[a]	25.12±3.20[a]	17.98±3.30[a]	15.58±6.2[a]

Values are expressed as mean ± S.D (n = 6). [a] - represents statistical significance versus normal control ($p<0.05$). [b] - represents statistical significance versus diabetic control ($p<0.05$).

Table 3: Effect of ABZ on Serum insulin, cholesterol and triglyceride in normal and diabetic rats after 45 days of treatment

Group	Insulin (µg/L)	C-peptide (µg/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)
NC	0.3±0.08	0.82±0.19	1.5±0.3	1.0±0.3
NVCO	0.34±0.03	1.19±0.17	1.4±0.3	0.7±0.3
NABZ	0.22±0.08	0.85±0.2	1.4±0.2	1.4±0.2
NABZVCO	0.24±0.04	0.90±0.2	1.5±0.2	1.2±0.2
DC	0.5±0.23	1.19±0.17	1.7±0.3	1.6±0.6
DVCO	0.3±0.09	0.85±0.20	1.6±0.3	0.9±0.4
DABZ	0.24±0.08	0.67±0.1	1.6±0.3	1.4±0.4
DABZVCO	0.24±0.07	0.52±0.19	1.5±0.2	0.9±0.2
DG	0.28±0.08	0.70±1.2	1.4±0.2	1.3±0.3

NC, normal control; NVCO, normal+VCO (2mg/kg); NABZ, normal+ABZ (5mg/kg); NABZVCO, normal+ABZ (5mg/kg)+VCO (2mg/kg); DC, diabetic control; DVCO, diabetic+VCO (2mg/kg); DABZ, diabetic+ABZ (5mg/kg); DABZVCO, diabetic+ABZ (5mg/kg)+VCO (2mg/kg); DG, diabetic+Glibenclamide (5mg/kg); values are mean ± SEM (n=6). *- represents statistical significance versus normal control ($p<0.05$).

Effect of ABZ and VCO on Glucose Levels

In the diabetic control group, the fasting blood glucose level was consistently high (> 13.8 mmol/L) throughout the study and is significantly increased ($p<0.05$) when compared to the normal control rats. In the diabetic group treated with ABZ aqueous bark extract at 5mg/kg, CO at 2 mg/kg, and ABZ and VCO simultaneously, significant hypoglycemic activities were seen. While in the diabetic group treated with the synthetic anti diabetic drug glibenclamide at 5 mg/kg did not show any significant reduction in fasting blood glucose level at day 0, 15, 30 and 45 when compared to the diabetic control group ($p<0.05$) (Table 2).

Effect of ABZ and VCO on Insulin, C-peptide level, Cholesterol and Triglyceride Levels

No significant difference was observed in serum insulin, C-peptide, cholesterol and triglyceride level in diabetic rats. There was no statistical significance in parameters estimated in normal animals (Table 3).

DISCUSSION

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintanence of good health was widely observed [16]. Over 50% of currently available drugs are derivatives of plant [17]. The present study was undertaken to assess the antihyhyperglycemic activity of aqueous bark extract of *Albizia myriophylla* and virgin coconut oil. Previously it was reported that the ethanol extract of stem, leaves, branch and bark of *Albizia* spesies has displayed high α-glucosidase inhibitory effect through in vitro investigations [10]. In virgin coconut oil, it has shown that it improves utilization of blood glucose and improves insulin secretion [11]. Thus both have been proven to have antihyperglycemic properties but without sufficient in vivo investigations being done.

In all diabetic patients, treatment should aim to lower blood glucose to near normal level. The present investigation fulfills this statement by producing a significant fall in blood glucose levels especially seen in rats treated with *Albizia myriophylla* and virgin coconut oil simultaneously. This could be due to synergistic effects of anti hyperglycaemic properties which both have been proven to have. Besides this, the possibility of the presence of phenolics in *albizia* [18] and virgin coconut oil [19] may have added effect as phenolics are found to be effective antihyperglycaemic agents [20]. It denotes the anti diabetic effect of aqueous extract of *Albizia myriophylla* and virgin coconut oil may be due to the presence of more than one antihyperglycemic principle and their synergistic effects.

Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting [21] and loss of tissue proteins. Diabetic rats treated with the *Albizia myriophylla* extract and virgin coconut oil both showed an increase in body weight as compared to the diabetic control, which may be due to its effect in controlling muscle wasting, i.e., by reversal of antagonizing [22].

Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurements of both insulin and C-peptide levels have been reported to be a valuable index of insulin secretion rather than insulin alone [23]. Even though, in this study, the insulin and C-peptide level did not show any significant changes within the group, the diabetic control group did however showed slightly elevated levels of insulin and C-peptide. Thus, in diabetes mellitus, ABZ and VCO might be able to increase the sensitivity of insulin, rather than increasing the insulin secretion. The mechanism which may be involved in this process would be possibly by increasing the number or raising the sensitivity of insulin receptor site to insulin [24]. As a result, increases the uptake of glucose, consequently leading to a reduction of glucose level. This mechanism of action is similarly seen in the Chinese traditional medicine which is *Panax Ginseng* [25].

In diabetes, the level of serum lipids are usually elevated, which represents a high risk factor for coronary heart disease. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in the diabetic state, lipoprotein lipase is not activated in sufficient amount due to insulin deficiency resulting in hypertriglyceridemia[26]. Even though, in this study, no significant cholesterol or triglyceride level changes were seen within groups, the diabetic control group did show a slight elevation of cholesterol and triglyceride level when compared to the other groups. The treatment with ABZ and VCO did show a slight reduction of serum cholesterol and triglyceride which was however insignificant, compared to the diabetic control group. Further evaluation and studies need to be done, to determine ABZ's hypolipidemic effect.

The aqueous bark extract of *Albizia myriophylla* and virgin coconut oil has anti diabetic activity as it lowers serum glucose levels in diabetic rats and significantly increases glucose tolerance. The treatment also increases the body weight of diabetic rats. Hence, long term studies of *Albizia myriophylla* and virgin coconut oil and its isolated compounds are necessary to elucidate the exact mechanism of action so as to develop it as a potent anti diabetic drug.

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REFERENCES

1. Rout SP, Choudary KA, Kar DM, Das L, Jain A. Plants in traditional medicinal system-future source of new drugs. Int J Pharm Pharm Sci 2009;1 Suppl 1:1-23.
2. Velmurugan C, Sundaram T, Kumar RS, Vivek B, Shekar S, Kumar BSA. Anti diabetic and hypolipidemic activity of bark of ethanolic extract of *OugeiniaOojeinensis*. Med J Malaysia 2011; 66 Suppl 1:22-26.

3. Mafauzy M. Diabetes mellitus in Malaysia. Med J Malaysia 2006; 61 Suppl 4:397-398.
4. Grover JK, Yadav SP, Vats V. Effect of feeding *Murrayakoeingii* and *Brassica juncea* diet kidney functions and glucose levels in streptozotocin diabetic mice. J Ethnopharmacol 2003; 2806:1-5.
5. Gomes A, Vedasiromoni JR, Das M, Sharma RM, Ganguly DK. Anti hyperglycemic effect of black tea (*Camellia sinensis*) in rat. J Ethnopharmacol 1995; 45:223-226.
6. Afifi F, Saket M, Jaghabir M, Al-Eisawi D. Effect of *Varthemiaiphionoides* on blood glucose level of normal rats and rats with streptozocin-induced diabetes mellitus. Curr Ther Res 1997; 58:888-892.
7. Saat A, Syakroni N, Rosli R. Potential hypoglycemic property of *Albiziamyriophylla* in streptozotocin-nicotinamide induced diabetic rats. Int J Pharm Pharm Sci 2012; 4 Suppl 3: 427-431.
8. Amornchat C, Kraivaphan P, Dhanabumi C, Tandhachoon K, Trirattana T, Choonharcongdej S. Effect of cha-em Thai mouthwash on salivary levels of mutans streptococci and total Ig A. Southeast Asian J Trop Med Public Health 2006; 37:528-531.
9. Panmei C, Singh PK, Gautam S, Variyar PS, Shantibala DGA, Sharma A. Phenolic acids in *Albizia* bark used as a starter for rice fermentation in Zouprepararation. J Food Agri Environ 2007; 5:147-150.
10. Tunsaringkarn T, Rungsiyothin A, Ruangrungsi N. α-Glucosidase inhibitory activity of Thai Mimosaceous plant extracts. J Health Res 2008;22 Suppl 1:29-33.
11. Garfinkel M. Insulinotropic potency of lauric acid: a metabolic rational for medium chain fatty acids (MCF) in TPN formulation. J Surgical Res 1992; 52:328.
12. Ginsberg BH. Effect of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. Biochem. Biophys. Acta 1982; 690 Suppl 2:15.
13. Yost TJ, Eckel RH. Hypocaloric feeding in obese women: metabolic effects of medium-chain triglyceride substitute. Am. J. Clin. Nutr 1989; 49 Suppl 2:326.
14. Nevin KG, Rajamohan T. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. ClinBiochem 2004; 37:830-835.
15. Pellegrino M, Christopher B, Michelle M, Gerard R. Development of a new model of type II diabetes in adult rats administered with streptozotocin and nicotinamide. Diabetes 1998; 47: 224-230.
16. Bhattaram VA, Ceraefe M, Koshlset C, Vest M, Deundorf H. Pharmacokinetics and bioavailability of herbal medicinal products. Phytomedicine 2002; 9 Suppl 3:1-36.
17. Jia W, Gao WY, Xia PG. Antidiabetic drugs of plant origin used in China: Compositions. Pharmacology and hypoglycemic mechanism. Zhongguo Zhong Yao Za Zhi 2003; 28:108-113.
18. Marina AM, Man YB, Nazimah SA, Amin I. Antioxidant capacity and phenolic acids of virgin coconut oil. Int. J. Food Sci. Nutrition 2008;1-10.
19. Manickam M, Ramanathan M, Jahromi MAF, Chansouria JPN, Ray AB. Antihyperglycemic activity of phenolics from Pterocarpusmarsupium. J. Nat. Prod 1997; 60:609-610.
20. Swanston-Flat SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatments for diabetes: studies in normal and streptozotocin diabetic mice. Diabetologica 1990; 33:462–464.
21. Whitton PD, Hems DA. Glycogen synthesis in perfused liver of streptozotocin diabetic rats. Biochem J 1975; 21:150-153.
22. Kaleem M, Medha P, Ahmed QU, Asif M, Bano B. Beneficial effects of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Singapore Med J 2008; 49 Suppl 10:800-804.
23. Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol 2004; 92:1-21.
24. Suzuki Y, Hikino H. Mechanisms of hypoglycemic activity of panaxans A and B, glycans of *Panax ginseng* roots: effects on plasma level, secretion, sensitivity and binding of insulin in mice.Phytother Res J 1989; 3:20-24.
25. Shivanand P. Treatment of diabetes and hyperlipidemia with extracts of Euginea jambolena seed and *Aeglemarmelos* leaf extracts in alloxan induced diabetic rats. Int J Pharmaco Bioscience 2010; 1:1-10.