ANTIDIABETIC ACTIVITIES OF MALAYSIAN AGARWOOD (AQUILARIA SPP.) LEAVES EXTRACT

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Aquilaria spp. or agarwood were reported to have pharmacological activities. There was a report of one diabetic patient who drank water infusion of agarwood leaf was found to have blood sugar return to normal. However, still, there is no evidence or systemic clinical study to comfirm the activities of antidiabetic agents. This study is undertaken to evaluate the effects of methanol and aqueous crude extracts of agarwood leaf in streptozotocin-induced diabetic rats with 20% glucose water consumption for 6 weeks. Aquilaria spp. crude methanolic and aqueous extracts was administered orally (250 and 500 mg/kg) to the respective treatment groups and compared to metformin (0.25 mg/kg). The blood glucose level, body weight, glycosylated hemoglobin, muscle and liver glycogen, lipid profile status were measured and histopathology of pancreas was performed after 6 weeks of treatment and compared to the control. In in vitro experiment, the effects of the methanol and aqueous crude extract at the concentration of 100µg/mL to 1000µg/mL are subjected to α-glucosidase and α-amylase inhibitory activity. For comparable, Acarbose is used at the same concentration as a standard. This expected result suggest that Malaysian Aquilaria spp. leaves extract will represent potential dietary supplements that may be useful for allowing flexibility in meal planning and automatically will reduce the number of diabetic patients in worldwide population.

Key words: Aquilaria spp., Antidiabetic, Streptozotocin-induced diabetic rats, Blood glucose.

1. Introduction

Diabetes mellitus is a disorder of metabolism which defects of body's ability to convert glucose (sugar) to energy. Diabetes can cause serious health complications including heart disease, blindness, kidney failure from nephropathia, nervous system disease, foot ulcers, and lower-extremity amputations. According to Rother (2007), a major metabolic defect associated with diabetes is the failure of peripheral tissues in the body to properly utilize glucose, thereby resulting in chronic hyperglycemia. Fujisawa et al. (2005) report that management of diabetes by modern medicines exerts serious side effects such as hepatoxicity, abdominal pain, flatulence, diarrhea, and hypoglycemia. Therefore, apart from these modern medicines options, many herbal medicines are recommended for treatment of diabetes. Traditional herbal medicines have been used throughout the world for a range of diabetes (Odhav et al, 2010).
Aquilaria or agarwood (Thymelaeaceae) has been reported has the potential for antidiabetic. This plants is now widely cultivated for its resin. Various parts of Aquilaria spp. were reported to have several pharmacological activities to decrease hypersensitivity, antipyretic, and anti-inflammatory (Zhou et al., 2008). This current research will focus on antidiabetic potential and its activities from Malaysian Aquilaria spp. leaf extract which involve the mechanism of lowering the glucose level. Thus, in this research, we investigated the inhibitory effect of aqueous and methanol extracts of Malaysian Aquilaria spp. on α-glucosidase and α-amylase activity and compared it with the commercially available inhibitor, acarbose. Further, we investigated the effect of both extract on levels of blood glucose in streptotozin-induced diabetic rats.

2. Methods

2.1 Plant Material Leaves of four Aquilaria spp. available in Malaysia including A. malaccensis, A. hirta, A. beccariana and A. rostrata will be collected from Malaysia's tropical forest and plantations in the vicinity of Pahang.

2.2 Preparation of Plant Extract The fresh leaves (1 kg) of each species will dry on a

with methanol solvent extraction and aqueous extraction. For methanol extract, the extract will be concentrated on a rotary vacuum evaporator. Whilst for aqueous extract, it will be freeze dry for four to five days to yield a dry powder.

2.3 In-vitro α-glucosidase Inhibition Study
The enzyme α- glucosidase inhibitory activity is determined by incubating 1.0mL solution of an enzyme preparation with 1.0mL 0.2M Tris buffer, pH 8.0 containing various concentrations of extract at 37°C for 60 minutes by using glucose as working standard. The reaction mixture is heated for two minutes in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidation method. (Assay condition 37°C±0.1°C, pH-8.0; O.D at 540 nm).

2.4 In-vitro α-amylase Inhibition Study
A starch solution is obtained by 0.1g stirring potato starch in 100mL of 16mM sodium acetate buffer. The enzyme solution is prepared by mixing 27.5mg α-amylase in 100mL distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution (DNS) 96mM. Both control (Acarbose) and plant extracts is added with starch solution and left to react with α-amylase solution under alkaline conditions at 25°C. The reaction is measure over three minutes. The generation of maltose is quantifying by the reduction of 3, 5 dinitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm. (Temperature 25°C±0.1 °C, pH 4.8; O.D. at 540 nm).

2.5 Calculation of 50% Inhibitory Concentration (IC50) Calculate the concentration of the plant extracts require to scavenge 50% of the radicals (IC50) by using the percentage scavenging activities at five different concentrations of the extract. Formula for Percentage inhibition (I %):

$$I\ \% = (Ac-As)/Ac \times 100$$

Where; Ac is the absorbance of the control and As is the absorbance of the sample.

2.6 Acute Oral Toxicity – Fixed Dose Procedure Acute oral toxicity studies will carry out for methanol and aqueous extracts using acute toxic method described as per OECD Test Guidelines [420]. Groups of rats of single sex are dosed using fixed doses 250, 500, 1000, and 2000 mg/kg body weight. Rats are

the laboratory conditions.
Rats were fasted prior to dosing (food but no water should be withheld overnight. The extracts are given 1ml/100g (body weight) and were administered in a single dose by gavage using a stomach tube. Following the period of fasting, rats were weighted and extracts administered. After the extracts administered, food were withheld for a further 3 to 4 hours. Animal are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total 14 days. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and behavior pattern.

2.7 Repeated Dose 28-days Oral Toxicity
Fasten rats are dosed with extracts daily for a period of 28 days. When the extracts is administrated by gavage, it was done in a single dose to the rats using stomach tube and maximum volume were 1ml/100g body weight. Observation was made along 28 days to detect delayed occurrence, or recovery from toxicity. All rats were weight once a week and food consumption (feed residue) measurements was made twice a week. The following haemaological examinations are made at the end of the test period; haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count and a measure of blood clotting.

2.8 STZ induced diabetic rats
Male Sprague-Dawley rats (200-280g) were purchased and maintained in an air-conditioned room (25±1oC), with a 12 hours light – 12 hours dark cycle and fed with standard diet and distilled water. Rats were acclimitised for 7 days before starting the experiment. Rats were diabetes-induced by a single intraperitoneal injection of 45mg/kg body weight of streptozotocin (STZ) dissolved in 0.1M citrate buffer (pH 4.5). After 7 days of STZ injection, wenous blood was collected from rat tail to determine fasting bloog glucose level. Only the rats with fasting blood glucose over 200mg/dl, were considered diabetic and were induced in the experiments.

2.9 Experimental design The animals were randomly divided into 7 groups of six animals each after the induction diabetes. Groups as follow:

Group 1: Non-diabetic control rats received carrier (distilled water/DMSO)
Group 2: Diabetic control rats received carrier (distilled water/DMSO)
Group 3: Diabetic rats received standard drug Glibenclamide, 10mg/kg bodyweight
Group 4: Diabetic rats given ethanolic extracts 250mg/kg body weight
Group 5: Diabetic rats given ethanolic extracts 500mg/kg body weight
Group 6: Diabetic rats given aqueous extracts 250mg/kg body weight
Group 7: Diabetic rats given aqueous extracts 500mg/kg body weight

The blood samples were collected from the retro orbital plexus at 1, 3, 5, 7, 24 hours and at the end of 1, 3, 5 weeks. Blood glucose levels were recorded using glucometer.

2.9.1 Histopathology Animals will sacrifices on during prolonged treatment. Pancreas, liver and kidney will remove and wash with cold saline and preserve in 10% formalin in buffer form. Blocks from tissues are routinely process and embed in paraffin. Thin sections will be cut using rotary microtome and stain with hematoxilin and eosin for histomorphology evaluation. Full histopathology will be carried out on preserved organs and tissue of all animals in the control and all dose groups. All gross leions will be examined.

2.9.2 Biochemistry test Clinical biochemistry determinations to investigate major toxic effects in tissues and specifically, effects on kidney and liver, will be performed on blood samples. Investigation of plasma or serum include sodium, potassium, glucose, total cholesterol, urea, total protein and albumin and enzymes indicative of hepatocellular effects.

2.9.3 Data Analysis Data will be expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) and the value of $P<0.001$ and $P<0.05$ will be considered statistically significant.

3. Results

Table 1: Inhibitory activity of methanol extract of Malaysian Aquilaria spp. and acarbose against α-glucosidase

SAMPLE	[ ] (µg/mL)	% INHIBITION (± SD)	IC_{50} (µg/mL)
Acarbose (standard)	200	30.81 ± 2.80	823.94
	400	39.98 ± 2.37	
	600	45.81 ± 2.19	
	800	49.77 ± 1.98	
	1000	54.62 ± 1.78	
A. malaccensis	200	33.82 ± 2.59*	375.50
	400	58.91 ± 1.63*	
	600	83.44 ± 0.65*	
	800	88.78 ± 0.44*	
	1000	89.76 ± 0.40*	
A. hirta	200	27.58 ± 2.92*	452.82
	400	42.75 ± 2.31*	
	600	77.47 ± 0.88*	
	800	89.11 ± 0.43*	
	1000	91.04 ± 0.35*	

Table 2: Inhibitory activity of methanol extract of Malaysian *Aquilaria spp.* and acarbose against α-amylase

SAMPLE	[ ] (µg/mL)	% INHIBITION (± SD)	IC_{50} (µg/mL)
Acarbose (standard)	200	37.78 ± 0.372	940.11
	400	38.43 ± 0.346	
	600	39.24 ± 0.309	
	800	43.69 ± 0.288	
	1000	55.44 ± 0.240	
A. malaccensis	200	45.86 ± 0.624*	397.23
	400	50.32 ± 0.519*	
	600	66.66 ± 0.442*	
	800	70.95 ± 0.404*	
	1000	78.48 ± 0.381*	
A. hirta	200	43.28 ± 0.835*	301.99
	400	59.92 ± 0.744*	
	600	64.62 ± 0.683*	
	800	70.92 ± 0.598*	
	1000	73.67 ± 0.497*	

*$p<0.05$ compared to standard (Acarbose). All determination were carried out in triplicate manner and values are expressed as the mean ± SD. The IC50 value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The results shown both Malaysian Aquilaria spp. efficiently inhibits α-glucosidase and α-amylase enzyme in vitro.

may be useful for allowing flexibility in meal planning and thus can be promising source for antidiabetic agent. Base information for further investigation to uncover the exact mechanism of agarwood extracts as well as to isolate compound(s) responsible for the activity and effects on animal

References

[1] Akrarapholchote, S., (2008) Krisna leaf tea. First News and Media Group, Bangkok. pp 66-67.

[2] Barden, A., N.A. Anak, T. Mulliken and M. Song, (2000). Heart of the Matter: Agarwood use and Trade and CITES Implementation for Aquilaria malaccensis. Traffic International, Cambridge, ISBN-13: 9781858501772.

[3] Day, C., Bailey, C.J., (1988) Hypoglycemic agents from traditional plant treatments or diabetes. International Journal of Industrial Biotechnology. 8, 5-8.

[4] Dennis, D. (2006). Type 1 Diabetes. The Lancet Journal, vol. 367(9513) pp 847-858

[5] Fujisawa, T., Ikegami, H., Inoue, Y., Kawabata, Y., and Ogihara, T., (2005). Effect of two α-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. Metabolism: Clinical and Experimental, vol. 54(4), pp. 415-420.

[6] Gunasekera, S,P., Kinghorn, A.D., Cordell, G.A., Farnsworth, N.R., (1981). Plants anticancer agents. XIX. Constituents of Aquilaria Malacensis. Journal of Natural Products. 44(5), 569-572.

[7] Malaysian Diabetes Association. What is diabetes?. 20 May 2012; Available from: URL: http:// http://www.diabetes.org.my/article.php?aid=5

[8] Mandrup-Poulsen, T. (1998) Diabetes. British Medical Journal. 316, 1221-5.

[9] Naef, R., (2011). The volatile and semi-volatile constituents of agarwood, the infected heartwood of Aquilaria species: A review. Flavour Fragr. J., 26: 73-78.

[10] Neel, J.V. (1962). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet; vol.14, pp. 353-362

[11] Odhav, B., Kandasamy, T., Khumalo, N., and Baijnath, H. (2010). Screening of pp. 1502-1507.

[12] OECD Guideline for testing of chemicals 423 [online]. Acute oral Toxicity. Acute classic Method. 25 Dec 2012; Available from: URL: Http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD.pdf

[13] OECD Guideline for testing of chemicals 420 [online]. Acute oral Toxicity. Acute classic Method. 25 Dec 2012; Available from: URL: Http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD.pdf

[14] OECD Guideline for testing of chemicals 407 [online]. Acute oral Toxicity. Acute classic Method. 25 Dec 2012; Available from: URL: Http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD.pdf

[15] Pranakhon, R., Pannangpetch, P., Aromdee, C., (2011). Antihyperglycemic activity of agarwood leaf extracts in STZ-induced diabetic rats and glucose uptake enhancement activity in rat adipocytes. Songklanakarin Journal of Science and Technology. 33(4), 405-410.

[16] R. Mohamed, P.L.Jong, M.S. Zali (2010) Fungal diversity in wounded stems of Aquilaria malacensis. Fungal Diversity 43: 67-74.

[17] Rother, K.I., 2007. Diabetes treatment-bridging the divide. New England Journal of Medicine. 356, 1499-1501

[18] Sofowara, A., (1993). Medicinal Plants and Traditional Medicine in Africa. 2nd edition. Ibadan: Spetrum Books Ltd. p150.

[19] Sustainable Agarwood Production in Aquilaria Trees. 23 May 2012; Available from: URL: http://forestpathology.cfans.umn.edu/agarwood.htm

[20] Zhou, M., Wang, H., Suolangjiba, Kou, J., and Yu, B., (2008). Antinociceptive and anti-inflammatory activities of Aquilaria sinensis leaves extract. Journal of Ethnopharmacology. 117, 345-350.Tom E., David G. and Mark A., (2010), "Paper title"; *Journal Name*, **67**, 2234 - 2237.