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NUTRITIONAL EVALUATION AND INFLUENCE ON CARBOHYDRATE AND LIPID METABOLISM OF PORRIDGE MADE WITH GREEN LEAFY VEGETABLES

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By

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2014

DECLARATION BY THE CANDIDATE

The work described in this thesis was carried out by me under the supervision of Prof. Sagarika Ekanayake (Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura) and Dr. Chandanie Wanigatunge (Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura) and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/ Diploma.

21-10-2014
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Date

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Signature of the candidate

CERTIFICATION BY SUPERVISORS

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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Prof. Sagarika Ekanayake

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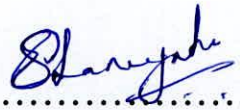
Dr. Chandanie Wanigatunge

CERTIFICATION BY SUPERVISORS

We certify that the candidate has incorporated all corrections, additions and amendments recommended by the examiners.



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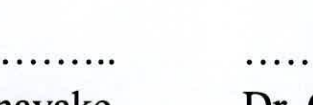
Prof. Sagarika Ekanayake



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Abbreviations

ABCC8		ATP-binding cassette transporter sub-family C member 8
ABTS		2,2'-azino bis 3-ethylbenzthiazoline-6-sulfonic acid
ADA		American Diabetes Association
ADIC		Alcohol and Drug Information Center of Sri Lanka
ADP		Adinosine Diphosphate
AGE		advanced glycation end product
AI		Atherogenic Index
Alk		Alkaloids
ALP		Alkaline Phosphatase
ALT		Alanine Transaminase
AMPK		activated protein kinase
ANOVA		Analysis of variance
AOAC		Association of Official Analytical Chemists
AR		*Asparagus racemosus* porridge
AST		Aspartate Transaminase
ATP		Adenosinetriphosphate
BHT		Butylated Hydroxy Toluene
BMI		Body Mass Index
BW		Body Weight
CALPN10	**or**	Calpain10
CAPN10		
CCH		cell culture extract of *H. indicus*
CCK		Cholecystokinine
CKD		Chronic Kidney Disease
CE		Catechin equivalence
CHD		coronary heart disease
CM		Coconut milk porridge
CRP		C- Reactive Protein
CSTH		Colombo South Teaching Hospital
CV		coefficient of variation
CVD		Cardio Vascular Diseases

DLA	Daltons lymphoma ascites
DM	Diabetes mellitus or Diabetic control group of rats
DPPH	di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium
DW	Dry Weight
EC50	half maximal effective concentration
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme Linked Immuno Sorbant Assay
FAO	Food and Agriculture Organization
FBG	Fasting Blood Glucose
Flav	Flavanoids
FW	Fresh Weight
GAE	Gallic Acid Equivalent
GFR	Glomerular Filtration Rate
GGT	Gamma Glutamyl Transferase
GI	Glycaemic Index
GL	Glycaemic Load
GLP	Green Leafy Porridges
GLP-1	Glucagon Like Peptide-1
GLUT4	Glucose Transporter 4
GPx	glutathione peroxidase
GSH	reduced glutathione
GST	glutathione-S-transferase
HbA1c	Glycated Haemoglobin
HC	Hip circumference
HDL	High Density Lipoprotein
HepG2	Hepatic G2 cells
HI	*Hemidesmus inducus* porridge
HMG Co-A	3-hydroxy-3-methylglutaryl Coenzyme A
IAUC	Incremental Area Under the Curve
IC50	half maximal inhibitory concentration
IDF	International Diabetes Federation
IDF	Insoluble Dietary Fibre

IFN	Interferon
IL	Interleukine
KCNJ11	potassium inwardly-rectifying channel, subfamily J, member 11
KDOQI	*Kidney Disease Outcomes Quality Initiative*
LBW	Low Birth Weight
LCAT	lecithin cholesterol acyl transferase
LD 50	50% concentration of the lethal dose
LDL	Low Density Lipoprotein
MBG	Mean blood glucose
MCF-7	Michigan Cancer Foundation-7
MIDD	Maternally inherited diabetes and deafness
MN	Moderately negative
MP	moderate positive
mRNA	Messenger Ribo Nucleic Acid
NADPH	Nicotinamide Adinine hydrogen Phosphate
NATA	National Authority on Tobacco and Alcohol
NHLBI	National Heart, Lung, and Blood Institute
NHSL	National Hospital of Sri Lanka
NO	Nitric Oxide
NR	Blood glucose level not reached to basal level after 120 minutes
Ob	Obese
OGTT	oral glucose tolerance test
OW	Overweight
Phen	Phenolic compounds
PPAR	peroxisome proliferator-activated receptors
PUFA	Polyunsaturated fatty acids
PYY	Peptide YY
RINm5F	rat insulinoma m5F
ROS	reactive oxygen species
SD	Standard Deviation
SDC	*Scoparia dulcis* porridge
SDF	Soluble Dietary Fibre

SEM	Standard Error of Mean
SLCTR	Sri Lanka Clinical Trials Registry
SOD	Super Oxide Dismutase
SP	strong positive
SR-B1	scavenger receptor type B1
STZ	Streptozotocin
T2DM	Type 2 Diabetes Mellitus
Tann	Tannins
TBARS	thiobarbituric acid reactive substances
TC	Total Cholesterol
TEAC	Trolox equivalent antioxidant capacity
Terp	Terpinoids
TG	Triglycerides
TNF	Tumour Necrosis Factor
USA	United States of America
VLDL	Very Low Density Lipoprotein
W:H or WHR	Waist to Hip ratio
WC	Waist circumference
WHO	World Health Organization
WN	weak negative
WP	weak positive

Nutritional evaluation and influence on carbohydrate and lipid metabolism of porridge made with green leafy vegetables

Senadheera S.P.A.S

ABSTRACT

Among Sri Lankans, 20% are diagnosed as disglycaemic and 27.1% suffer from metabolic syndrome (MS). Along with western medical treatments, 90% of Sri Lankan patients consume herbal remedies owing to their nutritive value, natural origin, availability and less side effects. Rice-based herbal porridge (*kola kenda*) is a popular method of consumption of herbal extracts due to high palatability and high satiety compared to water extracts. Most leaf extracts used for porridges have scientifically proven anti-diabetic effects. However, the potency when these leaves are incorporated in to porridges is not known. The endeavor of the present study was to evaluate the nutrient composition, glycaemic indices (GI), antioxidant potential and toxic effects and hypoglycaemic effects of herbal porridges, with the intention of developing a marketable herbal porridge suitable for diabetics.

Rice: leaves: scraped coconut were used in 20-30g: 5-20g: 10-15g [w/w/w] ratio for the porridge preparation. The leaf varieties used were *Murraya koenigii spreng* (Karapincha), *Asparagus racemosus* (Haathawariya), *Hemidesmus indicus* (Iramusu), *Aegle marmelos* (Beli), *Cassia auriculata Linn* (Ranawara), *Cardiospermum halicacabum* (Wel Penela), *Aerva lanata* (Polpala), *Clitoria ternatea Linn.* (Ela katarolu), *Scoparia dulcis* (Walkoththamalli), *Atlantia zeylanica Linn.* (Yaki narang), *Osbeckia octandra* (Heen bovitiya) and *Coccinia grandis* (previous name: *Cephalandra indica*) (Kowakka). Porridge made with rice only and coconut milk and rice only were

used to evaluate the effects of other ingredients of the porridge other than the leaf extract. Digestible carbohydrate, fat, insoluble and soluble dietary fibre, crude protein, ash and moisture contents of the porridges were estimated by accepted standard methods. GI, antioxidant effects (ABTS free radical scavenging assay) and total phenol content were estimated for all 14 porridges. Using the porridges with lowest GI [*A. racemosus* (AR), *H. indicus* (HI) and *S. dulcis* (SDC)] an animal bioassay was conducted with Streptozotocine-induced diabetic male albino Wistar rats for 3 months to evaluate the hypoglycaemic and toxic effects of these porridges. *S. dulcis* porridge which elicited a significant hypoglycaemic effect in the above study was prepared in large scale (GI for normal 58±11, diabetics 61±11). A crossover clinical trial with type 2 diabetic patients (n=35; group 1=16; group 2=19) was conducted to observe the hypoglycaemic effects and capability of this porridge on lowering risk factors of diabetes. Dietary habits of Sri Lankan type 2 diabetics were assessed by an interviewer administered questionnaire.

Moisture was the major component in all porridges (89% - 93%). Digestible carbohydrate contents in leafy porridges were not significantly different ($p \geq 0.05$). Total dietary fibre contents, crude protein content and fat content varied between 5% - 10%, 4.1% – 9.5% and 2.5 % - 27%, respectively on dry weight basis. The lowest fat amount was in rice porridge due to absence of coconut milk. All porridges except *C. auriculata* were in the low GI category (31-50). The lowest GIs were found in coconut milk (31±5), *Aerva lanata* (32±5), *Hemidesmus indicus* (40±8), *Scoparia dulcis* (39±8) and *Asparagus racemosus* (37±4). All leafy porridges elicited antioxidant effects (5 – 73 TEAC (µg)/ 100g) and high phenol contents (1.9 – 34.2 GAE g/ 100g) with lowest

values for rice and coconut milk porridges, indicating that antioxidant effect was mainly due to the leaf extract.

In the animal study, although not significant ($p \geq 0.05$), SDC group had lower mean blood glucose levels (194±73, 283±98 mg/dL in second and third months, respectively) compared to diabetic control group (298±94, 385±55 mg/dL). A significantly higher ($p \leq 0.05$) mean weight gain was observed in SDC porridge fed group (39 g), when compared to the other diabetic groups (DM 3, HI -8, CM 7 g). HbA1c level in SDC group (5.8±2.1%) was significantly lower ($p=0.018$) than the DM group (8.0±1.5%) while other porridge groups elicited no significant changes when compared with DM group. Although not significant ($p \geq 0.05$) when compared to other diabetic groups, the lowest total cholesterol (119±20.6 mg/dL) and highest HDL (33±6.3 mg/dL) against NC was also observed in SDC group. The CRP (<6 mg/dL), creatinine (range - 0.41±0.1-0.51±0.1 mmol/L), AST (range - 69.7±10.2 - 88.9±15.1 IU/L), ALT (range - 23.7±2.1 - 27.6±3.6 IU/L) and gamma glutamyl transferase (GGT) (range - 3.0±1.7 - 4.6±0.9 IU/L) concentrations of the diabetic groups were comparable with the normal control group indicating no adverse effects on liver and kidney due to porridge consumption.

Mean age of patients who participated in the questionnaire study was 55±9 years. In the study population 44 were males and 56 were females. Only 72% of the patients attended diabetes clinics regularly. Out of all, 44% of patients were either overweight or obese. Majority (83%) consumed red rice/ nadu/ parboiled rice which are suitable for diabetics. Only 67% consumed fruits at least once a day. High GI meals for 5 or more than five days/ week as the breakfast was consumed by 17.8% while for 3 or more than 3 days/

week as dinner was 13.3%. From the study population 87%, 93% and 92% did not consume pork, beef and mutton respectively. Thirty three percent consume high fat diets (fried foods) twice or more than twice/ week. Full cream milk was consumed by 71% and 22% consumed non fat milk. Normal sweetened foods were consumed by 99% and non caloric sweetener usage was nonexistent. From the population, 40%, 50%, 69% and 11% had been detected to have hypertension, high cholesterol, visual defects and heart diseases respectively. Only 14% exercised daily while 69% never exercised.

During the crossover clinical trial with commercially prepared SDC porridge being ingested three times per week, HbA1c of group 1 decreased from 7.9±0.46 to 6.5±0.30 ($p=0.003$) while HbA1c of group 2 decreased from 7.0±0.31 to 6.7±0.24 while they were in the test group. Therefore, both test groups (1 and 2) elicited a decrease in HbA1c compared to control groups. Likewise though not significant, both test groups elicited a decrease in FBG following the intervention (group 1 - from 174±14 to 160±10 mg/dL; group 2 - from 183±13 to 160±7 mg/dL). No significant differences ($p>0.05$) in insulin levels between or within groups were observed during study periods. When considering the lipidaemic measurements no significant differences between or within the groups 1 and 2 were observed for total cholesterol, triglycerides, LDL and HDL measurements and atherogenic index. All liver enzymes, creatinine, urea, cortisol, ferritin and CRP measurements were not significantly different between or within groups during both study periods (1 and 2) indicating absence of liver or kidney toxicity due to ingestion of SDC porridge.

Study revealed, all leafy porridges elicit low GI, have considerable antioxidant potential and therefore are suitable as a meal for diabetics. The *S. dulcis* porridge with positive anti-diabetic properties was commercially produced by changing the particle size of rice grains. The porridge contributed to decrease HbA1c significantly with a non significant decline in fasting blood glucose with no toxicity in diabetics, due to long term consumption.