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EFFECTS OF LONG TERM CONSUMPTION OF SELECTED GREEN LEAFY PORRIDGES ON GLYCAEMIC AND LIPIDAEMIC PARAMETERS OF DIABETIC WISTAR RATS

S. P. A. S. Senadheera and Sagarika Ekanayake

Department of Biochemistry, Faculty of Medical Sciences, University Of Sri Jayewardenepura

INTRODUCTION

Among the Sri Lankan diabetic patients 90% use herbal plant extracts as dietary remedies to treat diabetes with or without prior advice from a physician (Ediriweera and Ratnasooriya, 2009). Most of the ethanolic or water extracts of these leaves have proven hypoglycaemic and hypolipidaemic effects (Sivanandham, 2007).

Consumption of green leafy porridges is a reputed dietary remedy among Sri Lankans. Porridge is made by incorporating a leaf extract to rice porridge and is more palatable than the water extract. Although there is proven data on hypoglycaemic and hypolipidaemic effects of the leaf extracts, no data is available on the effects when the extract is incorporated in to porridge. A previous study (Senadheera and Ekanayake, 2011) indicated low glycaemic indices for common green leafy porridges. Hence the objective of the present study was to evaluate the long term effect of consumption of green leafy porridges with the lowest observed glycaemic indices (*Asparagus racemosus* [*Hathawariya*] (AR), *Scoparia dulcis* [*Wal koththamalli*] (SD) and *Hemidesmus indicus* [*Iramusu*]) on glycaemic (fasting serum glucose, HbA1c, C reactive protein) and lipidaemic (total cholesterol, triglycerides and high density lipoproteins) parameters in diabetic Wistar rats.

METHODOLOGY

The study was a randomized controlled (interventional) study. Porridges of the above mentioned leaves were prepared according to a standard recipe considering the palatability (in final porridge - leaves: coconut milk: rice = 13:90:25). All porridges had similar ingredients except for the leaf variety. The portion size used for the Glyceamic index study contained 35mg/Kg body weight (BW) of solid leaf. Ethical approval for the study was obtained from the Ethics Review committee (Approval No.476/09) of Faculty of Medical Sciences, University of Sri Jayewardenepura. Study was carried out with male albino Wistar rats (28 days old, weight 150-200g) purchased from the Medical Research Institute, Sri Lanka (n=42). Diabetes was induced in 35 rats using streptozotocin (STZ). Rats (n=35) with fasting blood glucose level above 126mg/dL or random blood glucose above 300mg/dL were divided in to five groups with 6 or 7 rats in each [3 green leafy porridge groups, coconut milk porridge group (CM) and diabetic control (DM)]. Remaining normoglycaemic rats (n=7) were used as the normal control. Coconut milk porridge was included for the study to determine whether the effects were due to coconut milk. Porridges were incorporated in to the normal recommended iso-caloric diets of the rats in order to contain a 6 times high dose of porridge than human dose (Reagan-Shaw *et al*, 2008) as metabolic rates of rats are higher than in humans. Normal control and diabetic control groups were provided with a normal diet without porridge. Study was continued for three months. Fasting blood glucose was measured at the end of each month. After three months HbA1c, C reactive protein, total cholesterol and high density lipoprotein (HDL) levels were measured. All assays were carried out using photometric assay kit methods. Weights, feed intake and the water consumption were monitored throughout the study period.

Sagarika Ekanayake (Corresponding author), *Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka* Tel. 0716875891, +94 11 2803578, +94 11 2758584 E.mail : sagarikae@hotmail.com

RESULTS AND DISCUSSION

All the rats fed SD and normal control groups gained weight while most of the rats in other diabetic groups lost weight. Mean weight gains in groups are given in table 1. The mean feed intake (26g – 30g /day / rat) and water consumption (150ml – 200ml / day / rat) of diabetic rats in all groups were not significantly different but were significantly higher ($p \leq 0.05$) when compared with the normal control group (15g – 20g of food/day/rat, 25 ml of water/day/rat). As the feed intake was comparable among the diabetic groups, the observed significant weight gain ($p \leq 0.05$) in SD group compared to other diabetic groups could be due to the hypoglycaemic effects exerted by the leaf extract incorporated in to the porridge.

The mean fasting blood glucose levels and blood glucose increment percentages are given in table 1. All diabetic groups had significantly high mean blood glucose levels compared to normal control ($p \leq 0.05$). Although not significant, SD fed group had lower mean blood glucose levels in the first, second and third months compared to other diabetic groups.

When considering the first 2 months, the highest blood glucose increment percentage was in DM group (77%) and lowest in SD group (1%). However, within the third month this increment was highest in SD (50%) and lowest in IM (15%) group. The reason could be the approach of blood glucose levels in rats to maximum levels within first 2 months in all other diabetic groups except in SD group. As there was a slow increase in blood glucose levels within the first 2 months a significant increment in SD group was observed in the third month. This revealed that SD might have an ability to slow down the STZ induced pancreatic derangement. Since three rats in AR group died within 2 months due to hyperglycaemia the group was excluded from the study.

During 1st to 3rd month a blood glucose increment in all groups including the normal control group was observed. When considering the total study period, the blood glucose increments were not significantly different ($p \geq 0.05$) between normal and SD groups, but was significantly higher ($p \leq 0.05$) in all other diabetic groups. Mean HbA1c level was lowest in the normal control group and among the diabetic groups SD had the lowest level.

C reactive protein (CRP) is an inflammatory marker as well as an indicator of diabetes. In the present study, CRP levels among the groups were not significantly different ($p \geq 0.05$) and were within the normal range indicating an insignificant change in CRP in diabetic rats (table 2).

Most studies carried out with water or ethanolic extracts of SD revealed a decrease in blood sugar levels, urine sugar levels (Das, 2011), plasma glycoproteins and tissue sialic acid levels, an inflammatory marker which is highly associated with the state of diabetes (Khurshid and Ibrahim, 2008). Increase in body weights and plasma insulin levels (Latha and Pari, 2005) when SD was administered at a dose of 250 mg/kg BW was reported. A glycoside, 'amellin' (Nath, 1943) and Scoparic acid D, a diterpenoid and flavonoids of leaves (Latha *et al.*, 2009) increased the uptake of glucose at peripheral tissue level and increased pancreatic β-cell function (Beh *et al*, 2010) and inhibited intestinal glucose absorption by inhibiting Na^+ - K^+ ATPase activator (Sharma and Shah, 2010).

SD extracts also reduce cellular oxidative stress by increasing glutathione peroxidase (GPx), glutathione-S-transferase (GST) and reduced-glutathione (GSH) activities and decreasing thiobarbituric acid reactive substances (TBARS) (Latha and Pari, 2004) which further reduce diabetic complications.

Total cholesterol levels were significantly high ($p \leq 0.05$) in all diabetic groups compared to

the normal control (table 2). Although not significant ($p \geq 0.05$) the lowest value among the diabetic groups was observed in SD group. Though not significantly different ($p \geq 0.05$) a higher HDL cholesterol level comparable to normal control group was also observed in SD group. The higher high HDL and low total cholesterol levels of SD fed group in the present study could be due to the effects on polyol pathway and lipid peroxidation (Latha and Pari, 2004). Another study revealed the hypolipidaemic effects of SD by reducing tissue cholesterol, triglycerides, free fatty acids, phospholipids, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase activity, and very low-density lipoprotein and low-density lipoprotein cholesterol levels in diabetic rats when SD was administered at a dose of 200mg/ KgBW for 6 weeks (Latha and Pari, 2006).

There was no observed hypoglycaemic or hypolipidaemic effect in AR or HI porridges fed rats in the present study. However there is scientific evidence for both hypoglycaemic and hyperglycaemic effects of AR extract leaving a query of the real effect of this plant. A study has shown significantly decreased blood glucose ($P<0.05$), thiobarbituric acid reactive substances (TBARS) and significantly increased reduced glutathione, superoxide dismutase and catalase with oral administration of AR ethanolic extract at a dose of 200 and 400 mg/kg/BW for 21 days (Vadivelan *et al*, 2011). In Ayurveda, AR (Shatavari) is used for dyspepsia (amlapitta) as AR can aid digestion by increasing the levels of amylase and lipase (Dange *et al*, 1969) which on the other hand could increase postprandial blood glucose level aggravating diabetes.

Extracts and alkaloids from the leaves of *Hemidesmus indicus* have proven evidence on hypoglycaemic (Murshed, 2005) and hypolipidaemic effects (Bopanna, 1997). Most of these effects were gained by administering 400mg/ kg BW of HI extract powder to rats.

Although scientific data prove the above effects, the amount of leaf solids in porridge is much lower (35mg/kg BW) than the amounts used for above mentioned studies. Hence the observed hypoglycaemic and hypolipidaemic effects of SD in the present study could be due to synergistic effects of above activities and /or other actions of the compounds of SD, when present in small amounts. However the amount of active compounds in AR and HI may not have been sufficient to cause beneficial effects.

The present study reveals that consumption of leafy porridges will not increase the cholesterol levels in diabetic individuals although porridges contain coconut milk and this may be due to the low amounts of coconut fat present in porridge.

CONCLUSIONS/RECOMMENDATIONS

It can be concluded from the present study that long term consumption of SD porridge reduces weight loss, elicits antihyperglycaemic effect without a significant change in total cholesterol, but contributes to increase HDL in mild and moderate diabetic Wistar rats.

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Table 1 **Changes in weight gain/ loss and blood glucose levels within 3 months (±standard deviation)**

Group	Weight gain/ loss (g)	Mean blood glucose (mg/dL)- end of 1st month	Mean blood glucose (mg/dL)- end of 2nd month	Increment % (from 1-2 month)	Mean blood glucose (mg/dL) - end of 3rd month	Increment % (from 2-3 month)	Increment % (from 1-3 month)
DM##	3^{ade}	168±37	298±94	77^{ade}	385±55	36^{abcde}	133^{ae}
SD#	39^{be}	194±59	194±73	1^{bc}	282±98	50^{abc}	45^{bc}
HI##	-8^{ade}	221±72	364±59	74^{ade}	377±16	6^{abcde}	86^{ad}
AR##	3*	290±101	400±30	49^{ade}	-	-	-
CM##	7^{abde}	174±21	285±117	60^{ade}	337±102	23^{abcde}	89^{ae}
Normal #	114^{c}	90±10	96±12	8^{bc}	124±7	30^{abcde}	38^{bc}

(#n=7, ##n=6); * weight gain in AR group after 2nd month; Some data of AR group was not included as 3 rats died during the first 2 months of the study

Different superscripts along a column indicate a significant difference ($P<0.05$).

a – not significant with DM group; b - not significant with SD group; c - not significant with normal group; d - not significant with HI group; e - not significant with CM group

Table 2 **Changes in HbA1c, Total cholesterol, HDL, C reactive protein and from 1st to 3rd month in rats (±standard deviation)**

	HbA1c	Total cholesterol (mg/dL)	HDL (mg/dL)	C reactive protein
DM##	8.0 ± 1.5^{ad}	121 ± 18.5^{abde}	30 ± 12.2^{abde}	□6mg/dL
SD#	5.8 ± 2.1^{bcde}	119 ± 20.6^{abde}	33 ± 6.3^{abce}	□6mg/dL
HI##	6.2 ± 2.6^{abcde}	133 ± 18.0^{abde}	24.4 ± 4.2^{ad}	□6mg/dL
CM##	6.1 ± 0.8^{abde}	121 ± 21.6^{abde}	22.3 ± 5.2^{ade}	□6mg/dL
Normal#	4.7 ± 0.7^{bcd}	87 ± 6.5^{c}	37.8 ± 7.4^{bc}	□6mg/dL

(#n=7, ##n=6); Different superscripts along a column indicate a significant difference ($P<0.05$); a – not significant with DM group; b - not significant with SD group; c - not significant with normal group; d - not significant with HI group; e - not significant with CM group

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