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Modulation of Glycemic Responses by "Nutricare-DM"- A Functional Food Formulation for Type 2 Diabetic Subjects

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ABSTRACT

Introduction: The incidence of diabetes has reached alarming levels worldwide, and there is a high risk of developing associated disorders in diabetic subjects. An effective approach to combat type 2 diabetes is through dietary management. **Methods**:A functional food was formulated, namely "Nutricare DM" (N-DM), its nutritionally important starch fractions were determined (*in-vitro*), and its glucose lowering effect was studied by supplementing 50 g carbohydrate portion of the test food for a period of 4 months in type 2 diabetic subjects. Subjects who met the inclusion criteria were recruited based on willingness to participate. Anthropometric measurements, blood glucose levels, lipid profile and hepatic enzyme levels were studied before and after the study period. **Results:** The addition of functional ingredients, namely oats, barley, and rice bran as fibre sources positively influenced the Starch Digestibility Index (SDI). The SDI of Nutricare DM *chapathi* (13±1.01) was significantly (p<0.05) lower than that of the control *chapathi* ((20±1.00). Supplementation of Nutricare DM for 3 months decreased glycated haemoglobin (HbA_1C) from 7.1±1.38 to 6.1 ± 0.95, while a gradual and consistent decrease in fasting blood glucose from 129 mg/dl to 99 mg/dl was observed. A significant decrease in the liver enzymes alanine aminotransferase (ALT) (from 47.69± 7.84 to 36.06±4.35IU/l) and aspartate aminotransferase (AST)(from 61.07±16.46 to 34.20±8.95 IU/l) indicated a protective effect of the nutritional intervention against liver damage. **Conclusion:** Results suggest that long term supplementation would be beneficial in modulating the glycaemic responses and hence serve as an effective dietary management strategy for type 2 diabetic subjects.

Key words: Blood glucose, dietary fibre, functional food, hypoglycaemic effects, digestibility index, type 2 diabetes

INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality (Rang, Dale & Ritters, 1991). These may be prevented by maintaining, as closely as possible, a normal blood glucose level. The increasing aged population, consumption of calorie rich diets, obesity, and sedentary lifestyles have led to a tremendous increase in the prevalence of diabetes world wide. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million individuals diagnosed with diabetes (Joshi & Parikh 2007, Kumar *et al.*, 2013. The prevalence of diabetes is predicted to more than double globally from 171 million in 2000 to 366 million in 2030 with the largest increase being in India.

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Statistical projections for India suggest that by 2030 up to 79.4 million individuals may be afflicted with diabetes mellitus (Boyle *et al.*, 2001; Wild *et al.*, 2004). Since, dietary management is the cornerstone of diabetes therapy, it is necessary to identify functional foods to manage the disorder.

In recent years, there has been a tremendous increase in the formulation of health foods that are, to some extent, disease specific, including those for diabetics. In recent years, there has been growing evidence that functional foods and their bioactive compounds, due to their biological properties, may be used as a complementary treatment for type 2 diabetes mellitus (Mirmiran, Bahadoran & Azizi, 2014). Studies report that both the amount of carbohydrates consumed and its source influence the glycaemic index (GI), post-prandial plasma glucose, and insulin responses (Arathi, Urooj & Puttaraj, 2003). Low-GI food with added dietary fibre, has been shown to have reduced post-prandial blood glucose and insulin responses, and improved the overall blood glucose and lipid concentrations in both normal subjects and patients with diabetes mellitus (Jenkins *et al.*, 1987; Brand *et al.*, 1991; Wolever, Jenkins & Vuksan, 1992; Post *et al.*, 2012). In the last decade, much research has been devoted to developing high fibre and high protein flours. These flours are excellent carriers for fibre and protein enrichment, because of their centralised production and the convenience of admixing functional ingredients in the production (De Rui Ter, 1974). Much of the efforts have been successful in creating nutritionally valuable flours, but their use in the production of human food is only in its very early stages. This study was planned to formulate a cost effective functional food prepared with commonly used ingredients with low-GI food, to determine the nutritionally important starch fractions by *in-vitro* technique, and to evaluate its impact on glycaemic control in Type 2 diabetic subjects.

METHODS

All the food ingredients were purchased from local stores on a fresh basis. Amyloglucosidase (Sigma, A9913), Pancreatine Porcine (Sigma P1750), Invertase (Himedi, RM 5983), Glucose oxidase-peroxidase reagent kit, Total cholesterol, Triglycerides, Alkaline phosphatase(ALP), Aspartate aminotransferase (AST) and Aalanine aminotransferase (ALT) (Agappe, India) were used. All other chemicals used were of analytical grade.

Formulation of composite flour

Food ingredients such as *Triticum aestivum* L (wheat-16%), *Hordeum vulgare* (barley-16%), *Zea mays* L (maize -16%), and *Sorghum bicolour* L (jowar - 16%) flours, *Avena sativa* (Oats - 16%), *Glycine max* (soy flour-11%), *Cinnamomunn zeylanicum* (cinnamon - 2%), and *Plantago ovata* husk (psyllium - 7%) were mixed well and sieved through a 60-mesh sieve (BS). The formulation was named 'Nutricare-DM' (N-DM). *Chapathi* (unleavened Indian flat bread) were made using N-DM and subjected to sensory studies to compare them with control *chapathi* prepared from wheat flour. The product was analysed for functional properties like bulk density, and water and oil absorption capacities (Sosulki, 1962).

Starch digestibility characteristics of *chapathi* made with N-DM

Total starch (TS), and different starch fractions such as rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and rapidly available glucose (RAG) were measured in triplicate by the method of Englyst, Kingman & Cumming (1992) in the freshly prepared *chapathi* samples. A summary of the analytical strategy used is shown in Figure 1. Glucose was determined in all the samples using glucose oxidase–peroxidase diagnostic kit.

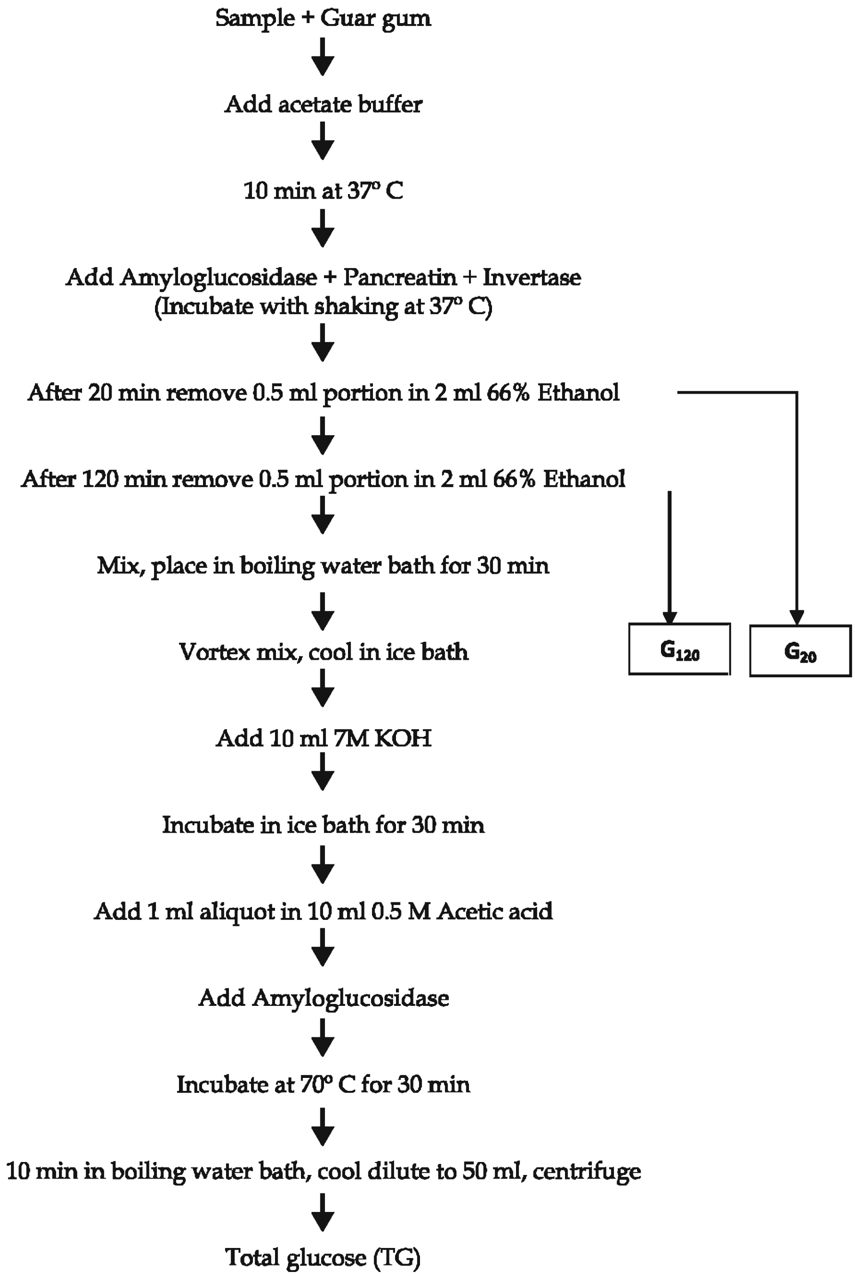


Figure 1. Summary of the analytical strategy for the measurement of starch fractions

Table 1. Dough characteristics and rheological properties of *chapathi* prepared from 'Nutricare-DM'

Variations	*Water used (ml)*	*Weight of dough (g)*	*Weight of chapathi (g)*	*Moisture content (%)*	*WAC (g/g)*	*OAC (g/g)*	*BD (g/cc)*
Control	70	154	120	40.0	2.1	0.05	1.5
N-DM	85	190	150	47.1	1.0	1.0	1.8

Control – wheat flour (100%); N-DM – 'Nutricare-DM'
BD- Bulk density; WAC – Water absorption capacity; OAC – Oil absorption capacity
Results are expressed as mean ±SD of three replicates

Treatment of data
The values for TS, RDS, slow digestible starch (SDS), and RS were calculated from the values of G_{20}, G_{120}, FG and TG follows

1. TS = (TG-FG) x 0.9
2. RDS = (G_{20}–FG) x 0.9
3. SDS = (G_{120}-G_{20}) x 0.9
4. RS = TS–(RDS+SDS) or (TG–G_{120}) x 0.9

The relative rate of starch digestion was calculated as follows

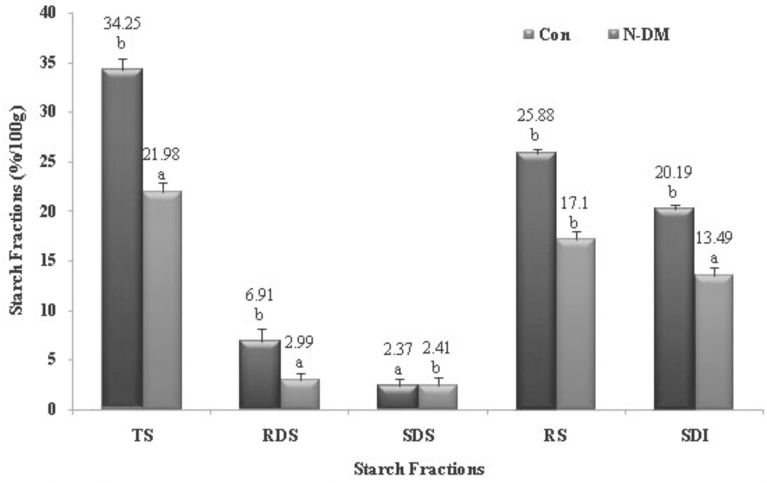
$$SDI = \frac{RDS}{TS} \times 100$$

Effect of supplementation of Nutricare-DM on glycaemic control in type 2 diabetic subjects
This study used a free living, randomised design, and was approved by Institutional Human Ethics Committee (IHEC-UOM No.14 /Res/2009-10). Type 2 diabetic subjects managed on oral hypoglycaemic agents (OHA) aged between 40 to 60 years, with fasting blood glucose (FBS) ≤140 mg/dl, without any complications such as retinopathy, nephropathy, neuropathy and cardiomyopathy and not on hypolipidemic drugs, were selected for the study from the Health Centre of the University of Mysore. The subjects (n=10) were provided with Nutricare-DM flour (≈50 g carbohydrate portion) for a period of 4 months. The subjects were instructed to prepare *chapathi* using the flour. Anthropometric measurements were taken before and after the study period by standard methods and using a body composition analyser. Biochemical parameters like fasting blood glucose (FBS), glycated haemoglobin content (GHB), total cholesterol (TC), and triglycerides (TGL) were analysed on the first day and at the end of the study. Additionally, the hepatic enzymes like Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated in serum.

Statistical analysis
Analysis of variance (ANOVA) was performed on the data, followed by Tukey's multiple comparisons test for significant differences using SPSS version 11.0. The values were considered significant at $p \leq 0.05$.

RESULTS

The proximate composition of N-DM was 15.15 g/100g total dietary fibre, 18 g/100g protein, and 3.2 g/100g fat. The dough characteristics and rheological properties of the *chapathi* prepared using Nutricare-DM are shown in Table 1. The water used for dough preparation was higher in N-DM than in the control, and similar characteristics and property trends were observed in moisture content, weights of dough and *chapathi*. The rheological properties of N-DM dough were higher



TS – Total starch; RDS – rapidly digestible starch; SDS-slow digestible starch; RS-resistant starch; SDI-starch digestibility index. Control – wheat flour *chapathi* (100%), N-DM- 'Nutricare-DM' chapathi.
Mean values carrying different superscripts a &, b, c….., in columns differ significantly (P≤0.05).
The results are expressed as mean ±SD of three replicates.

Figure 2. Starch digestibility characteristics of the formulated *chapathi* (%/100g)

than that of the control. The functional properties of the products were in response to the presence of dietary fibre (oats, barley, psyllium, and rice bran).

Starch digestibility characteristics of *chapathi* made of control and N-DM flours

The total starch and its fractions(RDS, SDS and RS) in the *chapathi* prepared from wheat flour (control) and N-DM flour are shown in Figure 2. The starch fraction profile differed according to the composition and compared to the control, the N-DM flour had significantly lower (p≤0.05) values for TS, RDS, RS, and SDI. The N-DM flour showed better starch digestibility characteristics with significantly low (p≤0.05) SDI. Measurement of different starch fractions provide a means for predicting the rate and extent of starch digestion in the human small intestine (Englyst *et al.* 1992; Englyst & Cummings, 1996). Functional foods with such a nutritional profile are suitable for diabetics as absorption of glucose will be delayed resulting in lower glycaemic responses that will consequently blunt the postprandial glycaemia.

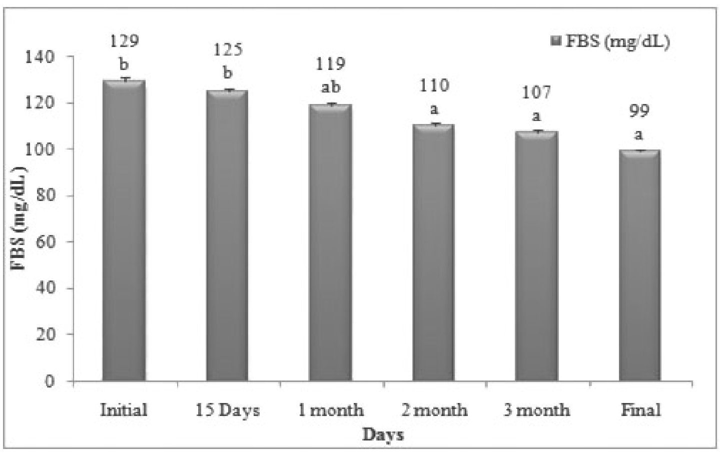
Effect of supplementation of N-DM on glycaemic control in type I diabetic subjects

The glycaemic responses of the subjects supplemented with N-DM was studied. The subjects were recruited based on their compliance, after consenting to participate. All the sensory parameters like appearance, taste, texture, colour, and the overall quality of *chapathi* prepared from N-DM flour received similar scores as the controls, and therefore were well accepted. The subjects consumed one serving of N-DM *chapathi* (equivalent to 50 g carbohydrate) each day, during the study period. The anthropometric measurements and indices of subjects are shown in Table 2. The

Table 2. Mean anthropometric measurements and indices of Type 2 diabetic subjects

Parameters	*Subjects (n=10)*
Age (yrs)	54 ± 9.91
Weight(kg)	64 ± 14.7
Height (cm)	165 ± 10.2
BMI	26.17 ± 3.99
MUAC(cm)	27.33 ± 3.6
SFT(cm)	12 ± 5.2
MUAMC	15.49 ± 2.53
Waist (cm)	97.1 ± 12.51
Hip(cm)	93.5 ± 9.26
WHR	1.03 ± 0.06
TF (%)	33.8 ± 10.23
VF (%)	10.2 ± 4.5
BMR	1331.7 ± 474

BMI- Body mass index, MUAC- Mid upper arm circumference, SFT-Skin fold thickness, MUAMC- Mid upper arm muscle circumference, WHR-waist hip ratio, TF-Total fat, VF-visceral fat,
BMR – Basal metabolic rate



FBS- fasting blood glucose.
Mean values carrying different superscript letters a &, b, ….., on bars differ significantly (P≤0.05).

Figure 3. Changes in mean FBS (mg/dL) levels of type 2 diabetic subjects (n=10) during the study period

body mass index (BMI) of most subjects was above the normal range. The effect of supplementation of N-DM on fasting glucose levels and biochemical parameters are presented in Figure 3 and Table 3, respectively. Supplementation resulted in a gradual, but significant ($p \geq 0.05$) decrease in the fasting blood glucose. A similar trend was observed with respect to the total cholesterol and triglycerides levels. There was a significant decrease ($p<0.05$) in the hepatic enzymes [ALP and AST]post-

Table 3. Comparison of mean biochemical parameters of type 2 diabetic subjects (n=10)

Biochemical parameters	Initial	Final
FBS (mg/dl)	$129^{a} \pm 28.00$	$99^{b} \pm 13.18$
HbA1C	$7.1^{a} \pm 1.38$	$6.1^{a} \pm 0.95$
T.Ccholesterol (mg/dl)	$170^{a} \pm 21.14$	$163^{a} \pm 18.22$
TGL (mg/dl)	$148^{a} \pm 43.60$	$140^{a} \pm 23.67$
Hepatic enzymes (IU/L)		
ALP	$177^{b} \pm 6.44$	$154^{a} \pm 11.60$
AST	$61.07^{b} \pm 16.46$	$34.20^{a} \pm 8.95$
ALT	$47.69^{a} \pm 7.84$	$36.06^{b} \pm 4.35$

FBS- fasting blood glucose; T cholesterol- total cholesterol; TGL-triglycerides; AST-aspartate aminotransferase; ALT-alanine aminotransferase; ALP- alkaline phosphatase.
Mean values carrying different superscript letters a &, b, ….., in rows differ significantly as per Tukey's test ($P \leq 0.05$).

supplementation. Additionally, N-DM flour was well accepted in the preparation of the *chapathi* and received a positive response from the subjects. Subjects also reported improvement in their bowel movement, which mighy be due to the fibre composition. The results show that e-DM supplementation exhibited a significant blood glucose lowering effect, and helped in the stabilisation of blood glucose levels as evidenced by the reduction in fasting blood glucose and HbA_1C levels.

DISCUSSION

The effect of functional food as an anti-hyperglycaemic agent was studied by supplementing N-DM in the regular diet of Type 2 diabetic subjects for four months. The addition of cereal β-glucans displayed all the physiological properties that have been attributed to dietary fibre like water holding capacity, swelling, diffusion-suppressing ability (viscous, gel formation), and binding properties. Therefore, grains with high levels of soluble β-glucans, such as oats and barley are generally more effective in improving blood glucose levels, insulin responses, and serum cholesterol levels than wheat, which contains predominantly insoluble dietary fibre. Clinical studies with diets containing foods enriched with oats and barley β-glucans revealed a reduction of GI and insulinaemic index (GII) (Billiaderis & Marta, 2007). Psyllium's mechanism of action for glucose reduction in diabetic patient's is probably similar to that of other soluble fibres because it forms a viscous gel in aqueous solution, or delays gastric emptying, or that it may sequester carbohydrates ingested with the meal thereby retarding carbohydrates' access to digestive enzymes. Reports indicate that psyllium can exert these effects hours after its administration, and can produce a significant reduction in glucose after a second meal (Layce *et al.*, 1991)

Studies also suggest that the inhibition of glucose diffusion in the small intestine is due to the adsorption or inclusion of the smaller sugar molecules within the structure of the fibre particles (Lopez *et al.*, 1996; Nishimune *et al.*, 1991). Administration of psyllium fibre to diabetic rats is reported to reduce glycaemia (Ahmed *et al.*, 2010). Similarly, Rodriguez, Guerrero & Lazcano (1998) observed a reduction in glucose and cholesterol levels in diabetic patients without significant adverse effect.

Clinical studies have demonstrated that β-glucan decreases plasma glucose and insulin concentrations following a single meal in both healthy individuals and individuals with type 2 diabetes (Battilana *et al.*, 2001; Wood *et al.*, 1994). These observations emphasise that there may be the adsorption of glucose / inhibition of α-amylase, or both, which are the probable mechanisms through which the N-DM exerts its hypoglycaemic effect, which in turn helps in the lowering of blood glucose levels or to maintain the normo-glycaemic condition in type 2 diabetic subjects.

It is well known that elevated liver enzyme levels indicate inflammation of, and damage to the liver. The occurrence of liver disease and raised liver enzymes is common in type 2 diabetes, and may be multi-factorial in origin. ALT has been suggested as a surrogate marker for non-alcoholic fatty liver disease (NAFLD) in diabetic subjects (Saligram, Williams & Masding, 2012). NAFLD is associated with type 2 diabetes and metabolic syndrome, and can progress to chronic liver disease (Schindhelm *et al.*, 2006, Pagano *et al.*, 2002). The subjects that showed higher levels of liver enzymes at the start of this study had significantly decreased levels at the end of the study period. This observation, along with the reductions in lipid profile suggest a beneficial effect of supplementation in the prevention of liver damage.

CONCLUSION

Dietary and lifestyle factors contribute to liver fat accumulation through multiple pathways. It is reported that dietary composition affects development of NAFLD (Tanaka *et al.*, 2005, Hernandaz-Rodaz, Valenzuela & Videla, 2015). Therefore manipulating macronutrient composition appears to be a practical approach in the prevention of NAFLD among type 2 diabetic subjects. Hence, the formulated functional food N-DM can be supplemented in the regular diet as the ingredients used are all natural, commonly consumed, with no side effects, provide nutritional support, and help in effective management of type 2 diabetes.

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Conflict of Interest

There is no conflict of interest to be declared.

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