CHAPTER IV

Ameliorating effect on diabetic status of slow carbohydrate digestible noodles in streptozotocin-induced diabetic rats.
4.1. INTRODUCTION

Diabetes mellitus is one of the major metabolic disorders, afflicting a large proportion of the population worldwide (Zimmet et al., 2001; Report of WHO, 1985). The disease knows no barrier of developing or developed country, rural or urban area, rich or poor as it is entering alarmingly in all sections of society. WHO has identified India as capital of diabetes as more and more people are coming under the girth of diabetes. Once thought as a single disease, diabetes serves as a ground for various other complications to pop up, and is the leading cause of an array of secondary complications including complications like kidney disorder, kidney failure, heart diseases, amputation, destructive periodontitis and blindness. Since, diabetes is a life-long disease, carrying diabetes throughout, with drugs is cumbersome and costly. So, it is vitally important to identify the most appropriate approach for the easy management of diabetes.

The diet is the most important and basic treatment of diabetes. Till today, diet remains in the cornerstone for the management of diabetes. Epidemiologic data suggest that a low-glycemic index diet plays a protective role against the development of type-2 diabetes (Nilsson et al., 2007). Foods providing equi-carbohydrate portions elicit different postprandial glycemic responses. The glycemic index of foods was developed in an attempt to systematically classify the carbohydrates in different foods according to the time integrated effects on postprandial glycaemia. The concept of GI has proved as a boon as far as diabetes mellitus is concerned and dietary guidelines were prepared keeping GI as major criteria. The WHO has categorized foods according to their GI values and are classified as low GI.
(55 or less), medium GI (56-69) and high GI (70 or more). Currently, the challenge is to identify hypoglycemic diet supplements to control blood glucose levels and to make such supplements easily accessible to the general public in a way, that it will be easy for them to follow the instructions. A meal consisting of a combination of cereal-pulse/legume mixes, are found to be more effective than the only cereal diet (Bijlani, 1993). If combinations of such foods are given in the form of a meal, it is better than giving an individual food, for long term adherence. The convenience in the form of supplementary food products should be provided, so that one can include these supplementary foods in a long term practice. Such supplementary foods will find place in the kitchen shelves only, if they will be based on the food habits of the people.

Food products made up of familiar, staple foods will have more chances of acceptability by the general public. So, formulation of such supplementary foods for diabetes is the need of the hour. Many hypoglycaemic ingredients have come into light through many researches. In this regards many researchers have focused cereals and pulses/legumes as foods having low GI. Many cereals like brown rice, finger millet and other millets are having low GI. Pulses/legumes, which are a very good source of protein are also a good source of dietary fiber and the carbohydrate of legume is being considered as slow release carbohydrate (Jenkins et al., 1982; 1983) which makes them foods of low glycemic values thus pulses/legumes occupies an important place in the list of low GI foods. Many other hypoglycaemic ingredients like various spices are found beneficial for the management of diabetes. Among all these, cereals draws the major
attention as they form the major bulk of the diet and are serving as staple foods for the population.

Hence, the present investigation were undertaken to make use of the health and nutritional benefits of brown rice/husked rice (pigmented and non-pigmented) and whole chick pea along with selected additives in the form of noodles on the glycemic response, as well as other biological responses in diabetic induced rats for the better and effective dietary management of diabetes.

4.2. MATERIALS AND METHODS

4.2.1a. Materials

Two types of noodles – Pigmented noodles (PN) and Non-pigmented noodles (NPN) were prepared using the combination of respective de-husked rice along with whole chick pea and fenugreek, along with selected additives, as explained in chapter II. A commercial white bread was purchased from local supermarket. The crust was separated and the fresh crumb was used as reference food in in-vivo studies.

4.2.1b. Chemicals

Streptozotocin (STZ) and p-dinitrosalicylic acid were obtained from Sigma Chemical Company (St Louis, Mo, USA). Bernhardt – Tommarelli–modified salt mixture were purchased from SISCO Research Laboratories, Mumbai. Casein was purchased from Nimesh Corporation, Mumbai, Refined pea nut oil were purchased from local market. All other chemicals and solvents used were of analytical grade from Qualigens Fine Chemicals, Mumbai.
4.2.2. Animals and diet

Three-months-old male Wistar rats weighing around 120 g were taken for the study. The study was conducted with due approval from the Institutional Animal Ethical Committee (CSIR‒CFTRI), taking all precautions to minimize pain or discomfort to the animals. The rats were divided into two groups: group I served as control (10 rats each) and the other group II, as diabetic (20 rats each). Rats were assigned to those fed starch, pigmented noodles, PN (pigmented husked rice, whole Bengal gram, fenugreek and additives- guar gum and xanthan gum) and non-pigmented noodles, NPN (non-pigmented husked/brown rice, whole Bengal gram, fenugreek and additives- guar gum and xanthan gum) 6 groups of rats to the following diets: starch-fed control (SFC), starch-fed diabetic (SFD), pigmented noodles (PN)–fed control (PNFC), pigmented noodles (PN)–fed diabetic (PNFD), non-pigmented noodles (NPN)–fed control (NPNFC) and non-pigmented noodles (NPN)–fed diabetic (NPNFD). The rats were fed with the American Institute of Nutrition (AIN)-76 basal diet as presented in Table 13 (Bieri et al., 1997). A powder form of pigmented noodles and non-pigmented noodles was added at a 10% level at the expense of starch to the AIN-76 diet and stored at 4°C and fresh diet was given every day.
### Table 13. Composition of the diets fed to rats.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ingredients</th>
<th>Dietary groups (g/kg Diet)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>SFC/SFD</td>
</tr>
<tr>
<td>1</td>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>AIN-76 Vitamin mix</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>AIN-76 Mineral mix</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>Choline chloride</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Refined groundnut oil</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Corn starch</td>
<td>653</td>
</tr>
<tr>
<td>7</td>
<td>Methionine</td>
<td>Little</td>
</tr>
<tr>
<td>8</td>
<td>Pigmented noodles</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Non-Pigmented noodles</td>
<td>-</td>
</tr>
</tbody>
</table>

4.2.3. Induction of diabetes

Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin at 45 mg/kg body weight in freshly prepared citrate buffer (pH 4.5, 0.1 mol/L) injected in a volume of about 1 mL. Control rats received only citrate buffer. Soon after streptozotocin injection, rats were provided with 5% glucose solution in place of water for the first 48 h to prevent the initial drug-induced hypoglycaemic mortality (Hatch, 1995). At 3 d, after the injection, blood was withdrawn from overnight fasted rat’s tail veins and was used to assess their hyperglycaemic status by the estimation of blood glucose levels. The rats with the fasting blood glucose levels higher than 250 mg/dL were considered as diabetic.

4.2.4. Collection of urine samples

Rats were maintained in individual metabolic cages and a 24-hour urine sample was collected under a layer of toluene. This was done on a weekly basis to examine the amount of sugar excreted in the urine. The urine samples were filtered, the volume was noted and stored frozen until further analysis.

4.2.5. Measurement of urine sugar

The content of reducing sugar present in the urine was measured by the dinitrosalicylic acid method (Miller, 1959).

4.2.6. In-vivo glycemic response in animal models

At the end of six weeks, rats were fasted for overnight (12 h), rats were intubated with 2 g diet. The control rats received the white bread (2g) and the experimental received 2 g carbohydrate based noodles (PN and
NPN) respectively. Blood samples were drawn from the rats tail veins and analysed with commercially available Accu check, gluco meter sensor, for glucose level at time intervals of 0, 20, 40, 80 and 120 min. Area under curve shows the incremental blood glucose area in relation to the corresponding area obtained after white bread used as reference food. At the end of the experiment, all animals were sacrificed under ether anaesthesia by extrangulation.

4.2.7. Statistical analysis

Data were analyzed using Minitab 17 statistical software. Each experiment was performed in triplicate and the results were expressed as the mean values ± standard deviation. Results were analyzed and significance level was calculated using Tukey–Kramer multiple comparison test by means of one way ANOVA. Values with $p< 0.05$ were considered statistically significant. Area under curve was calculated using Graph pad prism-3.
4.3. RESULTS AND DISCUSSIONS

4.3a. RESULTS

4.3.1. Influence of pigmented and non-pigmented noodles on water intake in diabetic and non-diabetic rats.

High water intake is a characteristic symptom of diabetes. The control rats consumed about 20 to 40 mL/d of water compared with 75-125 mL/d for the SFD rats (Fig 20). Both the PNFD and NPNFD groups consumed around 80 to 130 mL of water per day.

4.3.2. Influence of pigmented and non-pigmented noodles on feed intake in diabetic and non-diabetic rats.

All diabetic rats fed with the experimental diets (PNFC, PNFD, NPNFC and NPNFD) survived the experimental period of 6 weeks, whereas 30% of the diabetic rats fed with control diet (SFD) group died in the course of the experimental period. The cause of mortality is presumed to be severe hyperglycaemic and associated complications. The rats consumed the control as well as the experimental diets normally and dietary intake was comparatively higher in the diabetic group compared with the non-diabetic groups. The SFC rats consumed about 12 g/d diet, whereas the SFD group was statistically higher (Fig 21). Consumption of diet was higher in the PNFC group when compared with SFC and was about 14 g/d and the diabetic group (PNFD) consumed about 19 g/d. The NPNFD group consumed about 18 g/d and was not different from the SFD. Dietary fiber-rich foods are known to be consumed in higher amounts, which may also be due to greater palatability (Nandini et al., 2000; Plaami, 1997).
Fig 20. Influence of pigmented and non-pigmented noodles on water intake in diabetic and non-diabetic rats.

Values are mean ± standard deviation of (n=6 control; n=9 diabetic groups).

Fig 21. Influence of pigmented and non-pigmented noodles on food intake in diabetic and non-diabetic rats.

Values are mean ± standard deviation of (n=6 control; n=9 diabetic groups).

4.3.3. Influence of pigmented and non-pigmented noodles on body weights in diabetic and non-diabetic rats.

The body weights of the diabetic rats remained much lower than those of corresponding non-diabetic rats. There was no difference in the body weight gain pattern among the non-diabetic group of rats, whereas significant (p<0.05) weight loss was observed in the diabetic control group of rats (Fig 22). Diabetic rats maintained on experimental diets (PNFD/NPNFD) showed improvement in the body weight throughout the experimental duration as compared to the rats with control diet (SFD).

4.3.4. Influence of pigmented and non-pigmented noodles on urine excretion in diabetic and non-diabetic rats.

Excretion of urine was monitored weekly in rats and the controls excreted about 20 mL/d. Polyurea condition prevailed in the SFD rats and the urine output was about 70 mL/d (Fig 23). Both pigmented and non-pigmented noodles fed diabetic rats resulted in improvement of urine output (about 110 mL/d) in the diabetic groups (PNFD/NPNFD).

4.3.5. Influence of pigmented and non-pigmented noodles on urine sugar in diabetic and non-diabetic rats.

Urine sugar in rats was measured weekly (Fig 24). The control rats demonstrated low levels of sugar in the urine (mg quantities). The diabetic rats (SFD) excreted around 7 g/d urine sugar during the experimental period. The excretion of sugar in the urine was reduced in both of the PNFD and NPND rats, which was around 5 g/d.
Fig 22. Influence of pigmented and non-pigmented noodles on body weight in diabetic and non-diabetic rats.

Values are mean ± standard deviation of (n=6 control; n=9 diabetic groups

SFC-Starch Fed Control, SFD-Starch Fed Diabetic, PNFC-Pigmented Noodles Fed Control,
PNFD-Pigmented Noodles Fed Diabetic, NPNFC-Non-Pigmented Noodles Fed Control and
NPNFD-Non-Pigmented Noodles Fed Diabetic.
**Fig 23.** Influence of pigmented and non-pigmented noodles on urine volume in diabetic and non-diabetic rats.

Values are mean ± standard deviation of (n=6 control; n=9 diabetic groups).
Fig 24. Influence of pigmented and non-pigmented noodles on urine sugar in diabetic and non-diabetic rats.

Values are mean ± standard deviation of (n=6 control; n=9 diabetic groups).

4.3.6. Glycemic response of pigmented and non-pigmented noodles in rat model.

Glycemic response following the oral administration of pigmented and non-pigmented noodles compared to that of white bread are shown in (Fig 25). Plasma glucose concentrations at 40, 80 and 120 min following the oral administration of pigmented and non-pigmented noodles were significantly lower compared to white bread.

The mean fasting blood glucose concentration of the diabetic control animals was 220 mg/dL at the beginning of the study and it changed appreciably till the end of the study to 340 mg/dL (Fig 25). The mean fasting blood glucose of the diabetic experimental diet (PNFD/NPNFD) groups of animals were significantly (P<0.05) lowered compared to diabetic control (SFD) at the end of the 6 weeks of feeding.

These results were reflected in the area under the curve (Fig 26) also of plasma glucose. Area under the curve of glucose was significantly lower (P<0.05) in pigmented noodles followed by non-pigmented noodles compared to control (SFD).

The results indicated that, feeding diabetic animals with the diet containing pigmented and non-pigmented noodles helps in controlling the hyperglycemia.
Fig 25. Influence of pigmented and non-pigmented noodles on plasma glucose in diabetic and non-diabetic rats.

Values are mean ± standard deviation of (n=6 control; n=9 diabetic groups).

Fig 26. Influence of pigmented and non-pigmented noodles on area under plasma glucose curve in diabetic rats.

Values are mean ± standard deviation of (n=6 control; n=9 diabetic groups).

4.3b. DISCUSSION

The present animal study indicated that, the diabetic associated complications were significantly ameliorated by feeding a diet containing noodles of two types, respectively. PN and NPN were prepared with the combination of red-pigmented husked rice/brown rice-whole chick pea and fenugreek along with selected additives (xanthan gum and guar gum) by cold extrusion method. It is likely that the beneficial effect of these noodles probably may be due to the health-promoting functional food ingredients used in the formulations.

The lower glycemic response in rats fed with PN containing red-pigmented husked rice is probably attributed to high amylose, dietary fiber and resistant starch content. Starches with high amylase content are less susceptible to hydrolysis by α-amylase because of linear structure of amylase (Fitzgerald et al., 2004). There are many studies that suggest that resistant starch (RS) intake decrease postprandial glycemic and insulinemic responses (Behall et al., 2006). Polyphenols present in pigmented rice are known to decrease the digestibility of starch. Anthocyanin-rich purple corn is shown to ameliorate the hyperglycemia and insulin resistance (Tsuda, 2003). It has been reported that polyphenols reduce fasting hyperglycemia and attenuate the postprandial blood glucose response in rats (Scalbert et al., 2005). Hence the observed health benefits in the diabetic experimental group may possibly be attributed to the synergistic effect of these phenolic compounds present in the PN.

In several countries, legumes have increasingly been used in diabetic formulations to prevent diabetes (Brand et al., 1990). The two types on
noodles- PN and NPN had similar high proportions of protein (~14%), but the association of cereal and chickpea improves the level of quality protein in the noodles. When cereals and legumes are combined, the quality score of the combined proteins may be much higher than each of the individual values (Hegarty, 1995). Several studies have been published on the in vitro and in vivo digestion of starch in legumes and the resistant starch (RS) formation during cooking and storage of legumes-based food products (Bravo et al., 1998; 1999; Hoover and Zhou, 2003; Jenkins et al., 1982; Osorio-Diaz et al., 2005; Tovar et al., 1992a; Tovar and Melito, 1996; Velasco et al., 1997). The high RS content in legumes explains, at least partly, why the starch digestion rate and therefore the release of glucose into the blood stream are slower after the ingestion of legumes, resulting in reduced glycemic and insulinemic postprandial responses (Jenkins et al., 1982; Tovar et al., 1992a; Tovar et al., 1992b). Starch-containing cells in whole cooked legumes such as chickpea, possess remarkably rigid cell walls, whose mechanical resistance persists even in boiled and mildly homogenized samples (Tovar et al., 1990; Tovar et al., 1992a; Tovar et al., 1991) which in addition to the particular features of their starch constituents, result in low digestion rates (Bjorck et al., 1994; Garcia-Alonso et al., 1998; Granfeldt et al., 1992). Jenkins et al., 1987 studied the effect of starch-protein interaction in wheat and its effect on starch digestibility. Their reports suggested that the occurrence of a starch-protein interaction in white flour may account for the decreased glycemic response and reduced rate of digestion.

Dietary fiber is well established as a beneficial food component for improving the complications of many diseases, including diabetes
(Cummings, 1985; Wolver and Jenkins, 1986). Legumes also have high amounts of dietary fiber in a form that confers high mechanical resistance to the cell walls, thus minimizing the seed disintegration during cooking (Melito and Tovar, 1995; Tovar et al., 1992a; Tovar et al., 1992b; Wursch et al., 1986). This, along with the presence of certain anti-nutrients, may also account for the slow and relatively low digestibility of starch in legumes containing foods. Dietary fiber in these prepared noodles were (~23%), is important due to its functional effects in the gut. For instance, viscous fiber-containing foods may elicit low postprandial glycemic responses due to delayed glucose absorption (Bravo et al., 1999; Jenkins et al., 1982; Tovar et al., 1992b). In addition to the intrinsic properties of legume starches, their cognate viscous dietary fibers have been suggested to slow down diffusion of amylolytic products to the absorptive mucosa (Bjorck et al., 1994; Jenkins et al., 1987; Tovar, 1994; Wursch et al., 1986). A possibly that might therefore decrease the diffusion rate of cereal-legume based noodle starch digests.

Insoluble fiber has been reported to be effective in the glycemic control in dogs with insulin-dependent diabetes mellitus (Kimmel et al., 2000). Insoluble fiber of pre-germinated brown rice has been reported to lower both postprandial blood glucose and insulinemic responses in normal Wistar rats (Seki et al., 2005). Fiber concentrates of rice bran were effective in lowering serum glucose and cholesterol in both type 1 and 2 diabetics (Qureshi et al., 2002). It is likely that the beneficial effect of fenugreek seed mucilage is due to some of the bioactive compound present in the mucilage, known to facilitate insulin secretion (Sauvaire et al., 1998). Dietary fiber present in the noodles (PN and NPN) is mostly insoluble in nature (~15-17%)
and hence the anti-hyperglycemic action of the PN and NPN in the present study could also have been contributed by dietary fiber present in the noodles.

Polysaccharide based gums belong to the water-soluble non-starch polysaccharides and their effects on the human metabolism are considered to be beneficial because they decrease postprandial glycemic following ingestion of starchy food (Kaur and Singh, 2009). Ellis et al., (1995) studied the effect of adding guar gum (at different concentrations) on the net apparent glucose absorption in growing pigs. The pig meals containing guar gum resulted in increased zero shear viscosity of jejuna digesta along with a significant reduction in the rate of glucose absorption. This postprandial effect of guar gum results because of the gum’s capacity to increase the viscosity of digesta within the gastrointestinal tract due to the enlargement of fully hydrated galactomannan chains. This whole phenomenon reduces the rate of digestion and absorption of carbohydrates and therefore lowers the postprandial rise in blood glucose (Jaspreet Singh et al., 2010). Brennan et al., (1996) studied the addition of guar gum in white bread and studied its microstructure along with in vitro and in vivo digestibility. It was observed that the blood glucose lowering of gum is due to its ability to act as a physical barrier to starch digestion along with increasing the viscosity of digesta. The association between guar galactomannan and starch has also been confirmed through the microstructure analysis of pig digesta. The high level of viscosity slows down many of the physiological processes associated with the digestion of foods and absorption of nutrients and thus helps in improving the management of glucose intolerance. Hence, guar gum and xanthan gum
used in the formulation of noodles, acts as a barrier to starch digestion along with increasing the viscosity of digesta,

Low values of digestibilities from the extrusion cooked have also been seen sometimes which may be attributed to the formation of amylose-lipid complexation, starch-protein interaction and limited water availability which prolongs the starch digestion during enzymatic hydrolysis (Guha et al., 1997). Thermal processing methods have been reported (Reman and Shah, 2005) to act by thermal degradation of the anti-nutrient molecules, changes in their chemical reactivity and formation of insoluble complexes. With the help of in vitro and in vivo digestibility studies on amylase-lipid complexes, Holm et al 1983-4 observed that complexed amylase is hydrolysed and absorbed in the gastrointestinal tract to the same extent as free amylase but at a somewhat slower rate.

4.4. CONCLUSION

Factors acting on starch digestibility are multiple and not necessarily unrelated to one or more of the others. The effects of galactomannan such as guar gum in lowering postprandial rice in blood glucose not only include actions on intestine physiology but also include a direct inhibition of the first step of the biochemical degradation of starch. Other factors such as processing techniques, the presence of other food components like proteins, lipids, anti-nutrients/inhibitors also affect starch digestibility to significant extent.

The results presented in this study provide some evidence that feeding prepared noodles (Pigmented/Non-pigmented) to the diabetic rat improved diabetic status, as assessed by water consumption, gain in body weight,
urine output, urine sugar and fasting blood glucose. A 41% in PNFD and 38% in NPNFD, improvement in fasting blood glucose was observed at the end of the experiment in rats compared with the starch control diabetic rats.

The present investigation indicates that the incorporation of PN and NPN at the 10% dietary level confers beneficial effects in STZ-induced diabetic rats with respect to plasma glucose and other metabolic parameters. A detailed study on the mechanism of these beneficial effects exerted by these noodles may be conducted on diabetic patients, would be challenging and merits further investigation.

In conclusion, low carbohydrate digestible noodles prepared from the combination of husked rice (pigmented/non-pigmented), whole chick pea and fenugreek, along with selected additives is best suited in the dietary management of diabetics and disorders of carbohydrate metabolism.