Effect of Feeding Functional Snack “Khakhra” on Blood Glucose and Antioxidant Status of Type II Diabetic Males Residing in Anand

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ABSTRACT:
Type II diabetes mellitus is characterized by chronic hyperglycemia. Hyperglycemia is a major cause of secondary complications like coronary and peripheral arterial disease, nephropathy, neuropathy and retinopathy. Dietary approach is one of the ways to prevent hyperglycemia. The present study was planned to find out the effect of functional snack “Khakhra” on blood glucose levels, glycated haemoglobin and blood antioxidant status. Functional khakhra was developed by using wheat, amaranth seed, ragi, oats, soyabean, flaxseed, jambu seed, kadijiri, coriander, tomato and spices at different levels. Sensory evaluation as well as α-amylase inhibitory capacity of control and experimental “Khakhra” was carried out. 70 gm of khakhra (having highest overall acceptability) was fed to type II diabetic males (N = 22) for 90 days. Before and after feeding of khakhra whole blood was analysed for glutathione, vitamin C, glucose and glycated haemoglobin, while serum was estimated for total antioxidant capacity (using FRAP method). Significantly (p ≤ 0.05) higher α-amylase inhibitory activity was found in experimental khakhra compared to control. A significant reduction (p ≤ 0.05) was observed in blood glucose concentration (18.24 %) while a non-significant (p ≤ 0.05) reduction in HbA1c (1.20 %) was observed after feeding khakhra. Blood glutathione and FRAP were non-significantly (p ≤ 0.05) increased by 1.44 % and 19.79 % respectively. No significant change was observed in blood vitamin C. Present study concludes that the experimental “Khakhra” could be an effective combination in improving blood glucose levels and antioxidants status of type II diabetic males.

Key words: Type II diabetes mellitus, Antioxidants, α-amylase, Glycated haemoglobin
INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic disorders worldwide with an estimated 143 million people suffering from the disease [1]. This number may double by 2030 [2].

In India, statistical analysis revealed that the number of diabetics will rise to 57 million in the year of 2025 compared to 15 million diabetics in 1995 [3]. Diabetes mellitus is characterized by chronic hyperglycemia (very high blood glucose levels) and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action [4-5]. The effects of DM include long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, livers, hearts, and blood vessels. In the treatment of diabetes, many oral hypoglycemic agents like D-phenylalanine and α-glucosidase inhibitors are used in addition to insulin treatment along with appropriate diet and exercise [6]. However, none can be termed as an ideal one, due to their toxic side effects and sometimes diminution in response after prolonged use [7]. The limitations and side effects associated with existing synthetic oral hypoglycemic agents had necessitated the search for newer ways to cure hyperglycemia. Dietary approach is one of the ways to prevent hyperglycemia. As a result, natural agents from plants and plant products have been the alternative target to source for new antioxidant and antidiabetic agents based on their traditional use. Shobana et al [8] formulated four different foods from whole wheat, decorticated ragi and others. Foods in the form of porridge were provided to eight healthy adult subjects and the postprandial blood glucose response was determined. The alloxan induced rats fed with finger millet enriched diet showed a greater reduction in blood glucose (36%) [9]. Pancreatic α-amylase breaks down the carbohydrates into oligosaccharides, which are subsequently broken down to monosaccharide by α-glucosidase. Hence retardation of starch digestion by inhibition of these enzymes viz., pancreatic α-amylase and α-glucosidase would play a key role in the control of hyperglycemia. However, the discovery of safe, specific high-affinity inhibitors of these digestive enzymes for the development of therapeutics has remained elusive [10].

The present study was planned to investigate the effect of feeding Functional snack “Khakhra” on blood glucose level of type 2 diabetic males of Anand. Khakhra was prepared by using different ingredients like wheat, amaranth seed, soyabean, ragi, oats, kadijiri and jambu seed powder etc at different levels.
Material and methods

The present study was planned to investigate the effect of feeding functional snack “Khakhra” on blood glucose level of type II diabetic males of Anand and Vidhyanagar. Khakhra was prepared by using wheat, amaranth seed, soyabean, ragi, oats, kadijiri and jambu seed powder etc in different amount. Sensory evaluation of control and experimental khakhra was carried out. Control and experimental khakhra (with highest overall acceptability) were evaluated for α-amylase inhibition. The experimental khakhra having highest overall acceptability was used for feeding trial. Four khakhra per day each weighing 17 gm was fed to type II diabetic male subjects (no=22) for 90 days. Before and after feeding of khakhra whole blood was analysed for glutathione, vitamin - C, glucose and HbA1c while serum was estimated for antioxidant activity (using FRAP).

A) Locale of Study:

The research work was conducted at the laboratory of Foods and Nutrition, JASHBHAII KHODABHAII PATEL, Department of Home Science, Sardar Patel University, Vallab Vidhyanagar, Anand.

B) Experimental design:

Development of control and experimental Functional Snack (Khakhra):

**Procurement of raw materials:** All the raw materials like wheat flour, amaranth seed, finger millet, oat, soya bean, flax seed, jambu seed powder, kadijiri, salt & spices were purchased from the local market of Anand and Vallab Vidhyanagar.

**Preparation of control “Khakhra”:** Wheat flour and salt were mixed. Oil and water was added and kneaded to make soft dough. This dough was then rolled into small balls and were flattened similar to the chapattis. Then heated and roasted on slow heat and pressed via wooden press, till it become crispy and light brown in color.

**Preparation of Experimental Khakhra:** Experimental Khakhra was prepared by incorporating amaranth seed, finger millet, oats, soya bean, flax seed, jambu seed, kadijiri, salt and spices in powdered form in different proportion to the control khakhra.
Studying organoleptic characteristics of Functional Snack (Khakhra):

Control and experimental khakhra was subjected to sensory evaluation using Composite Scoring Test. The organoleptic qualities studied were appearance, texture, flavor and overall acceptability. The experimental khakhra containing highest overall acceptability was chosen for feeding trial.

Inclusion criteria:

Generally healthy males, age 40 to 70 years diagnosed type II diabetics in the last 5 years on medication, males having blood pressure with diabetes were also included.

Exclusion criteria:

Subjects were excluded if they had food allergy or sensitivity to study product ingredients, current or resent history of drug chemical or alcohol abuse.

Statistical Analysis:

The observations obtained were compared using paired ‘t’ test. One way ANOVA for sensory score was carried out using SPSS (Version 20).

Results and discussions

The results obtained are categorized into three major parts:

Sensory evaluation of control and experimental functional snack “khakhra” as well as evaluation of colour and texture attributes, Chemical analysis, Feeding trial.

Part 1:- Sensory Evaluation

Table 1 shows the mean value of various sensory attributes of control and experimental khakhra.

Sensory evaluation

When the quality of food product is assessed by means of human sensory organs, the evaluation is said to be sensory. Composite scoring test was carried out for acceptability of khakhra. Three samples were served to the panelist. Plate – 1 shows the appearance of control and experimental khakhra.

The mean score of appearance ranged from 7.69 to 8.80. The highest score was observed in control khakhra (8.80) which showed a significant difference (p ≤ 0.05) with experimental khakhra I (7.65) and experimental khakhra II (7.69). No significant difference was observed in the appearance score of experimental khakhra I and II. The mean score of colour ranged from 7.61 to 8.85. Control khakhra that contained wheat flour had significantly
(p ≤ 0.05) higher score (8.85) followed by experimental khakhra II (7.69) and khakhra I (7.61). Experimental khakhra I and II did not differ significantly in their colour score. The mean score of texture ranged from 17.20 to 18.48. The highest texture score was observed in control khakhra (18.48) followed by experimental khakhra I (17.20) and II (17.48). A significant difference (p ≤ 0.05) was observed in the texture score of control and experimental khakhra. No significant difference was observed between experimental khakhra I and II in the texture score. A score of flavour 8.26, 7.67 and 7.40 was observed in control, experimental I and experimental II khakhra respectively. Control and experimental khakhra differ significantly (p ≤ 0.05) while experimental khakhra I and II did not differ significantly (p ≤ 0.05) in their flavour score. The mean score of taste ranged from 16.15 to 18.22. The highest score was observed in control khakhra (18.22) which showed a significant difference (p ≤ 0.05) with experimental khakhra I (16.25) and II (16.15). No significant difference was observed between experimental khakhra I and II. Control khakhra had significantly (p ≤ 0.05) higher score (18.19) compared to experimental khakhra II (17.07) and non significantly higher than experimental khakhra I (17.68) in the overall acceptability. No significant difference was observed in the overall acceptability score of experimental khakhra I and II.

Table no. 1: Sensory score of control and experimental khakhra

<table>
<thead>
<tr>
<th></th>
<th>Appearance</th>
<th>Colour</th>
<th>Texture</th>
<th>Flavour</th>
<th>Taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Khakhra</td>
<td>8.80(^b)</td>
<td>8.85(^b)</td>
<td>18.48(^b)</td>
<td>8.26(^b)</td>
<td>18.22(^b)</td>
<td>18.19(^b)</td>
</tr>
<tr>
<td></td>
<td>± 0.78</td>
<td>± 0.89</td>
<td>± 1.33</td>
<td>± 1.08</td>
<td>± 1.34</td>
<td>± 1.41</td>
</tr>
<tr>
<td>Experimental khakhra – I</td>
<td>7.65(^a)</td>
<td>7.61(^a)</td>
<td>17.20(^a)</td>
<td>7.67(^a)</td>
<td>16.25(^a)</td>
<td>17.68(^ab)</td>
</tr>
<tr>
<td></td>
<td>± 1.25</td>
<td>± 1.17</td>
<td>± 2.40</td>
<td>± 1.30</td>
<td>± 3.47</td>
<td>± 1.14</td>
</tr>
<tr>
<td>Experimental khakhra – II</td>
<td>7.69(^a)</td>
<td>7.69(^a)</td>
<td>17.48(^a)</td>
<td>7.40(^a)</td>
<td>16.15(^a)</td>
<td>17.07(^a)</td>
</tr>
<tr>
<td></td>
<td>± 1.19</td>
<td>± 1.06</td>
<td>± 1.88</td>
<td>± 1.18</td>
<td>± 2.90</td>
<td>± 2.02</td>
</tr>
<tr>
<td>F – value</td>
<td>16.30 *</td>
<td>19.94 *</td>
<td>5.63 *</td>
<td>6.18 *</td>
<td>8.46 *</td>
<td>5.84 *</td>
</tr>
</tbody>
</table>

Values are Mean ± SD score of a composite scoring test by a panel of 12 judges x 3 replications

* Indicates significant difference (p ≤ 0.05)

Mean values with the different superscripts within the column differ significantly (p ≤ 0.05)
The above data indicated that the panel members preferred the control khakhra containing 100% wheat flour compared to the experimental khakhra. Among the experimental khakhra highest preference was given to the experimental khakhra I than II. The panel members rated less to the experimental khakhra because of the bitter taste of certain ingredients like kadijiri and jambu seed powder incorporated to it.

Part 2: Chemical Analysis
Table 2 shows the mean value of α-Amylase inhibitory activity of control and experimental khakhra.

The experimental khakhra showed significantly higher \( (p \leq 0.05) \) α-amylase inhibitory activity (9.09 % inhibition) compared to control khakhra (1.43 % inhibition). A percentage increase of 535.66 was found in the α-amylase inhibition of experimental khakhra as compared to control khakhra.

Experimental khakhra had higher α-amylase inhibitory activity compared to the control sample. This may be due to presence of various ingredients in experimental khakhra that
contain α-amylase inhibitory activity as evident from the studies of various researchers. Ani et al [11] reported that the inhibitory activity of the C. anthelminticum (kalijiri) extract against human salivary α-amylase was 185.5 μg in rats. The inhibitory components of the extract were determined as a mixture of polyphenolic compounds such as, gallic acid, protocatechuic acid, caffeic acid, ellagic acid, ferulic acid, quercetin and kaempferol. They suggested that the C. anthelminticum exhibit antihyperglycemic effect by reducing postprandial glucose in rats through the modulation of α-amylase and glucosidases (sucrase and maltase) activity.

Table no. 2: α-amylase inhibitory activity of control and experimental khakhra I

<table>
<thead>
<tr>
<th></th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>± 0.84</td>
</tr>
<tr>
<td>Experiment</td>
<td>9.09</td>
</tr>
<tr>
<td></td>
<td>± 1.01</td>
</tr>
</tbody>
</table>

T – value -7.16 *

Values are Mean ± SD of three observations

* Indicates significant difference (p ≤ 0.05)

Part 3: Feeding Trial

Table 3 shows the mean values of initial and final levels of glucose, HbA1C of subjects fed functional snack khakhra I

Glucose:

The initial glucose level was found to be 180.95 mg/dl which significantly (p ≤ 0.05) decreased to 147.93 mg/dl after 90 days feeding of khakhra. The percentage decrease in the glucose levels was found to be 18.24 after feeding the khakhra.

In the present study a significant reduction in the blood glucose level was observed after supplementation of khakhra for a period of 3 months to males suffering from type-II diabetes. This may be due to several hypoglycemic foods incorporated in experimental
khakhra. One of the several ingredients incorporated in khakhra was soyabean. Amer [12] reported a significant decrease in blood glucose concentration in soyabean treated diabetic rats. Further soyabean has been shown to promote serum insulin production [13].

Table no. 3: Glucose and HbA1c level of subjects (n=22) fed Functional Snack khakhra I

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial level</th>
<th>Final level</th>
<th>T – value</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUCOSE (mg/dl)</td>
<td>180.95 ± 68.40</td>
<td>147.93 ± 57.26</td>
<td>4.19 *</td>
<td>70-110</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.31 ± 1.66</td>
<td>8.21 ± 1.59</td>
<td>0.61</td>
<td>4.2-5.8</td>
</tr>
</tbody>
</table>

Values are Mean ± SD

* Indicates significant difference (p ≤ 0.05)

HbA1c:

The initial level of HbA1c was found to be 8.31 % which was decreased non significantly (p ≤ 0.05) to 8.21 % after 90 days feeding of khakhra. The percentage in the HbA1c levels was found to be 1.20 after feeding the khakhra. As mentioned earlier the HbA1c is a marker of blood glucose for past 90 days. In the present study the khakhra supplementation for 3 months significantly reduced the blood glucose concentration which ultimately had lessened the HbA1c of the subjects. Barre et al [14] reported in human study that HbA1c dropped down due to presence of flaxseed lignan complex. Pan et al [15] also found in their study that 12-week supplementation of a flaxseed-derived lignan complex, which provide 360 mg/day SDG (secoisolariciresinol diglucoside), statistically reduced HbA1c concentrations by 0.10% in type II diabetic patients.

Table 4 shows the mean values of initial and final levels of glutathione, FRAP and ascorbic acid of subject fed functional snack khakhra I.

Glutathione:

The initial level of whole blood glutathione was found to be 32.60 mg/dl which non significantly (p ≤ 0.05) increased to 33.07 mg/dl after 90 days feeding of khakhra. A percentage increase in the whole blood glutathione level was found to be 1.44 after feeding
the khakhra. Ramchandran et al [16] reported an increase in glutathione levels from 108.68 to 128.46 after supplementation of ragi plus soyabeanc.

Table no. 4: Glutathione, TAC (using FRAP assay) and vitamin-C level of subjects (n=22) fed functional snack khakhra

<table>
<thead>
<tr>
<th></th>
<th>Initial level</th>
<th>Final level</th>
<th>T – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione</td>
<td>32.60</td>
<td>33.07</td>
<td>-0.11</td>
</tr>
<tr>
<td>(mg %)</td>
<td>± 16.88</td>
<td>± 9.62</td>
<td></td>
</tr>
<tr>
<td>Vitamin – C</td>
<td>3.57</td>
<td>1.29</td>
<td>5.46</td>
</tr>
<tr>
<td>(mg %)</td>
<td>± 1.93</td>
<td>± 0.46</td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>25.17</td>
<td>30.15</td>
<td>-4.57</td>
</tr>
<tr>
<td>(mg %)</td>
<td>± 8.30</td>
<td>± 6.70</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean ± SD

**FRAP:**

The initial level of serum total antioxidant capacity was found to be 25.17 mg TE/100 ml which non significantly (p ≤ 0.05) increased to 30.15 mg TE/100 ml after 90 days feeding of khakhra. A percentage increase in the serum total antioxidant capacity was found to be 19.78 after feeding the khakhra.

**Ascorbic acid:**

The initial level of serum ascorbic acid was found to be 3.57 mg % which non significantly (p ≤ 0.05) decreased to 1.29 mg % after 90 days feeding of functional snack khakhra. A percentage increase in the serum total antioxidant capacity was found to be 63.87 after feeding the functional snack khakhra.
Conclusion

In conclusion the results revealed that khakhra containing wheat, ragi, amaranth seed, oats, flaxseed, soyabean, kadijiri and jambu seed powder proved to significantly reduce blood glucose as well as HbA1c level by supplementation to male diabetic patients for 90 days. Thus the functional khakhra could be an effective combination to improve blood glucose level and antioxidant status of diabetic subjects with type II diabetic mellitus.

Acknowledgment

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References


