HYPOGLYCEMIC EFFECT OF DIETARY FIBER FEEDS MADE FROM DIFFERENT VARIETIES OF MILLET ON STREPTOZOTOCIN INDUCED DIABETIC RATS

A. Hamid and J. Hussain [*]

College of Home Economics Lahore, Pakistan
[*]School of Nutrition and Dietetics, University of Faisalabad
Corresponding author's Email: asmaabilal2001@yahoo.com

ABSTRACT: The ground samples of Pearl millet, Finger millet, Foxtail millet and Proso millet were evaluated biochemically and following nutrients were determined i.e. moisture, ash, protein, fat, crude fiber, lignin and cellulose. The fiber feeds were prepared with some necessary alterations to the Basic Purified Diet 93-G for rats, recommended by American Institute of Nutrition (AIN). The rats (n=36) were divided equally into 6 groups. There were two control groups of rats comprising of normal rats and Streptozotocin induced diabetic rats which were fed on fed on Basic Purified Diet AIN-93-G, while the 4 other groups were fed on Dietary Fiber Feeds (DFF) prepared with different types of millet. All of these groups were monitored for random blood glucose (mg/dl) for a six-week time period.It was found that both Pearl Millet and Finger Millet had a significant effect on the lowering the random blood glucose (RBS) level in experimental animals. However, Finger Millet proved to have a significantly ($p<0.05$) greater effect on lowering RBS than Pearl Millet.

Key words: Dietary fiber feed, Millet, Random blood sugar, Streptozotocin induced diabetes.

(Received 28-12-2016 Accepted 31-07-2017).

INTRODUCTION

The cereal crop Millet belongs to the grass family, Graminae. “Millet” is a generic term used for a number of small-seeded annual grasses belonging to species under the five genera in the tribe Paniceae, namely Panicum, Setaria, Echinochloa, Pennisetum and Paspalum, and one genus, Eleusine, in the tribe Chlorideae (Karuppasamy, 2015) . There are various genera of Millet that are grown in tropical and subtropical areas across the world. Millet is consumed as a staple food by a large human population across Asian and African countries and some parts of Europe (Nuss and Tanumihardjo, 2011). Millet can roughly be divided into four different types i.e. Pearl Millet (*Pennisetum glaucum*),Finger Millet,(*Eleusine coracana*), Foxtail Millet (*Setaria italic*) and Proso Millet (*Panicum miliaceum*).

The local name of Pearl Millet is bajra, Finger Millet is ragi, Foxtail Millet is kangni and Proso Millet is cheena or moti kangani. Pakistan and India have a long history of serving these varieties of millet as a staple food and as delicacies. The Pearl Millet flour is mixed with wheat flour and is used to make a flat bread (bajaray kee rotti). Other than bread (rotti) a pudding (bajaray ki kheer) with milk is also made (Jha *et al.*, 2013). Finger Millet or Raagi has been cultivated and consumed in Indian subcontinent since centuries. Finger Millet is also eaten in the form of porridge known as Ragi kee kheer in some rural areas (Maqsood and Ali, 2007). Similarly, both Foxtail Millet and Proso Millet are ground coarsely and their flour is used mixed with wheat flour to make bread and kheer.

Due to the rapid shift in the dietary patterns and overall change in lifestyle of the rural population metabolic disorders like diabetes mellitus, cardiovascular diseases and obesity have increased over the years (Popkin *et al*., 2012). There is an impending need to investigate the possible benefits in these varieties of millet and other whole grains in regards to lowering the blood glucose level. Some studies have indicated that different varieties of millet are known to have a broad spectrum of health benefits such as anti-diabetic, anti-tumerogenic and atherosclerogenic agent (Sireesha *et al*., 2011 and Devi *et al.*, 2014). .

The present study was to investigate the effect of the dietary fiber contained in different millet variety feeds on the blood glucose level of streptozotocin induced diabetic albino rates.

MATERIALS AND METHODS

Sample Collection: The locally produced varieties of millet including Pearl Millet (Barani Bajra), Finger Millet (TZA-01), Foxtail Millet (C-47), Proso Millet (Moti Cheena) were obtained from Akbari Mandi (local seed market) Lahore. The seeds of millet were manually cleaned to remove foreign materials and later ground into coarse flour.

Proximate Analysis: Proximate composition of all the variety samples was carried out according to the AOAC methods (2005). The crude dietary fiber was analyzed by Weende Method (Van and Mc Queen, 1973). Cellulose was analyzed by Kurschner and Hank method (1930) while lignin was analyzed by the method of Calixto *et al.* (1983).

Formulation of Basic Purified Diet AIN-93-G and Millet Dietary Fiber Feeds: Basic Purified Diet (AIN-93-G) was used as a basal diet, whereas four experimental Dietary Fiber Feeds, i.e. Pearl Millet, Finger Millet, Foxtail Millet and Proso Millet were prepared by increasing the ratio of dietary fiber content of the Basic Purified Diet -93-G **(Table 4)**. The crude dietary fiber contents were increased from 50gm/kg to 252gm/kg (Reeves, 1997).

The coarsely ground seeds were sieved through 200-300 mesh to obtain the outer crust, the main source of crude fiber (Anonymous, 1989).

The feed ingredient were mixed and kneaded with distilled water to obtain the appropriate consistency for making pellets. The pellets were oven-dried on low heat (50°C) and stored at a cool and dark place until further use.

Induction of Diabetes: The rats were allowed to fast for 12 hours before diabetes was induced in them with a single dose of intraperitoneal injection of Streptozotocin (Abeeleh *et al*., 2009). The STZ dose was calculated as 40 mg/kg and was dissolved in 100 ml citrate buffer with a pH of 4.5 as reported by Yu *et al*., 2012 and Sharma *et al*., 2011.

Diabetes was confirmed in the rats on the third day of the administration of STZ using previously calibrated glucometer while blood samples were drawn through tail puncture.

Experimental Design: Thirty six (36) male white albino rats, aged at 6 to 12 months and weighing between 180gm to 220 gm were obtained from the Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories,) These animals were kept under controlled room temperature (20 - 26 °C). The feed and fresh water was offered *ad libitum* to the rats all the time. The exact amount of food and water consumed by each rat, kept individually in a hanging wire-mesh cage, was recorded regularly.

Grouping of the Rats: Completely Randomized Design (CRD) was employed for evaluating the effect of different feeds on the random blood glucose (RBG) levels of rats. The rats (n=36) were divided equally into 6 groups. Experimental design men timed in Table-1 was followed. The groups of rats were monitored for random blood glucose (mg/dl) for a six-week time period.

The various groups of rats **(Table-4)** were given no treatment for the first week (zero week) to acclimatize the rats. The different groups of rats were given the basal feed (BPD-AIN-93-G) and the four different Dietary Fiber Feeds (DFF) for a period of 6 weeks. The Mean ±SD random blood glucose values (mg/dl) in all the experimental rat groups were recorded.

Pattern of Diet Consumption: The experimental diets were prepared according to the standard formula given in **(Table-3).**Each prepared feed was weighed carefully before it was given to the animals. The left over feed obtained in the feed hoppers was also weighed to record the exact amount (g) consumed by each rat in the individual cage.

Collection and Analysis of Blood for RBG: The blood samples were drawn from the individual rat through tail prick method to estimate random blood sugar (RBG) using glucometer as above. The mean level of RBG was estimated in each rat group.

RESULT AND DISCUSSION

The mean values of various nutrients in different millet varieties have been presented in Table 2, Pearl Millet containing 9.57% moisture, 1.43 ash, 14.27% crude protein, 3.6% crude fat, 3.10% crude fiber, 1.79 lignin and 1.69% cellulose. Whereas, Finger Millet had10.34% moisture 1.93% ash 8.6% crude protein, 1.8% crude fat, 4.10% crude fiber, 2.20% lignin and 1.94% cellulose. Foxtail Millet exhibited the nutrient composition as moisture 8.99%, ash 2.01%, crude protein 9.98%, crude fat 1.38%, crude fiber 3.99%, lignin 1.76% and cellulose 1.75%.

While, Proso Millet had moisture 11.23%, ash 1.29%, crude protein 12.6%, crude fat 2.7%, crude fiber 3.99%, lignin 1.02% and cellulose 1.01%.

Previous studies reported similar range of nutrients in these varieties of millet, however there were some differences as well, which could be due to the difference in climatic and soil conditions (Choudhury *et al*. 2011; Saleh *et al*. 2013 and Dharmaraj *et al.* 2016)

Table-4 shows the week wise comparison of the changes in random blood glucose (RBG) level of the three diabetic groups as determined by ANOVA technique.

Group-I (normal group) shows non- significant change ($p>0.05$) in blood glucose level over the entire period of the experiment.

However, a significant ($p<0.05$) increase in the mean RBG value in Group II (Diabetic Group on BPD-AIN-93-G) was recorded in the first week after the induction of diabetes to be from 100.55±12.15 mg/dl to 273.33±12.22 mg/dl, which increased in the blood glucose level in the group which remained on the higher side almost throughout the experimental period of 6 weeks. However, a significant decrease ($p<0.05$) in the RBG level was noted in the 2^{nd} and 3^{rd} week (from 252.23±36.61 mg/dl to 240.26±15.12 mg/dl). The mean RBG level increased significantly towards the end of 4th week i.e. from 240.26±15.12 mg/dl to 258.12±12.3 mg/dl. In 5th week again there was a slight decrease ($p>0.05$) in the mean RBG level in this group to be from 258.12±12.3 mg/dl to 253.03±13.22mg/dl. Once again there was a significant increase in the RBG of Group II in the 6th i.e. from to 253.03±13.22mg/dl to 270.00±24.22 mg/dl. The blood glucose level in Diabetic Group III on Pearl Millet DFF, after the induction of diabetes in 1st week showed a great increase ($p<0.01$) in the blood glucose level as compared to the zero week (100.46±12.46 to 248.33±15.95). However, a signnificant decrease in blood glucose level was recorded during the 2nd and 3rd week i.e 235.15±18.62 mg/dl and 222.33±12.80 mg/dl, respectively. However, no significant change ($p>0.05$) in the blood gluocse level in that group was observed (220.32±12.00 mg/dl, 222.42±6.21 mg/dl and 219.13±19.45 mg/dl) in the 4th, 5th and 6th weeks of experimental period.

The Group IV (Diabetic Group on Finger Millet DFF) showed a significant ($p<0.05$) increase in the blood glucose level in the 1st week after the induction of diabetes (105.50±13.99 to 252.00±9.71). In 2nd and 3rd week, however, a significant ($p<0.05$) decrease in the values was observed i.e. 221.66±4.71 mg/dl to 203.16±15.36 mg/dl. A slight increas in the mean value of blood glucose level of this group occured in the 4th week i.e. from 203.16±15.36 mg/dl to 208.66±8.75 mg/dl. However, in the 5th week a significant increase ($p<0.05$) in the mean value of RBG level in that group (208.66±8.75 mg/dl to 212.00±10.41 mg/dl persisted at almost the same level till the 6th week, i.e. 214.83±19.51.

Group V (STZ- induced diabetic rat group reared on Foxtail millet DFF) exhibited a significant increase ($p<0.01$) in the RBG level as recorded after the induction of diabetes (99.88±21.44 mg/dl to 259.11±10.22mg/dl). By the end of the 2nd week a significant ($p<0.05$) decrease in the RBG level in that group was observed (259.11±10.22 to 238.23±21). In the 3rd week a slight increase ($p>0.05$) in the RBG level in that group was recorded (238.23±21 to 245.22±5.1.22). However, by the end of the 4th week there was a significant ($p<0.05$) decrease in the RBG level in that group (245.22±5.1.22 to 218.33±9.22) that went on unabated in the 5th (200.11±13.90) and during 6th week (198.12±19.22), though non-significantly ($p>0.05$).

There was a significant increase ($p<0.01$) in the mean blood glucose level of rats in Group VI (STZ induced diabetic rats fed on Proso Millet FF) during the 1st week of the induction of diabetes (103.15±9.32mg/dl to 257.67±17.47 mg/dl). However, a significant decrease ($p<0.01$) in the mean RBG level was recorded in the 2nd week (257.67±17.47 to mg/dl to 230.80±10.10 mg/dl). Similarly, there was a significant ($p<0.05$) decrease in the mean value of blood glucose level during the 3rd week as well (230.80±10.10mg/dl to 215.35±15.14 mg/dl). However, the mean RBG level of rats in that group increased through 4th and 6th week of the experimental period, though non-significantly, from 220.22±9.50 to 224.42±11.33 mg/dl.

It is observed in previous studies that dietary fibers from various whole grain cereals and vegetables were found beneficial in lowering random blood glucose levels in diabetic patients (Moharib and El-Batran, 2008; Díez *et al*. 2013). Whole grain cereals are the chief source of dietary fiber and a large percentage of rural population in Pakistan still consumes various varieties of whole grain millet flour along with other cereal grains (Jha *et al.*, 2013). Therefore it is very important to probe into the possible health benefits of these indigenously grown varieties of millet so that that the same can be used for therapeutic purpose especially for diabetes mellitus which is a very common metabolic disorder in Pakistan (WHO, 2014).

The present study showed very encouraging responses in the experimental animals where high fiber feed prepared from various varieties of millet had a significant glucose lowering effect. Choi *et al*. (2005) reported that Foxtail millet had (70%) decrease in serum glucose level in experimental rats. Likewise A significant decrease in fasting blood glucose, a significant improvement in glycemic index and lower levels of HbA1c in diabetic rats was also noted in another study carried out by (Sireesha *et al*., 2011). These results are inline to the present study as Foxtail millet FF proved to be the most effective feed in lowering the random blood glucose level (23.5%).

Similarly Thathola *et al.* (2011) and Ugare *et al.* (2014) recorded the effect of millet supplementation on serum glucose and glycosylated hemoglobin in type 2 diabetics. It was noted that diabetic patients who consumed millet biscuits had a marked improvement in the random blood glucose levels. These results are similar to the findings of this study as the high fiber feed prepared from Finger millet, Pearl millet and Proso millet dietary fiber also lowered the random blood glucose levels by 15%, 11% and 12% respectively.

It was noted in the present study that diabetic rats showed a positive response in lowering of the random blood glucose level after consuming these millet high fiber feeds for a period of six weeks. It can be inferred from the above results that these various varieties of millet dietary fiber can be used for intervention in diabetic patients on a long term basis.

Table-1. Groups of Diabetic and Normal Rats on Basic Purified Diet AIN-93-G, Pearl Millet FF, Finger Millet FF, Foxtail Millet FF & Pro Millet FF.

Groups	Animal Blood Profile	Treatments
I	Normal rats	Basic Purified Diet AIN-93-G
II	Streptozotocin induced Diabetic rats (blood glucose > 200mg/dl)	Basic Purified Diet AIN-93-G
III	Streptozotocin induced Diabetic rats (blood glucose > 200mg/dl)	Pearl Millet FF (50%)
IV	Streptozotocin induced Diabetic rats (blood glucose > 200mg/dl)	Finger Millet FF (50%)
V	Streptozotocin induced Diabetic rats (blood glucose > 200mg/dl)	Foxtail Millet FF (50%)
VI	Streptozotocin induced Diabetic rats (blood glucose > 200mg/dl)	Proso Millet FF (50%)

Table-2: Proximate composition of Different Varieties of Millet g/100 g.

	Pearl Millet	Finger Millet	Foxtail Millet	Proso Millet
Moisture	9.57±0.23	10.34±0.34	8.99±0.34	11.23±0.71
Ash	1.43±0.43	1.93±0.91	2.01±0.51	1.29±0.82
Protein	14.27±0.54	8.62±0.34	9.98±0.41	12.64±0.62
Fat	3.62±0.87	1.84±0.23	1.38±0.37	2.72±0.34
Crude Fiber	3.10±0.22	4.10±0.51	3.99±0.62	3.99±0.76
Cellulose	1.69±0.53	1.94±0.23	1.75±0.72	1.01±0.23
Lignin	1.79±0.41	2.20±0.83	1.76±0.43	1.02±0.77

Table-3: Composition (% gm/kg) Purified Diet AIN-93-G, Pearl Millet DFF, Finger Millet DFF, Foxtail Millet & Proso Millet DFF

	Cornstarch	Casein (85% protein)	Dextrinized cornstarch	Sucrose	corn oil	Crude fiber/ bran	Mineral mix	Vitamin mix	L- Cystine	Choline bitartrate	TBQ
Purified Diet AIN-9-G	397.486	200.000	132.000	100.00	72.00	50.000	35.000	10.000	3.00	2.500	0.014
Pearl Millet FF	195.000	200.500	130.000	100.00	72.00	252.00	35.000	10.000	3.00	2.500	0.014
Finger Millet FF	195.000	200.500	130.000	100.00	72.00	252.00	35.000	10.000	3.00	2.500	0.014
Foxtail Millet FF	195.000	200.500	130.000	100.00	72.00	252.00	35.000	10.000	3.00	2.500	0.014
Proso Millet FF	195.000	200.500	130.000	100.00	72.00	252.00	35.000	10.000	3.00	2.500	0.014

Table-4: Change in Blood Glucose Level /Week of Group I, Group II , Group II, Group IV, Group V & Group VI (Mean ±SD).

	Blood Glucose Level (mg/dl) in different weeks after induction of diabetes						
	Zero	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
Group I Normal BPD- AIN- 93-G	101.50±12.00	99.33±14.58	108.33±10.74	102.66±15.27	106.66±11.94	101.50±8.93	99.66±13.35
Group II Diabetic BPD- AIN- 93-G	100.55±12.15[a]	273.33±12.22[b]	252.23±36.61[c]	240.26±15.12 [d]	258.12±12.3 [eh]	253.03±13.22[fh]	270.00±24.22 [gh]
Group III Diabetic Expt. Pearl Millet FF	100.46±12.46[a]	248.33±15.95[b]	235.15±18.62	222.33±12.80	220.32±12.00	222.42±6.21	219.13±19.45
Group IV Diabetic Finger Millet FF	105.50±13.99[a]	252.00±9.71[b]	221.66±4.71[c]	203.16±15.36[dh]	208.66±8.75[eh]	212.00±10.41[fi]	214.83±19.51[gi]
Group V Diabetic Foxtail Millet FF	99.88±21.44[a]	259.11±10,22[bh]	238.23±21[ci]	245.22±5.1.22[di]	218.33±9.22[ej]	200.11±13.90[fj]	198.12±19.22[gj]
Group VI Diabetic Proso Millet FF	103.16±9.33[a]	257.76±17.47[b]	230.80±10.10[ch]	215.35±15.14[di]	220.22±9.50[ehij]	222.03±13.19[fij]	224.42±11.33[gij]

Conclusion: The nutritional evaluation of four different varieties of millet showed that Proso millet had highest moisture content, highest percentage of protein and fat was found in Pearl millet. The highest percentage of crude dietary fiber along with cellulose and lignin was found in finger millet. However when the rat feed prepared from the different varieties of millet were fed to the Streptozotocin induced diabetic rats, Foxtail millet FF proved to be the most effective feed in lowering the random blood glucose level. Finger millet FF was the second most effective millet variety to lower the blood glucose level in the experimental animals, whereas Pearl millet FF and Proso millet FF had almost the same results. In the light of the results of the present study it can be said that all the above mentioned varieties if incorporated in the daily diet of the diabetic patients may prove beneficial in lowering the random blood glucose level of the patients.

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