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EFFECTS OF WHEAT BRAN DIET AND MAIZE BRAN DIET ON THE RANDOM BLOOD GLUCOSE AND WEIGHT OF ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study was conducted to evaluate the effect of wheat and maize brans feeds on the random blood glucose of the rats. These feeds were prepared using the the Purified Diet AIN-93-G with some modifications. The experiment was laid out under completely randomized design. The rats (n=24) of almost uniform age and weight were divided into 4 groups each containing 6 rats. Group I comprised rats with normal blood glucose level reared on basal diet (AIN-93-G), Group II was Alloxan monohydrate (ALX monohydrate) induced diabetic rats reared on Purified Diet AIN-93G. Group III comprised Alloxan monohydrate induced diabetic rats fed a wheat bran diet and Group IV consisted of Alloxan monohydrate induced diabetic rats reared on a maize bran diet. The Random Blood Glucose (mg/dL) of these groups was monitored at the end of each week for a period of 6 weeks. Bran diets (maize and wheat) significantly lowered the Random Blood Glucose level in the experimental animals. However,the group of rats fed on maize bran diet had a significantly ($p<0.05$) more pronounced glucose lowering as compared to the group of rats fed on wheat bran diet. The results indicated that both wheat and maize bran exert anti-diabetic effects on the ALX monohydrate induced diabetes and therefore, can be a part of diet based therapy for the management of diabetes.

Key words: Wheat Bran, Maize Bran, random blood glucose, Purified Diet (PD AIN-93G).

INTRODUCTION

Wheat (*Triticum aestivum*) is the largest food crop used around the world and about 60% of the total caloric intake of Pakistani population comprises of wheat (Safdar *et al.,* 2009). The wheat grain contains 2-3% germ, 13-17% huskbran and 80-85% mealy endosperm before the removal of the husk (Belderok *et al.,* 2000), once the husk is removed the percentage of crude dietary fiber is 1·7 to 3.8% (Slavin, 2004; Stewart and Schroeder, 2013).

Although wheat bran is of very unique nutritional significance however, most of it is removed during milling. Nutrients like proteins and carbohydrates found scarcely in wheat bran, whereas it is rich in mineral content which is around 7.2% (Cornell, 2003). The two basic types of proteins present in the wheat grain storage protein (GSP) namely of gluten and gliadins. (Šramková *et al.,* 2009 and Jones, *et al.*2013).

Maize (*Zea mays) is* the third most important crop in order of cultivation after wheat and rice in Pakistan. The maize grain has a low protein contents and the quality of protein in maize is also poor due to the deficiency of some essential amino acids, mainly lysine (Hunt *et al.,* 1989). The nutritional composition of maize is as following crude protein 8.8% to 11.1%, and crude fat 4.7% to 6.5% and crude dietary fiber 3.3% to 5.4%**.**% (Pan *et al*., 1996 and Nascimento, *et al*., 2014).

In past few years the incidence of diabetes mellitus has increased many fold in Pakistan (5.1% in men and 6.8% in women in urban areas and 5.0% in men and 4.8% in women in rural areas). IGT in the urban versus the rural areas was 6.3% in men and 14.2% in women against 6.9% in men and 10.9% in women, respectively (Shera, *et al*., 2007). Many researchers have discussed the potential effect of different varieties of wheat and maize on physiological and metabolic functions such as hypoglycemia and weight reduction. Ou *et al*., (2001) studied the role of dietary fiber in lowering postprandial serum glucose. Similarly the hypoglycemic effect of maize dietary fiber has been a point of interest. Kendall et.al (2008) studied the effect of soluble maize fiber in the form of a drink on the total cholesterol level and on postprandial glycemic response of human subjects and reported that maize bran can reduce the glucose level in diabetic patients.

Hence there is a dire need to probe into the possible benefits of dietary fiber in lowering the blood glucose levels from the local perspective. Since both wheat and maize are widely used cereal grains in the urban and rural areas of Pakistan, the present study aimed to evaluate the effect of indigenous varieties of wheat and maize brans on blood glucose level and weight of normal and Alloxan monohydrate induced diabetic rats.

MATERIALS AND METHODS

Whole grain Wheat FD08 and Maize 30Y87 are local varieties of cereals procured from the Punjab Seed Corporation were nutritionally evaluated through proximate analysis (AOAC, 2005) in the present study.

Formulation of the Purified Diet AIN-93 G and High Fiber Diet: Basal Diet was made according to Reevees *et al.* (1993) the Purified Diet for Rats (PD-AIN-93G) (Table 1). To prepare the rat feeds, wheat and maize were coarsely ground to obtain the outer crust which is the main source of crude bran. The bran was separated by sieving the flour of seeds using a sieve of 200-300 pore size. The experimental Wheat Bran Feeds and Maize Bran Feed were made by increasing the ratio of dietary fiber content of the PD-AIN-93G (Table 2).

The experiment was laid out under Completely Randomized Design (CRD). White Albino Rats n= 24, ages between 12-14 weeks, weight 200-230 g were obtained from the animal house of PCSIR Laboratory, Lahore. These animals were randomly divided into 4 groups containing 6 rats each. The animals were housed in separate cages, so that exact amount of food consumed could be recorded (Table 3)

Diabetes was induced in Group II, Group III and IV with ALX monohydrate. Diabetes was induced after 12 hours of fasting (Kumar *et al*., 2012). Alloxan monohydrate was prepared in 0.9% saline solution and was administered to the rats in a single shot (70mg/kg) and was given to individual rat according to the calculation of body weight (Orsolic *et al*., 2011). The Alloxan monohydrate was administered intravenously at the coccygeal lateral vein of the rat (Thorington 1966; Young and Dawson 1982)

The rats were weighed regularly at the end of each week. The weighing was done before the withdrawal of blood for the RBG (Random Blood Glucose) estimation.

The weighed quatity of feed was offered to the rats in feed hoppers daily and the refusal was measured to determine the exact amount of feed consumed by each rat housed in a separate cage. The average feed intake was 10-15g/day/ rat.

The functional and metabolic effects of Purified Diet (AIN-93-G) and two Bran Feeds made from Wheat and Maize were used to determine the effect on blood glucose in the rats. Serum glucose was determined by enzymatic procedure (Trinder, 1969) and insulin was assayed by an immunoenzymatic method with the help of commercial kits (Enzyme test insulin, Boehringer Mannheim, Mannheim, FRG)

Statistical Analysis: Data on all the parameters studied were presented as descriptive statistics (percentage mean and standard deviations). Analysis of variance with repeated measures was used to compare the changes in RBG level and weekly weight of each group. Paired comparison post-hoc LSD test was used to compare the four groups within each week for all the six weeks of the experiment. For significant ($P \leq 0.05$) post-hoc analyses were carried out using Tukey Kramer test to compute pair-wise differences in the means.

RESULTS AND DISCUSSION

The results of proximate analysis AOAC (2005) of indigenous variety of wheat FD-08 and Maize are presented in Table 4. Wheat grains contained moisture 8.73%, ash 1.50%, crude protein 12.58%, fat 2.42% and crude dietary fiber 2.33%. In addition to these basic nutrients cellulose and lignin were found to be 1.91% and 1.79% respectively. The proximate analysis of Maize showed nutrients contents as moisture: 11.15%, ash 1.54%, crude protein, 11.92%, fat 4.31, crude dietary fiber 3.40%, cellulose 1.13% and lignin 2.17. As can be seen in Table 4 there was no significant ($p \leq 0.05$) difference in the Ash content of both wheat and maize (1.50% and 1.54%). Similarly, no significant difference was found in the percentage of protein in wheat and maize (12.58% and 11.92%). However the moisture (8.73 and11.15) and fat percentages (2.42 and 4.31) of wheat were significantly ($P \leq 0.05$) lower than maize. On the contrary, the overall percentage of dietary fiber was higher in maize (3.98) than wheat (2.33). Likewise the percentage of lignin in maize was higher than wheat (2.17and 1.79) cellulose (2.91 and 1.13).

The results of the proximate analysis of the local variety of wheat (FD08) are similar to the already reported studies of Randhawa *et al*. (2002), Ikhtiar and Alam (2007), Safdar *et al*. (2009) , Hussain *et al* (2010) and Satter *et al* (2013). They reported these nutrients in the range of 7.68 to 9.32% moisture, 1.32 to 1.72% ash, 2.15 to 2.55% fat, 1.72 to 1.85% dietary fibers and 11.82 to 14.10% crude protein %. This variety of wheat is usually used to for the production of whole wheat or refined flour locally.

The results of the proimate analysis ofindigenous variety of maize (30Y87) were i.e.crude protein 8.8% to 10.1%, crude fat7.7% to 8.5%, starch 68.4% to 68.9% and crude dietary fiber 3.3% to 3.5 and these results are similar to previous studied (Pan *et al*., 1996 and Nascimento, *et al*., 2014).

The results of the Repeated Measure ANOVA technique used to analyze the week wise comparison in the changes in Random Blood Glucose level of all the four groups are presented in Table 5. The Normal Group on PD AIN-93G showed no significant change in blood glucose level over the entire period of the experiment. The Diabetic Group on PD AIN-93G showed a significant increase in the blood glucose level in the first week after the Alloxan monohydrate induced diabetes (from 100.66±15.55 mg/dL to 264.33±19.52 mg/dL).

This increase in the blood glucose level of this group remained on the higher side almost throughout the observation period of 6 weeks. A significant decrease was observed in the 2nd (264.33±19.52 mg/dL to 253.66±46.66 mg/dL) and in 3rd week a further significant decrease from 253.66±46.66 mg/dL to 242.16±25.49 mg/dL was recorded. But then it increased significantly by the end of 4th week there was a slight decrease in the mean RBG of this group (262.16±14.8 mg/dL). In 5th week once again there was a slight decrease in the mean RBG to 260.00±10.52 mg/dL (which was statistically non-significant). Afterwards once again there was significant increase in the Random Blood Glucose (RBG) of this group occurred in the 6th or last week i.e. from 260.00±10.52 mg/dL to 267.00±14.12 mg/dL.

The diabetic group reared on wheat BD, showed a significant change in blood glucose level over the period of six weeks (Table5). In week zero which was the period of acclimatization, the mean of blood glucose level was 108.83±8.54 mg/dL. There was a significant increase in the blood glucose level of the experimental group in the 1st week after the induction of diabetes, RBG increased to 275.50±22.04 mg/dL from 108.83±8.54. In the 2nd week the mean value of RBG decreased from 275.50±22.04 mg/dL to 268.10±24.90 mg/d. The blood glucose level during the 3rd week of the experiment remained about the same as in the 2nd week i.e. 266.50±7.89 mg/dL. In 4th week again a significant decrease (266.50±7.89 mg/dL to 254.00±14.12 mg/dL) in the mean value of RBG occurred, which persisted during the 5th week (254.00±14.12 mg/dL to 256.00±10.48 mg/dL). However in the last week there was significant increase in the mean value of RBG (256.00±10.48 mg/dL to 265.00±10.48 mg/dL.

The RBG of Diabetic Group on Maize (*Zea mays)* BD showed a significant increase in the 1st week (259.00±22.10 mg/dL) as compared to zero week (109.16±11.83 mg/dL) the period.But a significant fall in the mean value of blood glucose occured in 2nd , 3rd , 4th, and 5th weeks. During this period an overall decrease from 266.50±23.69 mg/dL to 244.83±10.40 mg/dL was observed. In the 6th week again, a decrease was recorded in the RBG (244.83±10.40 mg/dL to 238.16±9.60 mg/d)l.

In the week-wise comparison of all the four groups within each week it can be seen in Table 5 that there was no significant difference ($p \leq 0.05$) in the mean RBG of all of the four groups in week Zero, the acclimatization week, before the induction of diabetes. In the first week after the induction of diabetes by Alloxan, the RBG of Normal Group on PD AIN-93G was significantly lower than the rest of the three diabetic groups and was the same till the 6th week of the experiment; while there was no significant ($p \leq 0.05$) difference in the RBG of the three diabetic groups in this week. In the 2nd and 3rd weeks after the induction of diabetes the RBG of the Diabetic Group fed on Wheat BD was higher than the Diabetic Experimental Group on PD AIN 93-G and Diabetic Experimental Group on Maize BD. In 4th, 5th and 6th weeks the RBG of the Diabetic Experimental Group on PD AIN 93-G was significantly ($p \leq 0.05$) higher and the RBG of the Diabetic Experimental Group fed on Maize BD was lowest in all the three diabetic groups. Ou *et al*., (2001) observed the effect of dietary fiber from various sources lowerd the postprandial serum glucose in vitro. It was noted that wheat bran lowered postprandial serum glucose levels to a minimal level as compared to soluble dietary fiber from guar gum due to its viscosity and binding glucose and hence lowering the concentration of available glucose in the small intestine. The results of the present study are in line to this study as wheat bran did not reduce the blood glucose level in the Alloxan induced diabetic rats significantly.

Kendall et.al (2008) also had similar result as the present research as they studied the effects of a maize-based high fiber diet on postprandial glycemia. In clinical study in which seven test beverages containing maize-based fiber ingredients (25g total carbohydrate) were given to healthy adults with normal blood glucose and cholesterol levels. The experimental group that consumed fibers had a significantly lower glycemic and insulinemic responses compared to the control group which was not given maize fiber-based beverage

As can be seen in Table 6, the Normal Group showed a significant increase in weight up to 4th week (220.66±10.63g to 235.00±8.17 g). However there was no significant increase in the weight of this group in the 5th and 6th week (236.00±8.35 g to 237.50±9.64g). The Diabetic Group on PD AIN-93G showed a significant increase in the 1st and 2nd week of the experiment as it was increase from 225.66±9.77g to 233.00±6.72g. In the 3rd week, increase in the weight of this group was not significant (233.00±6.72g to 236.00±5.81g) but a significant weight gain occurred in the 4th from 236.00±5.81g to 240.16±5.11g. In the 5th week no change in weight was observed while a significant decrease in weight of 241.66±5.46g to 234.00±8.48g was observed in the 6th week the weight was nearly the same as the Normal group on PD AIN-93 G.

The Diabetic group on the Wheat BD showed no significant ($p \leq 0.05$) increase in weight during 1st week. The weight of this group was increased from 229.33±10.51g to 234.83±7.38 g in the 2nd week.Whereas there was no change in weight in the 3rd week. In the 4th week there was a slight decrease in the weight. While in 5th week and 6th week a decrease of 231.83±5.19 to 230.66±3.66 g was recorded but not significant.

The mean weight of the Diabetic Expt. on Maize (*Zea mays)* BD increased significantly in the 1st week (221.00±6.81g to 225.33±4.76 g). The change in the weight of this group was not significant in the 2nd, 3rd, 4th

and 5th week (227.00±4.28 g, 226.50±6.41g, 224.16±4.87 g and 224.00±5.72 g). However there was a significant decrease in the mean value of weight of this group in the 6th i.e. last week of the experiment (224.00±5.72 g to 226.00±6.38 g).

It can be seen in Table 6 that in the week-wise comparison of the all the four groups that there was no significant difference in the weight of the Normal group fed on PD-AIN-93-G, Diabetic group fed on PD-AIN-93-G , Diabetic group fed on Wheat BD and Diabetic group fed on Maize BD in Zero, 1st, 2nd weeks and 3rd weeks. In 4th week however the weight of Diabetic Expt group fed on Maize BD was significantly lower than the rest of the groups. Whereas in the 5th and 6th week the RBG of Diabetic Expt group fed on Wheat BD was significantly ($p \leq 0.05$) lower than the Normal group and Diabetic group fed on PD-AIN-93-G, while the mean RBG of the Diabetic group fed on Maize BD was significantly ($p \leq 0.05$) lower than all the other three groups. Diez *et al*. (2002) noted the changes in weight of obese dogs on a High Protein, Low Carbohydrate and moderate dietary fiber diet. It was noted in the present study that there was an increase weight of the rats fed on Maize BD and Wheat BD in the period of six weeks though it was statistically not significant. This might be due to the fact that the Bran Diets were prepared from the balanced Basic Purified Diet (AIN-93G) which is a balanced diet. The weight loss recorded in the study of Diez *et al*. (2002) might be due to a low carbohydrate and higher protein percentage and not due to the dietary fiber.

Table 1. Purified Diet AIN-93G diet formulated and used for rats and rodents

Ingredients	g/kg
Cornstarch]	397.486
Casein |>85% protein	200.000
Dextrinized cornstarch (90-94% tetrasaccharides)	132.000
Sucrose	100.00
Soybean oil// corn oil	70.00
Fiber	50.000
Mineral mix (AIN-93G-MX]	35.000
Vitamin mix (AIN-93-VX)	10.000
L- Cystine	3.000
Choline bitartrate (41.1% choline)	2.500
Tert-butylhydroquinoneone	0.014

Table 2. Wheat bran and Maize bran diets prepared after the modification of Purified Diet AIN-93G

Ingredient	g/kg
Cornstarch	97.486
Casein >85% protein	200.000
Dextrinized cornstarch (90-94% tetrasaccharides)	132.000
Sucrose	100.00
Soybean oil// corn oil	70.00
Wheat bran/ maize bran	350.000
Mineral mix (AIN-93G-MX]	35.000
Vitamin mix (AIN-93-VX)	10.000
L- Cystine	3.000
Choline bitartrate (41.1% choline)	2.500
Tert-butylhydroquinoneone	0.014

Table 3. Groups of Diabetic and Normal Rats on Nornal and Bran Diets.

Groups	Animal Blood Profile	Treatment
I	Normal (Control Group)	PD- AIN-93-G
II	Alloxan induced Diabetic rats (RBG < 200 mg/dL)	PD-AIN-93-G
III	Alloxan induced Diabetic rats (RBG < 200 mg/dL)	Wheat BD (25%)
IV	Alloxan induced Diabetic rats (RBG < 200 mg/dL)	Maize BD (25%)

Table 4. Percentage of various nutrients according to the Proximate Analysis AOAC (2005).

	Wheat FD-08	Maize 30Y87)
Moisture %	8.73±0.12	11.15±0.21
Ash%	1.50±0.18	1.54±0.20
Protein%	12.58±0.25	11.92±0.12
Fat%	2.42±0.19	4.31±0.18
Dietary Fiber%	2.33±0.10	3.40±0.24
Lignin%	1.91±0.12	2.17±0.21
Cellulose%	1.79±0.17	1.13±0.23

Table 5. Random Blood Glucose of the Rats After Induction of diabetes in different Weeks

	Zero	1st	2nd	3rd	4th	5th	6th
Normal (PD AIN-93 G)	101.50±12.00	99.33±14.58	108.33±10.74	102.66±15.27	106.66±11.94	106.50±8.93	99.66±13.35
Diabetic (PD AIN-93G)	100.66±15.55a	264.33±19.52b	253.66±46.66c	242.16±25.49d	262.16±14.83eb	260.00±10.52e	267.00±14.12f
Diabetic Expt. Wheat BD	108.83±8.54a	275.50±22.04bh	268.10±24.90ch	266.50±7.89dh	254.00±14.12ei	256.00±10.48fi	265.00±10.19gh
Diabetic Expt. Maize BD	109.16±11.83a	259.00±22.10b	266.50±23.69c	251.16±24.41d	238.66±17.60e	244.83±10.40fh	238.16±9.60gh

Mean values followed by different letter in a row are significantly different at α=0.05
BD AIN-93 G: Purified Diet AIN-93G, Wheat BD: Wheat Bran Diet, Maize BD: Maize Bran Diet

Table 6. Weight (g) of the Rats After Induction of diabetes in different Weeks

	Zero	1st	2nd	3rd	4th	5th	6th
Normal (PD AIN-93 G)	220.66±10.63a	223.33±10.53a	230.16±8.18b	232.66±8.21c	235.00±8.17eh	236.00±8.35fh	237.50±9.64gh
Diabetic (PD AIN-93 G)	219.16±11.05a	225.66±9.77b	233.00±6.72ch	236.00±5.81dh	240.16±5.11e	241.66±5.46ef	234.00±8.48gh
Diabetic Expt. Wheat BD	223.33±10.72a	229.33±10.51af	234.83±7.38bf	234.16±4.79cf	231.83±5.19cf	231.00±4.14df	230.66±3.66ef
Diabetic Expt. Maize BD	221.00±6.81ac	225.33±4.76b	227.00±4.28b	226.50±6.41b	224.16±4.87b	224.00±5.72b	226.00±6.38ch

Mean values followed by different letter in a row are significantly different at α=0.05
BD AIN-93 G: Purified; Diet AIN-93G, Wheat BD: Wheat Bran Diet, Maize BD: Maize Bran Diet.

Conclusions: There was a minimal decrease (2.5%) observed in the mean value of Random Blood Glucose (RBG) of the diabetic rats fed on Wheat Bran Diet for the period of six weeks. Based on this, the claim of local producers that wheat bran breads are beneficial for the diabetic patients does not seem to be valid. On the contrary maize Bran Diet (BD) proved to be more effective in lowering the RBG of diabetic rats as there was an overall 8.1% decrease in the mean value of RBG of the diabetic rats who consumed this feed for a period of six week. In the light of the results of the present study, it can be said that the use of maize bran is more effective that whole wheat for lowering the blood glucose level of a patient suffering from hyperglycemia; while there is no significant effect on weight loss from any other type of bran.

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