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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: [editorpjn@gmail.com](mailto:editorpjn@gmail.com)

## Nutritional Composition and Metabolic Effects of Oat Dietary Fiber Extracts on Diabetic and Hypercholesterolemic Male Rats

Asmaa Bilal<sup>1</sup>, Riffat Parveen<sup>2</sup> and Samia Kalsoom<sup>1</sup>

<sup>1</sup>Department of Food and Nutrition, Faculty of Food and Nutrition,  
College of Home Economics, Lahore, Pakistan

<sup>2</sup>Institute of Chemistry, University of the Punjab, Lahore, Pakistan

**Abstract:** The nutritional analysis of raw, ground Oat grain was conducted according to AOAC, 2005. The mean percentage value of various nutrients in Oat was following, protein (12.32%), fat (4.49%), crude fiber (6.06%), ash (3.04%) and moisture 10.01%. The lignin and cellulose content were also assessed; cellulose content being estimated as (4.11%) and lignin as (2.69%). High fiber diet (HFD) for rats was prepared using dietary fiber isolated from raw ground seeds, with some necessary alteration of the basic purified diet AIN-76 (BPD AIN-76) for rats. The rats (n = 36) were equally divided into six groups to study the effect of Oat HFD on serum glucose and serum total cholesterol (TC). Group I comprised on rats with normal blood glucose level fed on basal diet (AIN-76), Group II was Alloxan (ALX monohydrate) induced diabetic rats fed on basic purified diet AIN-76. Group III comprised of diabetic rats fed on Oat HFD. The same research design was replicated to study the cholesterol level, hence Group IV comprised normal rats on BPD AIN-76, Group V was of induced Hypercholesterolemic rats on BPD AIN-76 and Group VI consisted of induced hypercholesterolemic rats on Oat HFD. The random blood glucose level (RBGL) (mg/dL) and TC (total cholesterol) level (mg/dL) were monitored for a period of six weeks. It was observed that Oat had a significant effect on the lowering of RBS level and total cholesterol in the experimental animals.

**Key words:** High fiber diet, random blood sugar, total cholesterol, basic purified diet AIN-76

### INTRODUCTION

Oat belongs to the kingdom Plantae and its binomial name is *Avena sativa* (Zhou *et al.*, 1999). It is a member of Poaceae family and is known as 'Jai' and 'Javi' in the Indian Subcontinent. It is a self-pollinating hexaploid crop (Leggett and Thomas, 1995). Due to its rich nutritional composition it is the sixth largest crop growing in the world following wheat, maize, rice, barley and sorghum (Butt *et al.*, 2008). Oat is 'Rabi' crop, mainly harvested in temperate regions across Pakistan. It is rapidly growing crop and is used as multi-cut fodder especially for the bovine cattle (Nawaz *et al.*, 2004; Dost, 2004). It has numerous uses in foods as rolled or crushed into oatmeal, which can be consumed as porridge and may be added in bakery products such as cookies and bread (Van Horn, 1988).

The average sized grain oat on the percentage dry matter contains about 15.9% protein, 7.0% fat, 9.7% dietary fiber and 63.2% starch (Pearson, 2009). Similarly Welch and McConnell (2002) and Mariotti (2006), reported protein as 11.2 to 16% and oil content 4.8 to 9.2%. Oat also contains a liberal amount of complex carbohydrate especially beta-glucan and dietary fibers like cellulose these qualities render it nutritionally beneficial for human use.

The polysaccharide content of oat comprise of starch, lignin cellulose, hemicellulose, gums, pectins and mucilages. Lignin which is highly indigestible carbohydrate is present in an abundant amount; it is almost 3% of the total grain in some varieties whereas in others it is as low as 1% (Rowe *et al.*, 2001). Oat has a high concentration of mono-unsaturated fatty acids like palmitic acid and linoleic acid and has considerable amount of oleic acid as well (Zhou *et al.*, 1999). The North American varieties of oats contain the highest amount of protein which is around 15-20%. Protein is chiefly present in the form of globulin (Peterson and Brinegar, 1986).

Oat can prove to be nutritionally beneficial as it has a lower glycemic index which helps in balancing the glucose levels in diabetic patients thus preventing from related complications by increasing the viscosity of the content of stomach, slowing digestion and delaying the absorption of glucose in blood (Lammert, 2008). Oat beta-glucan which is a soluble dietary fiber, slows down the rate of cholesterol absorption and thus facilitate the improvement of gastrointestinal functions and glucose metabolism (Aman *et al.*, 2004; Anttila *et al.*, 2004). Beta-glucan along with insoluble fiber delays the overall absorption of cholesterol and glucose (Beer *et al.*, 1997).

Diet rich in oat dietary fiber has proved to lower the blood cholesterol and glucose levels facilitate weight loss and increase fecal mass in human subjects (Anderson *et al.*, 1984; Chandalia *et al.*, 2000). The consumption of oat has also been related with the reduced risk of developing breast cancer in post-menopausal women (Iscovich, 1989). It has also been associated to prove beneficial in Celiac disease due to the lack most of the prolamines especially gluten (Hoffenberg, 2000). Though Oat is recognized to be a beneficial food around the world, yet it is rarely used as a part of the staple diet in Pakistan and not enough research has been done on this grain. The present research aimed to find out the nutritional characteristics of the indigenous Oat and also to determine its possible beneficial metabolic effects on two of the most commonly found metabolic disorders i.e., Diabetes Mellitus and Hypercholesterolemia.

## MATERIALS AND METHODS

**Samples:** Oat seeds were purchased from The National Seed Council (NSC) Pakistan and were stored in clean air tight polythene bags in laboratory for later use.

**Treatment:** The oat grains were cleaned in order to eliminate external material, immature seeds and damaged grains and were then ground raw into flour.

**Chemical evaluation:** The nutritional composition of the raw ground Oat samples was determined as per (AOAC, 2005).

**Composition and formulation of the basic purified diet and high fiber diet:** Basal Diet was made according to (Knapka *et al.*, 1983; Reeves *et al.*, 1993) called as Basic Purified Diet for Rats (AIN-76A). However in the high fiber diets the amount of oat fiber was increased to double the amount mentioned in the Basic Purified Diet (AIN-76A).

**Process of preparation of high fiber feed:** These seeds were ground and the outer crust which is the main source of crude fiber was separated by sieving of the flour of seeds using sieve of 220 mesh size of. (Anonymous, 1989).

**Mixing and storage of the feed:** To minimize the loss of micronutrients like minerals and vitamins the diet was mixed in a room with defused light. All the ingredient except oil, mineral and vitamins were mixed thoroughly with electric bowl mixer at slow speed. Later commercially prepared corn oil, with all the essential fatty acids was added. After the addition of oil distilled water was added sparingly to bring it in the consistency that was befitting for making pellets. The feed was then converted into rat feed in pellets. Once the pellets were prepared and dried they were stored in plastic container

with air tight fitted lids. These containers were later stored at a cool and dark place until further use. (Fullerton *et al.*, 1982).

**Experimental design:** A total of 36 White Albino Rats (male), weighing 200 to 250 gm. were obtained from the Animal House of PCSIR Laboratories. Animals were housed in a temperature (20 to 23°C) and humidity level (approximately 50%). To measure the exact amount of food consumption, the rats were housed conventionally in individual stainless steel hanging wire-mesh cages, with food and tap water provided according to the need. The rats were divided into the following groups:

- a) Group I (normal rats fed on Basic Purified Diet AIN-76)
- b) Group II (Alloxan induced Diabetic rats on Basic Purified Diet AIN-76)
- c) Group III (Alloxan induced Diabetic rats on Oat HFD)
- d) Group IV (normal rats fed on Basic Purified Diet AIN-76)
- e) Group V (Hypercholesterimic rats fed on Basic Purified Diet AIN-76)
- f) Group VI (Hypercholesterimic rat fed on Oat HFD)

**Induction of diabetes:** Diabetes was induced in Group II and Group II with ALX monohydrate (10 gm packing) of Simga-Aldrich Company. The rats were made diabetes after a fasting of 12 h (Kumar *et al.*, 2010). The freshly prepared dose of Alloxan (ALX monohydrate) 0.9 in saline solutions was given in a single shot after calculating the exact amount i.e., 70 mg/kg (Orsolic, 2011). The Alloxan dose was administered intravenously at the coccygeal lateral vein of the rat (Thorington, 1966; Young and Dawson, 1981).

**Blood glucose estimation:** One Touch Ultra 2 Glucometer of OneTouch Verio IQ. U.S.A. was used to assess the Random Blood Glucose Level mg/dL (RBGL) of rats.

**Induction of hypercholesterolemia:** The rats were induced with hypercholesterolemia by oral administration of 1% of cholesterol powder (1 gm/kg) for a period of 10 days as prescribed by Reeves *et al.* (1993) and Schurr *et al.* (1972) Hypercholesterolemia was confirmed at the 11th day of the treatment by using the diagnostic kit.

**Collection and analysis of blood samples:** A blood sample of 1 mL was collected from each Albino rat from the coccygeal vein which is a tail vein is a structure that carries blood back from the tail to the heart in a vertebrate. The vein leads to the posterior cardinal vein in the backside of an animal, a common feature of rodent anatomy Staszuk *et al.* (1995).

**Estimation of serum TC (total cholesterol):** The blood samples were centrifuged for about 15 min at 3000 rpm to separate the serum. After this Randox enzymatic kit was used according to the given protocol to estimate the serum cholesterol level using spectrophotometer. The cholesterol level was estimated by using Richmond (1973) method.

**Pattern of diet consumption:** The amount of feed consumption was determined by weighing feed in grams before putting it in the Feed Hoppers. Later the remaining feed was weighed once again to determine the exact amount of feed consumed by each rat in the individual cage (Jackson *et al.*, 1994). The average feed intake was 15 gm/day/rat.

**Metabolic effects of high fiber diet:** Two basic metabolic effects were considered in this study. The functional and metabolic effects of High Dietary Fiber feeds made from Oat bran, was used to determine its effect on blood glucose and lipid levels in the rats. The research was conducted in two different stages in the first stage the rats were rendered diabetic by using Alloxane monohydrate and later given Oat HFD (High Fiber Diet).

In the second stage the rats were rendered hypercholesteremic and were later given the same Oat HFD and then the antihyperlipidemic of the same was recorded.

**Statistical analysis:** Descriptive statistics were calculated for all the parameters studied. the Repeated Measure ANOVA technique and One-way ANOVA technique and multiple comparisons of means were made by Least Significant Difference (LSD) test were applied using SPSS version 15.

## RESULTS AND DISCUSSION

The Group I showed no significant ( $p < 0.05$ ) change in blood glucose level over the entire period of the experiment, whereas Group II showed a significant ( $p < 0.05$ ) increase in the blood glucose level in the first week after the Alloxan induced diabetes. After the induction however there was no significant change in the blood glucose level of this group and it remained on the higher side almost throughout the period of 6 weeks (Table 2).

Group III (Diabetic Expt. Oat HFD) showed a significant ( $p < 0.05$ ) change in blood glucose level over the period of six weeks. In week zero which was the period of acclimatization, the mean of blood glucose level was significantly ( $p < 0.05$ ) low as compared to the 1st week after the induction of diabetes in this group. The mean value of blood glucose level of Group III decreased significantly ( $p < 0.05$ ) in the 2nd and 3rd. However there was no significant ( $p < 0.05$ ) change in the 4th week.

While there was a significant increase in the 5th week which persisted to be almost the same in the 6th week. The One Way ANOVA, paired comparison post-hoc LSD test was applied (Fig. 1). Before Alloxan monohydrate induced diabetes, in week Zero there was no significant difference in the RBGL (random blood glucose level) of the rats of all the three groups. A significant difference continued to exist in Group I and Group II and Group III in from the 1st week to the 6th week. Further on when the two diabetic groups are compared it became evident the RBGL of the Diabetic group on BPD AIN-76 was significantly lower than Group III in the 1st and 2nd week of the experiment. However in the 3rd week a sudden significant decrease in Group III was recorded. This trend continued till the last week and the mean value of RBGL the Group III was significantly lower than Group I and Group II.

Therefore there was a total of 8.1% decrease in the blood glucose level in Alloxan induced diabetic rats in six weeks. The glucose lowering effect was most pronounced in the 3rd week after which oat HFD was more or less stable for the rest of the period. The results of the present study are similar to Chandalia *et al.* (2000) who did a crossover research to find out the effect of oat fiber on patients with type II diabetes. They compared the recommended dietary fiber i.e., 24 gm. with that of nearly its double amount i.e., 50 gm. of dietary fiber for six weeks. At the end six weeks span it was found out that the mean value of the daily pre-prandial plasma glucose level was 13 mg/dL lower with a (95% confidence interval) in the oat high fiber group as compared to the other group.

The serum cholesterol levels were also recorded for six weeks in the rats having induced hypercholesterolemia and were compared to that of rats fed on normal and Oat HFD. The results are shown in Table 3.

According to the results of the Repeated Measure ANOVA technique it is evident (Table 3) that there was no significant ( $p < 0.05$ ) change in the mean value of serum cholesterol level of the Group IV (Control group rats reared on BPD-AIN 76) through the period of 6 weeks.

On the other hand Group V (induce hypercholesteremic rats fed on BPD-AIN 76) showed significant difference ( $p < 0.05$ ) in week zero (before the induction of hypercholesterolemia) with all the other six weeks after the induction. The 1st and 2nd week showed no significant change in total serum cholesterol of this group. However there was a significant ( $p < 0.05$ ) decrease in the blood cholesterol of this group in the 3rd week after the induction of hypercholesterolemia. After 3rd week the cholesterol level of the group remained almost constant and there was no further significant decrease noted in this group over 4th, 5th and 6th week. Group VI (induced hypercholesteremic rats fed on Oat HFD) showed a significant change over the period of six weeks. Week zero had significantly ( $p < 0.05$ ) lower level

Table 1: Proximate Analysis of Oats % (Mean±SD)

	Moisture	Ash	Protein	Fat	Dietary fiber	Lignin	Cellulose
Whole oats	10.01±0.18	3.04±0.46	12.32±0.35	5.49±0.76	6.06±0.21	2.69±0.34	4.11±0.13
Oat bran	8.03±0.34	7.52±4.45	3.92±3.42	1.52±2.3	29.32±2.87	13.23±2.3	15.2±4.5

Table 1: illustrates the % of various nutrients present in Oat grain after the Proximate Analysis (AOAC, 2005)

Table 2: Change in Random Blood Glucose Level /Week of Different Groups on BPD AIN-76 and Oat HFD (Mean±SD)

	Blood glucose level (mg/dL) after induction of diabetes in different weeks						
	Zero	1st	2nd	3rd	4th	5th	6th
Group I	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
Group II	100.66±15.55 <sup>a</sup>	264.33±19.52 <sup>b</sup>	253.66±46.66 <sup>b</sup>	242.16±25.49 <sup>d</sup>	262.16±14.83 <sup>eh</sup>	260.00±10.52 <sup>h</sup>	267.00±14.12 <sup>h</sup>
Group III	111.50±10.74 <sup>a</sup>	282.83±21.80 <sup>b</sup>	270.66±22.60 <sup>b</sup>	214.66±11.93 <sup>d</sup>	219.83±11.08 <sup>d</sup>	225.50±12.67 <sup>a</sup>	226.50±11.76 <sup>a</sup>

Mean values followed by different letter in a rows are significantly different at p<0.05

Table 3: Change in Serum Cholesterol Level/Week of Different Groups on BPD AIN-76 and Oat HFD (Mean±SD)

	Blood Cholesterol Level (mg/dl) After Induction of Hypercholesterolemia in different Weeks						
	Zero	1st	2 <sup>nd</sup>	3 <sup>d</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>
Group IV	188.83±12.70	190.66±15.43	188.83±15.38	193.83±14.13	189.50±8.36	190.16±11.77	191.16±15.17
Group V	177.50±11.55 <sup>a</sup>	249.33±13.57 <sup>bh</sup>	244.83±14.26 <sup>bh</sup>	235.50±11.79 <sup>di</sup>	234.50±10.54 <sup>ei</sup>	231.50±11.37 <sup>h</sup>	228.00±9.3 <sup>h</sup>
Group VI	177.50±6.92 <sup>a</sup>	238.33±12.25 <sup>ba</sup>	226.50±5.12 <sup>ca</sup>	211.83±8.50 <sup>d</sup>	201.00±9.18 <sup>hi</sup>	190.00±6.69 <sup>hi</sup>	184.50±4.92 <sup>a</sup>

Mean values followed by different letter in a rows are significantly different at p<0.05

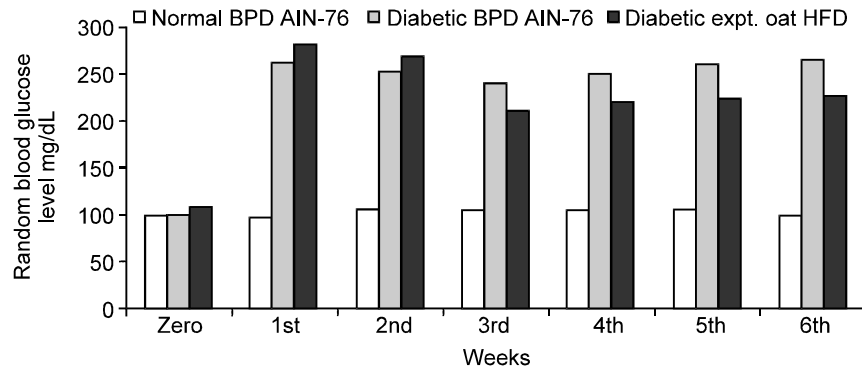


Fig. 1: Represents the week wise comparison of change in RBGL of different groups on BPD AIN-76 and Oat HFD

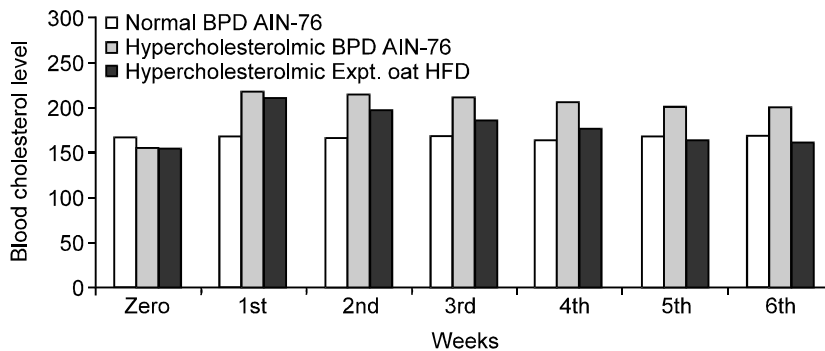


Fig. 2: Week wise comparison of change in serum cholesterol level (mg/dL) of different groups on BPD AIN-76 and Oat HFD

of cholesterol in comparison to all other weeks as it was prior to the induction of hypercholesterolemia. The 1st week showed a significant increase in the cholesterol as the reading were taken after the induction was confirmed. While there was no significant decrease ( $p < 0.05$ ) in the total serum cholesterol in first and 2nd week. However by the end of 3rd week there was a

significant decrease in the cholesterol. This trend of decrease continued in the 4th week. While during the last two weeks i.e., 5 and 6th the serum cholesterol level of this group remained almost constant.

It was evident after applying One Way ANOVA, post-hoc LSD test (Fig. 2) that in week zero there was no significant ( $p < 0.05$ ) difference in the blood cholesterol

level of the rats of different groups. However the difference was found significant between Group IV Group V and Group VI from 1st week till the 6th week. Whereas no significant difference was found between Group V and Group VI in the 1st week, after which the mean value of serum cholesterol of Group VI was significantly lower than the that of Group IV and Group V. Oats have been shown to decrease LDL-cholesterol which in-turn decreases the risk of heart attacks and it is known from a long time that health promoting activity of oat bran is equal or higher than the vegetable and fruits. (Braaten, 1994) Oats also contain antioxidant compound which are thought to help to prevent LDL-cholesterol damage. The increase in the percentage of Oats in the diet have proved beneficial in bringing down the VLDL and LDL cholesterol, as well as weight loss and increase in the fecal mass (Brown *et al.*, 1999).

The results of changes in the serum TC (Total Cholesterol) in the present research have also been very encouraging and it was noted that in comparison to the rats with Hypercholesterolemia fed on BPD AIN-76, the rats with Hypercholesterolemia fed on Oat HFD showed marked reduction in serum TC, especially in the last two weeks i.e., the 5th and 6th weeks of the experiment (Table 3) as the mean value of cholesterol of the Hypercholesterolemic group on Oat HFD was significantly lower than that of the Hypercholesterolemic group BPD. There was a total of 22% decrease in the mean value of cholesterol in the six week period in the present research.

**Conclusion:** The present research was conducted in two phases the first phase comprised of the nutritional estimation and extraction of crude dietary fiber from oat. In the second phase the metabolic impact of Oat High Fiber Diet (prepared from oat bran) was recorded on blood glucose level and total cholesterol level on groups of male rats with alloxan induced diabetes and induced hypercholesterolemia.

The proximate analysis of indigenous oat variety revealed that Oat grain has high dietary fiber content (Table 1). Hence Oat HFD (High Fiber Diet) was prepared for the experimental groups of rats. When the Oat HFD was given to the experimental groups of rats with alloxan induced diabetes it was noted that there was a significant level of decrease in the RBGL of this group in the period of six weeks as compared to the alloxan induced diabetic rats that were given the BPD AIN-76.

Likewise, when the rats with induced hypercholesterolemia fed on the same Oat HFD also had a gradual and significant lowering and stabilizing effect on the serum cholesterol level.

Therefore on the basis of results reported it can be safely recommended that the Oat fiber is a better and cheap source for lowering the blood glucose and cholesterol levels in different diseases.

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