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# Extraction of ß-glucan from Oat and its Interaction with Glucose and Lipoprotein Profile

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**Abstract:** ß-glucan was extracted and purified from oat, at various temperature and pH levels. Response surface methodology was applied to optimize the temperature and pH for extraction of ß-glucan gum pellets. Higher temperatures and neutral pH appeared to increase the yield of gum pellet and recovery of ß-glucan in extracted gum pellets. An extraction temperature of 50°C with a pH 7 was proved effective in removal of more of the impurities from the gum pellet. All the treatments extracted higher amounts of SDF (74.11-76.85%) and TDF (86.71-91.03%) in the extracted gum pellets. However, soluble dietary fiber and total dietary fiber content of gum pellets declined with increase in pH of extrcation medium. Serum glucose, total cholesterol, triglycerides and LDL cholesterol of albino rats decline with administration of increased doses of gum pellet extracted at temperature of 50°C with a pH 7. Incorporation of this gum pellet at 5% level in feed of rats increase the HDL by 37.74% over control group of rats. The reduction in lipoprotein fraction was directly associated with presence of SDF and TDF in the gum pellets.

**Key words:** Oat, ß-glucan, dietary fiber, cholesterol, lipoprotein, extraction method

## INTRODUCTION

The role of oat (*Avena sativa* L.) in daily diet has been well established since ancient times but its definite role as nutraceutical ingredient was much explored in last decade. These nutraceutical properties arise due to presence of dietary fiber in this cereal crop. Extraction of dietary fiber including ß-glucan is a difficult task. Numerous factors can affect its recovery from their sources. Among these factors, temperature and pH have an influence on recovery yield and chemical composition of ß-glucan (Temmelli, 1997). With the change in extraction condition composition of extracted dietary fiber is also affected, that may influence the health related issues of dietary fiber especially ß-glucan.

Dietary Fiber (DF) is the part of plant that resists digestion in small intestine of human being and other monogastric animals and thus, affect utilization of food by the body. Ingested DF is partly fermented in large intestine and this fermentation process largely dependent on physical characteristics such as solubility of DF. According to the solubility of Total Dietary Fiber (TDF) it can be classified into 2 groups, namely Insoluble (IDF) Dietary Fiber and Soluble (SDF). Insoluble DF is composed of non-starchy polysaccharides along with some quantity of lignin. IDF has a capacity to hold high volume of water thus, it add volume to feaces. Other benefits of IDF include reduction in bowel transit time (Shimotoyodome *et al*., 2001), prevention of constipation and lessening the risk of colorectal cancer (Bingham, 1990; Hill, 1997). Water-soluble fiber in cereals is mainly composed of ß-glucan and arabinoxylan and has a capacity to form viscous solutions. Increased viscosity in the intestine slows intestinal transit, delays gastric emptying (Wisker *et al*., 2000; Anderson and Chen, 1986) and slows glucose and sterol absorption by the intestine (Wood *et al*., 1990; Kahlon and Chow, 1997). Health benefits associated with intake of SDF include low chance of cardiovascular diseases, reduction in problem of obesity (Bourdon *et al*., 1999) lowering of blood cholesterol (Kahlon *et al*., 1993; Newman *et al*., 1992; Behall *et al*., 1997; Keogh *et al*., 2003), better control on diabetes (Wood, 1993; Newman *et al*., 1992; Brennan and Tudorica , 2003; Pick *et al*., 1996), hypercholesterolemia (Maki *et al*., 2003; Yang *et al*., 2003), cancer (Sier *et al*., 2004), hypertension (Anderson, 1983; Anderson, 1990) and support in growth of beneficial intestinal micro flora (Crittenden *et al*., 2002; Tungland, 2003).

The objective of this study was to better understand the relationship between the factors (temperature and pH) affecting the yield, recovery and composition of ß-glucan gum pellet and their subsequent effect on glucose and lipoprotein profile in animal model.

## MATERIALS AND METHODS

**Sample material and chemicals:** Oat grains of cultivar Avon was obtained from Fodder Research Institute, Sargodha. Grains were milled in a high-speed pin mill

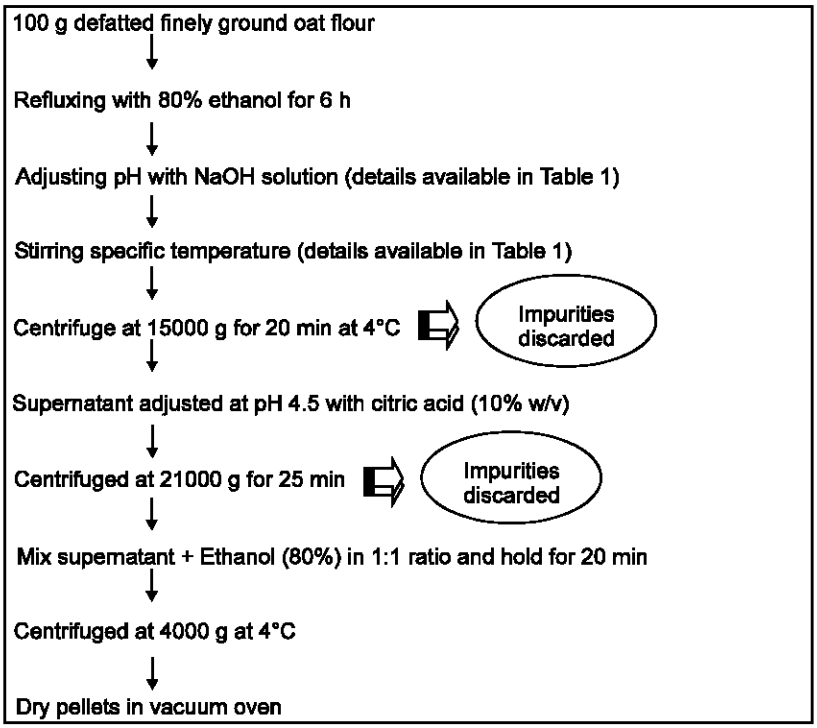


Fig. 1: Extraction and purification of ß-glucan gum pellet from oat

Table 1: Extraction Treatments from Oat

| Temperatures (°C) | pH 7.0 | 8.0 | 9.0 | 10.0 |
|---|---|---|---|---|
| 5 | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| 40 | $T_5$ | $T_6$ | $T_7$ | $T_8$ |
| 45 | $T_9$ | $T_{10}$ | $T_{11}$ | $T_{12}$ |
| 50 | $T_{13}$ | $T_{14}$ | $T_{15}$ | $T_{16}$ |

equipped with 0.50 mm screen. All other chemicals, reagents and solvents used in the present study were of analytical grade and were obtained from reputed companies.

**ß-glucan extraction:** ß-glucan was extracted in the form of gum pellets from oat grains by adopting hot water extraction process. In this process, various temperatures and pH was planned (Table 1). Detailed process for extraction and purification of ß-glucan gum pellet is presented in Fig. 1.

**Chemical analysis:** The chemical analysis of the oat grains and extracted ß-glucan for moisture content, ash, crude fat and crude fiber were performed using standard methods (Methods numbers 44-16 , 08-01, 46-10, 32-10, respectively) outlined in AACC (2000). The nitrogen content in samples were determined by Kjeldahl's method as described in AACC (2000) Method No. 46-10 and a conversion factor of 5.7 was used for calculating the protein content. The analytical kits for ß-glucan and dietary fibers were purchased from Megazyme International Ireland Ltd, Wicklow, Ireland and ß-glucan analysis was performed according to method of McCleary and Holmes (1985) whereas, TDF, SDF and IDF was determined by adopting the methods of AACC (2000).

**Glucose and lipoprotein profile of rats:** Albino Rats were obtained from the National Institute of Health (NIH), Islamabad to conduct biological assay studies. The young male albino rats (Sprague Dawley) of almost same age (6 weeks) were randomly divided into 6 groups: First group was fed on normal diet devoid of dietary fiber (Control group). Second group was fed on diet containing ß-glucan at 1%, third; fourth, fifth and sixth group was fed on diet containing ß-glucan at 2-5% level, respectively. ß-glucan extracted from $T_{13}$ was selected to be used in biological assay experiment based on highest TDF and SDF content.

The rats were housed at a constant temperature of 25±2°C, relative humidity of 60±2% and a 12:12 h light-dark cycle with no natural light. During first week of acclimatization, the rats were fed on a standard rat chow in meal. After that period, the rats were fed on the experimental diets and water ad libitum for 4 weeks. The experimental diets contain same level of protein, starch, fats, minerals and vitamins but different levels of extracted oat ß-glucan. During this period, feed intake, residues and weight of each individual rat were recorded once in a day (data not shown).

At the end of the experiment, the rats were decapitated and their blood was collected, centrifuged and serum was preserved at a temperature of 4°C. Plasma glucose was determined by the method of Thomas and Labor (1992)., Triglycerides, Total cholesterol and HDL were determined using commercial kits from Human (Wiesbaden, Germany). LDL was calculated as the difference between the serum cholesterol value and HDL (McNamara *et al*., 1990).

**Statistical analysis:** Analysis of variance was performed adopting GLM technique by using Minitab Software (Minitab Ver. 13.1) to test the effects of temperature and pH on yield (gum pellet) and recovery of ß-glucan in gum pellets. Least squares means and the standard errors of the least squares means were generated for later use in creating response surfaces. Results of chemical composition of gum pellets are presented with standard deviation and reduction in glucose and lipoprotein profile over control group is shown graphically.

## RESULTS AND DISCUSSION

Chemical analysis of oat flour (Fig. 1) indicated that it is a good source of Dietary Fiber (DF) and presence of higher amounts of DF in this cultivar showed its potential to be used functional ingredient in many foods. The TDF (11.09%), SDF (8.04%) and IDF (3.05%) content of tested oat cultivar in present study were comparable with TDF (7.5-16.8%), SDF (5.6-6.4%) and IDF (1.9-10.4%) content of promising oat cultivars as reported previously by several researchers (Lim *et al*., 1992; Manthey *et al*., 1999; Marketta *et al*., 2004). During extraction of gum pellet protein appeared to be major impurity, other minor impurities were crude fat and ash. Estimation of these impurities is helpful in design of extraction method. Temelli (1997) while, extracting ß-glucan from barley also observed presence of protein as impurity. This protein content can be efficiently removed by lowering the pH at isoelectric point of flour. At isoelectric points these protein has minimum interaction with other components and are easily removed from the extraction medium, this resulted in an increase in the percentage recovery of dietary fiber. The results substantiated that the protein content of ß-glucan gum pellets decreased progressively as a function of temperature and increased with increase in pH towards alkaline medium. Decrease in protein content of ß-glucan gum pellets with increase in temperature was well supported by the earlier findings of different workers (Wood *et al*., 1978; Dawkins and Nnanna, 1993; Temelli, 1997). The moisture contents (3.64-3.97%), crude fat (1.90-1.95%) and ash content (1.92-3.24%) of ß-glucan gum pellets varied non significantly in all samples. The crude fiber and NFE content of ß-glucan gum pellets were found temperature and pH dependent and affected significantly by these parameters.

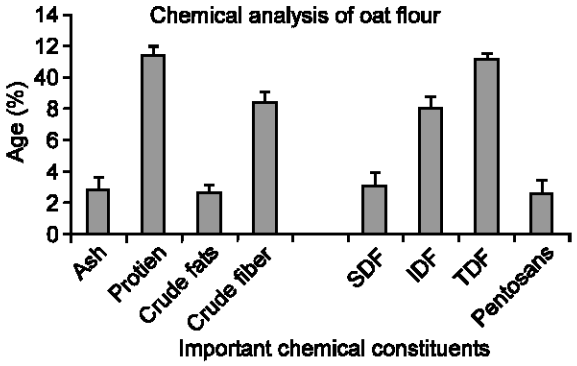


Fig. 2: Chemical analysis of oat flour

Based on chemical composition of oat flour, extraction method was designed to remove maximum amount of impurities for the extraction of ß-glucan gum pellets. Extracted ß-glucan gum pellets showed a significant variation in yield at various extraction temperatures and pH level. Higher temperature of extraction yielded more ß-glucan gum pellet where as an increase in pH towards alkaline reduced the yield of gum pellet (Fig. 4). This yield of gum pellet represents only the extracted gum pellet that was obtained from 100 g of oat flour. This did not indicate the amount of ß-glucan actually present in the gum. To determine the amount of ß-glucan in the gum pellet and to decide about the efficiency of extraction procedure to remove the impurities, the percent recovery of ß-glucan was calculated. Percent recovery of ß-glucan corresponds to the percentage ratio of weight of extracted ß-glucan in gum pellet (obtained from 100 g flour) to the weight of ß-glucan actually present in 100 g flour. The recovery of ß-glucan into gum pellets increased with increase in temperature of the extraction medium but it decline with rise in pH of the extraction medium (Fig. 5). Increase in recovery of ß-glucan is in line with previous study of Temmelli (1997) who observed positive effect of temperature for extraction of ß-glucan from barley. Estimated Regression Coefficients showed a significant effect of temperature and pH for yield and recovery (Table 3). The higher coefficient of determination $R^2$ indicates that both models explain variability in the data to a value of about 95 and 93% for yield and recovery, respectively.

On the basis of solubility, dietary fibres are classified as soluble and insoluble fibre. Soluble fibres include pectins, ß-glucan, galactomanan, gums and a large range of nondigestible oligosaccharides including inulin whereas insoluble fibres include lignin, cellulose and hemicelluloses. Interactive effect of temperature and pH on extraction of SDF, IDF and TDF is shown in Figure 2. Highest amount of TDF was observed in the extraction gum pellets when the temperature and pH of the extraction medium was maintained at 50°C and 7,

Table 2: Chemical composition of ß-glucan gum pellets

| Temp (°C) | pH | Moisture | Protein | Crude fiber | Crude fat | Ash | NFE |
|---|---|---|---|---|---|---|---|
| 35 | 7 | 3.76±0.23 | 11.57±0.45 | 9.34±0.43 | 1.91±0.23 | 2.29±0.28 | 71.127±1.62 |
| 35 | 8 | 3.97±0.25 | 12.44±0.48 | 10.22±0.47 | 1.94±0.35 | 2.60±0.21 | 68.83±1.76 |
| 35 | 9 | 3.93±0.21 | 13.55±0.43 | 11.32±0.34 | 1.90±0.21 | 2.92±0.27 | 66.37±1.46 |
| 35 | 10 | 3.78±0.19 | 14.93±0.35 | 12.83±0.41 | 1.95±0.28 | 3.24±0.26 | 63.263±1.49 |
| 40 | 7 | 3.85±0.24 | 10.12±0.42 | 8.48±0.36 | 1.94±0.26 | 2.15±0.31 | 73.463±1.59 |
| 40 | 8 | 3.96±0.21 | 11.78±0.34 | 9.37±0.38 | 1.95±0.26 | 2.50±0.28 | 70.437±1.47 |
| 40 | 9 | 3.78±0.18 | 12.78±0.32 | 10.56±0.28 | 1.93±0.29 | 2.74±0.24 | 68.22±1.31 |
| 40 | 10 | 3.80±0.23 | 13.78±0.35 | 11.70±0.31 | 1.92±0.21 | 3.02±0.21 | 65.777±1.31 |
| 45 | 7 | 3.91±0.21 | 9.54±0.37 | 7.60±0.37 | 1.95±0.34 | 2.04±0.25 | 74.95±1.54 |
| 45 | 8 | 3.94±0.21 | 10.75±0.34 | 8.47±0.38 | 1.92±0.26 | 2.35±0.32 | 72.573±1.51 |
| 45 | 9 | 3.83±0.21 | 11.84±0.36 | 9.64±0.39 | 1.92±0.29 | 2.63±0.21 | 70.137±1.46 |
| 45 | 10 | 3.84±0.24 | 12.98±0.42 | 10.89±0.45 | 1.94±0.23 | 2.72±0.23 | 67.627±1.57 |
| 50 | 7 | 3.90±0.18 | 7.78±0.39 | 6.11±0.35 | 1.92±0.27 | 1.92±0.27 | 78.37±1.46 |
| 50 | 8 | 3.87±0.23 | 9.03±0.31 | 7.35±0.41 | 1.94±0.31 | 2.18±0.21 | 75.623±1.47 |
| 50 | 9 | 3.96±0.24 | 10.33±0.34 | 8.67±0.39 | 1.92±0.28 | 2.48±0.27 | 72.64±1.52 |
| 50 | 10 | 3.95±0.21 | 11.58±0.36 | 9.63±0.45 | 1.90±0.32 | 2.64±0.26 | 70.303±1.60 |

Table 3: Estimated regression coefficients for yield and recovery

| Term | Yield | Recovery |
|---|---|---|
| Constant | 3.942** | 72.276** |
| Temperature | 0.607** | 1.633** |
| pH | -0.490** | -1.524** |
| Temp*Temp | 0.026 | 0.322* |
| pH*pH | 0.030 | 0.346* |
| Temp*pH | 0.057** | -0.110* |
| $R^2$ | 95.5% | 93.7% |

**p = 0.01, *p = 0.05

respectively. Lower temperature and higher pH (35°C and pH 10) adversely affected the TDF in extracted gum pellets. Lowest SDF (71.02%) and TDF (86.71%) were observed when ß-glucan was extracted at temperature of 35°C by maintaining pH of extraction medium at 10. The SDF range (74.11-76.85%) of ß-glucan gum pellet observed in present study is in close proximity with the results of Faraj *et al.* (2006) who reported SDF of purified ß-glucan concentrate in the range of 87.2-91.4% and unpurified concentrate in the range of 50.7-541%. This SDF content of ß-glucan gum pellets is also comparable with SDF content (75%) of guar gum as observed by Frias and Sgarbieri (1999).

**Glucose and Lipo protein profile:** Serum glucose was significantly ($p \leq 0.05$) reduced by administration of ß-glucan containing diet. A linear decline in serum glucose was observed with an increase in level of ß-glucan. Decrease in serum glucose was positively correlated with SDF (r = 0.87) and TDF (r = 0.81) of gum pellets. Incorporation of ß-glucan gum pellets at level of 5% decrease the serum glucose up to 18.55% as compare to control (Fig. 6). This decrease in serum glucose was due to increased intestinal viscosity that was an outcome of ingestion of ß-glucan containing diets, which resulted in slow absorption of glucose in the blood stream. The results of present study substantiate the previous study of Ostman *et al.* (2006) who observed lowering of serum cholesterol by consumption of ß-glucan rich barley in bread.

Serum lipids (total serum cholesterol, LDL and triglycerides) were significantly ($p \leq 0.05$) affected by ß-glucan level in the diets of the rats. Group of rats fed on diets prepared with 5% levels of ß-glucan had lowest ($p \leq 0.05$) total serum cholesterol (Fig. 7), triglycerides (Fig. 8) and LDL-cholesterol (Fig. 9) and than other groups. A strong negative correlation was observed between SDF of extracted ß-glucan gum pellet and total serum cholesterol (r = -0.77), LDL (r = -0.89) and triglycerides (r = -0.74). The reduction in these parameters was due to the capacity of extracted ß-glucan to control the micellar solubility of lipids and interfere with absorption of dietary cholesterol and lipids thus, changing the rate and site of absorption of cholesterol (Schweizer and Würsch, 1991; Marlett *et al.*, 1994). The viscous nature of SDF also resulted in decreased reabsorption of bile acids and increase their fecal excretion. This would result in more synthesis of bile acid from cholesterol in the liver (Horn, 1997). The inclusion of ß-glucan gum pellet in test diet at level of 5% also reduced serum LDL concentrations by 329% as compared to control. This observation is in close conformity with observation of Brown *et al.* (1999), who estimated that one gram of soluble fiber from oats can produce change in LDL cholesterol of -1.23 mg/dL. Therefore, our work extends these studies by showing that bioactive ß-glucan gum pellets when used at level of 5% can reduce total cholesterol and triglycerides up to 35.68 and 35.55%, respectively and increase in HDL (Fig. 10) by 37.74% as compare to control. The present study shows that extracted oat ß-glucan gum pellets are biologically active and have a prominent role in reducing serum cholesterol due to higher SDF and TDF content. Shinnick *et al.* (1991) also found that ß-glucan, which is

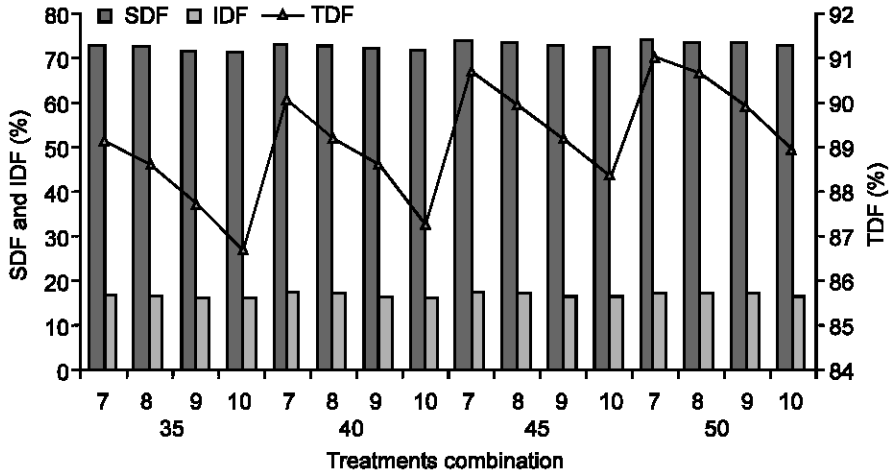


Fig. 3: Dietary fiber of ß-glucan gum pellet extracted at various temperature and pH

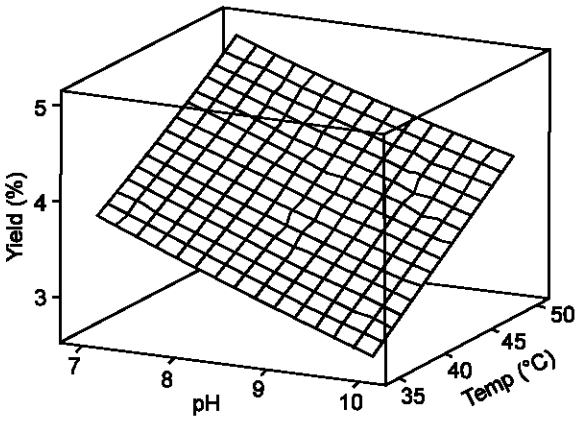


Fig. 4: Response surface for %yield of gum pellet as function of temperature and pH

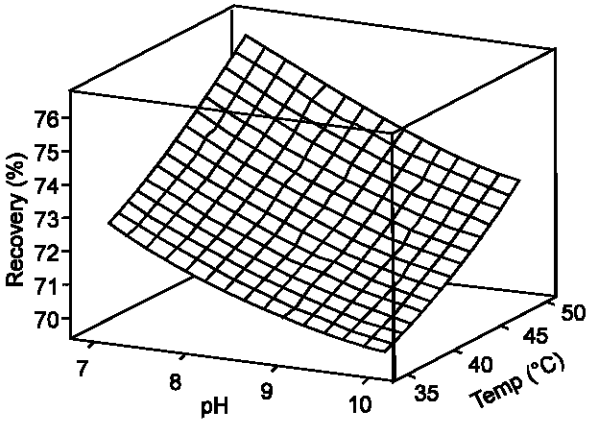


Fig. 5: Response surface for % recovery of ß-glucan as function of temperature and pH

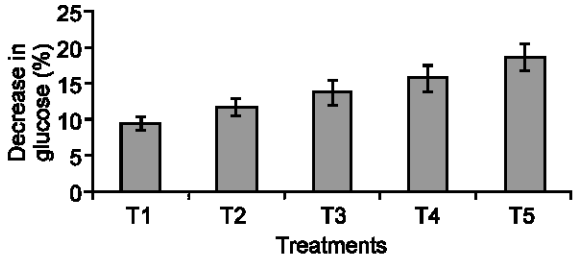


Fig. 6: Percent decrease in glucose by administration of ß-glucan gum pellets $T_1$ = 1%, $T_2$ = 2%, $T_3$ = 3%, $T_4$ = 4%, $T_5$ = 5%

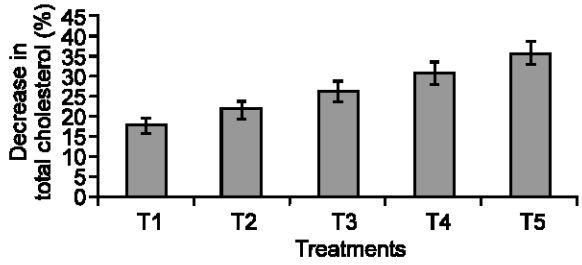


Fig. 7: Percent decrease in cholesterol by administration of ß-glucan gum pellets $T_1$ = 1%, $T_2$ = 2%, $T_3$ = 3%, $T_4$ = 4%, $T_5$ = 5%

the prevalent water-soluble fiber in oat and barley, could reduce serum cholesterol level. Taken together with our present research, these data strongly suggest that incorporation of oat ß-glucan gum pellets in the normal diet can be nutritionally important with respect to lipoprotein profile. Because, most of the cholesterol absorbed by mono gastric animals and humans is recirculating endogenous biliary cholesterol, ingestion of ß-glucan in these models will reduce cholesterol absorption that would tend to lower serum cholesterol regardless of the amount of dietary cholesterol taken in.

**Conclusion:** Tested oat variety proved to be an excellent source of dietary fiber. Temperature and pH exerted

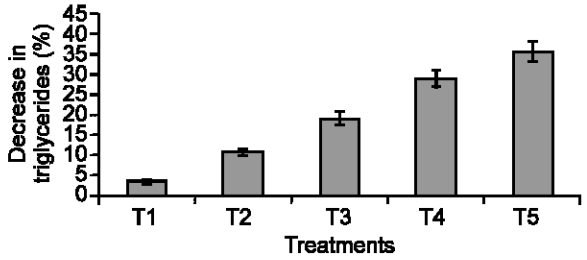


Fig. 8: Percent decrease in triglycerides by administration of ß-glucan gum pellets $T_1$ = 1%, $T_2$ = 2%, $T_3$ = 3%, $T_4$ = 4%, $T_5$ = 5%

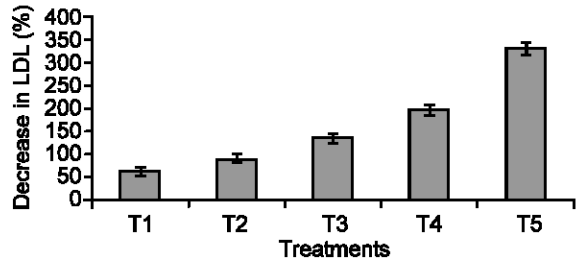


Fig. 9: Percent decrease in LDL by administration of ß-glucan gum pellets $T_1$ = 1%, $T_2$ = 2%, $T_3$ = 3%, $T_4$ = 4%, $T_5$ = 5%

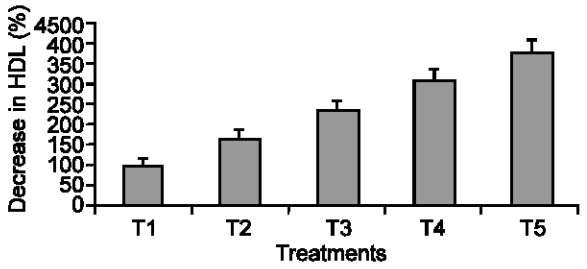


Fig. 10: Percent increase in HDL by administration of ß - glucan gum pellets $T_1$ = 1%, $T_2$ = 2%, $T_3$ = 3%, $T_4$ = 4%, $T_5$ = 5%

significant effect on yield, recovery as well as impurities of extracted ß-glucan gum pellets. Maximum gum pellet yield and ß-glucan recovery were achieved at temperature of 55°C and pH7. Much of the healthful effects of oat ß-glucan gum pellet was associated with soluble fiber.

A concomitant decline in glucose, triglycerides, total cholesterol and LDL can be achieved by increasing dose of ß-glucan up to 5%. Negative correlation between SDF and TDF with lipoprotein profile indicated that gum pellets extracted in present study under specific condition have a capacity to reduce total cholesterol, triglycerides and LDL. Extracted ß-glucan gum pellets was especially very effective in lowering of LDL cholesterol.

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