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Phenolic compounds and antioxidants capacity of sweet lupine derivatives-wheat Flour mixtures and the effects on diabetic rats

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Abstract: *The aim of this research was to study the chemical and nutritional properties of sweet lupine seeds (lupine flour and fiber) at concentrations (5, 10 and 15 % as a substitution of wheat flour) and effects of its addition on diabetic Rats. Lupine flour showed higher levels of moisture, crude protein, ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour showed higher levels of starch. The lupine flour showed higher levels of total phenolic and total flavonoids than the wheat flour. Conversely, wheat flour showed higher levels of total flavonols. Results clearly indicate that lupine flour exhibited higher antioxidant activity with Diphenyl-P-Picryl Hydrazyl (DPPH) and 2, 2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) than the wheat flour. Forty eight male albino rats were divided into two main groups (negative control \ 6 rats & Alloxan injected \ 42 rats). After hyperglycemic confirmation, they divided into seven subgroups (6/group); and fed on basal diet only (positive control) or basal diet with 5, 10 and 15% of lupine flour and 5, 10 and 15% of lupine fiber respectively. Diabetic rats fed on neither lupine flour nor fiber increased significantly in body weight gain like normal control group. Also, elevated glucose, cholesterol and total lipids of injected groups modulated significantly after treatments comparing with positive one. Finally, the addition of lupine flour or fiber reduced blood glucose, total cholesterol and total lipids in diabetic rats; it could be used successfully as hypoglycemic agents in feeding diabetic patients.*

Keywords: *Lupine; Wheat flour; Nutrition Value; Diabetic Rats; Cholesterols; Glucose and Total lipids.*

I. Introduction:

Legume is one of the three largest families of flowering plants, comprising nearly 700 genera and 18,000 species. The legumes used by humans are commonly called food legumes or grain legumes. The food legumes can be divided into two groups, the pulses and the oilseeds. Pulses group consists of dried seeds of cultivated legumes, which have been eaten for a long time **(Asian Productivity Organization, 2003).**

Animal proteins being more expensive, especially people in developing countries depend largely on plant to fulfill their protein requirements. In general, cereals and legumes take a large place of human food consumption. Grain legumes alone contribute to about 33 % of the dietary protein nitrogen needs of humans. Moreover, it is also a good source of minerals **(Kirmizi and Guleryuz, 2007**). Besides being a good source of nutrition, there is a considerable interest in the relationship between plant-based diets and the prevention of certain human diseases, in which increased levels of radicals are implicated. Likewise legumes seem to be responsible for improving health and can prevent chronic diseases (**Frias et al., 2005**). Cholesterol-free legumes in combination with their low sodium content form a good food stuff not only for people living in developing countries but also for those living in industrialized nations (**Sebastiá et al., 2001**).

Currently interest in a wider utilization of this legume seed is rising. Lupine has been used as a source of protein and oil since ancient times. Lupine is commonly consumed as a snack in the Middle East and is coming into use as a high-protein soy substitute in the other parts of the world (K**urzbaum et al., 2008**).This is mainly due to its similarity with soybeans as a high source of protein and to the fact that it can be grown in wider climatic range. Moreover; its adaptation to poor (i.e. leached) soil, makes it economically feasible (**Sujak et al., 2006)**.

Due to low glycemic index of lupine seeds, it was found that lupine kernel fibers have appetite suppression (**Archer et al., 2004**) and cholesterol lowering properties, that they lower blood glucose and insulin levels, and aid bowel health as a fecal bulking agent. Many researchers have paid more attention towards the possibility of using lupine as a human food (**Petterson and Mackintosh, 1997**) and their potential health benefits. However, little is known about their photochemistry and antioxidant activity (**Hall et al., 2005**).

Nevertheless, demand for wheat-based bakery products is increasing, particularly in developing countries where the major grain is wheat (**Quail, 1996**). The nutritional quality of wheat protein is lower than that of proteins from pulses and oilseeds due to its low levels of lysine, methionine, and threonine (**Kulp, 1988**). It could be improved by supplementation with non-wheat proteins such as those from pulses, including lupine, which would increase the protein content and improve the essential amino acid balance of the baked product.

The aim of this research was to study; the chemical and nutritional properties of sweet lupine flour and fiber, also the effects of their additions at different concentrations (5, 10 and 15 %) on diabetic Rats.

II. Materials and Methods

2.1. Materials

Local Egyptian breeds of lupine (Lupinus albus L. variety Giza) were obtained from the Agricultural Research Centre, Giza, Egypt. Lupine seeds were crashed in a local home millstone, and then hulls were separated from endosperm particles by aspiration. Both endosperm and hulls particles were milled separately in a laboratory hammer mill (Retsch - Germany) until they could pass through a 250 µm screen to obtain lupine flour and milled lupine hulls (expressed as lupine fiber). Commercial wheat flour was obtained from local market. All other chemical reagents used in the experimental analysis were of analytical grade.

2.2. Chemical analysis

Chemical analysis was carried out according to ICC Standard Methods (**ICC, 2001**). Moisture content was determined by drying the samples at 105 °C to constant weight (ICC 109/01). Ash content was determined by calcinations at 900 °C (ICC 104/1). Nitrogen content was determined by using "Kieldahl" method with factor of 5.7 to determine protein content (ICC 105/2). The total lipid content was determined by defeating in the "Soxhelt" apparatus with hexane (ICC 136). The determination of starch content was assessed using a polarimetric method according to Ewers, modified by **Davidek et al., (1981)**. Dietary fiber contents were determined by the method of **Goering and Van Soest, (1970)** as modified by **Baker, (1977).** All the measurements of analyzed samples were made in triplicate. Total phenolic content was determined by the Folin–Ciocalteu micro-method **(Arabshahi-Delouee and Urooj, 2007**). While total flavonoid content was determined by the method of **Ordoñez et al., (2006).** Also total flavonols content was determined by the method of **Kumaran and Joel Karunakaran, (2007)**.

Antioxidant capacity of extracts:

The antioxidant capacity of each extract was determined through two complementary assay procedures. Radical-scavenging activity was determined throw DPPH assay according to **Lee et al., (2003).** Also the ABTS assay according to method of **Re et al., (1999)** was adopted. Because of the differences among the various test systems available, the results of a single method can provide only a limited assessment of the antioxidant properties of a substance (**Sacchetti et al., 2005)**.

2.3. Experimental animals design:

Forty eight male albino rats (Sprague Dawley strain) ranged weight 110-120 g were obtained from National Research Center, Giza, Dokki, Egypt. They were housed in individual cages at room temperature. The animals were left to acclimatize for ten days before the start of experiment. They were fed with standard laboratory diet according to **(AOAC, 2002**) and randomly divided into two main groups. First group (6 rats) was fed on a basal diet without lupine flour or fiber for 35 days and considered as control rats (control A). The second main group (42 rats) was fasted overnight and injected with alloxan solution (120 mg/kg rat weight) to induce hyperglycemia (**Arbeeny and Bergquist, 1991**). After 48 h of injection, the second main group was divided into seven subgroups (6 rats each). The first subgroup represents the diabetic rats (control B) was fed on basal diet without lupine flour or fiber. The rats of 2nd, 3ed and 4th subgroups were fed separately on basal diet plus different levels (5, 10 and 15 %) of lupine flour respectively. The rats of 5nd, 6ed and 7th subgroup were fed separately on basal diet plus different levels (5, 10 and 15 %) of lupine fiber respectively. Animals were weighted weekly and sacrificed at the end of the experiment. Blood samples were collected from hepatic portal vein; serum was separated by centrifugation at 3000 rpm, for 15 minutes and kept at -5 °c tell analysis. Serum total cholesterols, lipids and glucose level were determined according to **Hewitt and pardue, (1973)**; **Frings and Dunn, (1970)**; **Trinder, (1969)** respectively.

2.4. Statistical analysis

Analysis of variance (ANOVA) was carried out using SAS program (Statistical Analysis System version. 9.1) SAS Institute Inc. (**SAS, 2004**). When the treatment factor effect was found significant, indicated by a significant F-test ($p < 0.05$), differences between the respective means were determined using **Duncan, (1957)** and considered significant when $p < 0.05$. Mean ± standard deviation of mean was used.

III. Results and Discussion

3.1. Chemical analysis

3.1.1. Lupine, wheat flour and their mixtures

The results for the chemical analysis of wheat flour (WF), lupine flour (LF), and their blends are shown in Table (1). The lupine flour shows higher levels of moisture, crude protein, ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour shows higher levels of starch. These results confirmed by statistical analysis, which highly significant differences ($P < 0.05$) were observed between the two types of flours. Mean protein and total dietary fiber increased with increasing amount of lupine flour added to be 12.73,

13.75, 15.28 and 5.66, 7.61, 9.57 g/100 g for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. There was no significant difference between wheat flour and supplemented flour with different concentration of lupine for moisture, ash and fat content. Lupine is a good source of nutrients, not only proteins but also lipids, dietary fibre, minerals, and vitamins (**Martínez-Villaluenga et al., 2009)**.

The chemical properties of wheat flours have been studied previously by several researchers and they found that moisture content ranged between 12.5 to 14.6 % crude protein content 8.23 to 12.71 % and ash content 0.42 to 0.66 **(Ahmad et al., 2001)**.

Table 1: Chemical analysis of lupine flour (LF), wheat flour (WF) and their mixtures (gm/100 gm)

Analysis	LF	WF	LF level (%)		
			5	10	15
Moisture	11.37 ± 0.46	10.27 ± 0.09	10.35 ± 0.19	10.42 ± 0.17	11 ± 1.16
Protein	37.6 ± 0.87	11.1 ± 0.20	12.73 ± 0.24	13.75 ± 0.27	15.28 ± 0.31
Ash	3.21 ± 0.03	0.42 ± 0.02	0.57 ± 0.01	0.74 ± 0.04	0.89 ± 0.03
Fat	9.84 ± 0.16	1.52 ± 0.19	1.98 ± 0.19	2.35 ± 0.17	2.77 ± 0.16
Starch	0.97 ± 0.034	68 ± 1.94	65 ± 3.92	62 ± 1.86	59 ± 2.82
* S. D. F	12 ± 1.36	1.8 ± 0.09	1.90 ± 0.14	2.69 ± 0.27	3.69 ± 0.31
** I. D. F	31 ± 2.45	1.9 ± 0.23	3.76 ± 0.31	4.92 ± 0.42	5.88 ± 0.53
*** T. D. F	43 ± 3.08	3.7 ± 0.15	5.66 ± 0.27	7.61 ± 0.43	9.57 ± 0.60

Mean ± Standard Deviation (* Soluble, ** Insoluble, *** Total Dietary Fiber)

Protein content of lupine (37.6 %) was higher than that of a lot of legumes. **Favier et al., (1995)** reported that haricot bean, lentil and soy bean contain 28.8 %, 26.7 % and 40.5 % protein, respectively. Because of the high protein content, lupine flour could be used in the human diet. Also, temperature of denaturation of these proteins is higher than animal protein, so they are technologically easier to handle **(Chapleau and de Lamballerie-Anton, 2003)**. Lupine flour had a high amount of crude fibre (16.2 %). These fibers have many desirable properties, including white color, high water-holding capacity (7.1 g H_2O/g) and beneficial effects on human health (**Huyghe, 1997**). Therefore, lupine flour can be incorporated into a wide range of foods to make dietary products.

3.1.2. Lupine fiber, wheat flour and their mixtures

The proximate compositions of lupine fiber (L-fiber), wheat flour (WF) and wheat flour substituted with different levels of lupine fiber in Table (2). The lupine fiber shows higher levels in ash, crude fat and dietary fiber than the wheat flour. Conversely, wheat flour shows higher levels of moisture, crude protein and starch. These results confirmed by statistical analysis, which highly significant differences ($P < 0.05$) were observed between wheat flour and lupine fiber. Mean dietary fiber increased with increasing amount of lupine fiber added to be 6.62, 11.95 and 16.07 for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. There was no significant difference between wheat flour and supplemented flour with different concentration of lupine fiber for moisture, ash and fat content.

Table 2: Chemical analysis of Lupine fiber (L-fiber), wheat flour (WF) and their mixtures (gm/100 gm)

Analysis	L-fiber	WF	L-fiber level (%)		
			5	10	15
Moisture	8.66 ± 0.06	10.27 ± 0.09	11.16 ± 0.08	11.12 ± 0.07	11.89 ± 0.16
Protein	4.9 ± 0.26	11.1 ± 0.20	11.72 ± 0.23	11.35 ± 0.25	10.91 ± 0.29
Ash	2.46 ± 0.18	0.42 ± 0.02	0.50 ± 0.11	0.61 ± 0.05	0.72 ± 0.43
Fat	2.2 ± 0.11	1.52 ± 0.19	1.65 ± 0.17	1.68 ± 0.14	1.7 ± 0.10
Starch	0.22 ± 0.04	69 ± 1.96	67 ± 1.91	64 ± 1.86	58 ± 3.82
* S. D. F	43 ± 2.19	1.8 ± 0.09	3.12 ± 0.15	6.38 ± 0.31	8.49 ± 0.45
** I. D. F	41 ± 1.12	1.9 ± 0.23	3.50 ± 0.25	5.57 ± 0.26	7.58 ± 0.34
*** T. D. F	84 ± 4.38	3.7 ± 0.15	6.62 ± 0.32	11.95 ± 0.43	16.07 ± 0.65

Mean ± Standard Deviation (* Soluble, ** Insoluble, *** Total Dietary Fiber)

3.1.4. Phenolic compounds and antioxidants capacity

Phenolic compounds ubiquitous in plants are key phytochemical drivers of the health and functional foods and nutraceutical industry. Research with polyphenol compounds from various crops has created a growing market for polyphenol-rich ingredients, estimated to be worth around $ 99 million in Europe in 2003 **(Nutraingredients, 2005**).

3.1.4.1. Lupine, wheat flour and their mixtures

Conventional solvent extraction has been reported in a laboratory scale using acetone, hexane, methanol and ethanol (**Kosar et al., 2004**). In this study, methanol was used for the extraction of antioxidant

compounds from wheat, lupine flour and their blends (Table 3). The extraction yield 13.4 and 35.1 g/100g dry weight for wheat and lupine flour respectively.

Table 3: Phenolic compounds and antioxidant capacity of lupine flour (LF), wheat flour (WF) and their mixtures

Analysis	LF	WF	LF level (%)		
			5	10	15
Yield extract (%)	35 ± 1.73	13.4 ± 3.99	11.7 ± 0.36	13.8 ± 1.34	113.8 ± 1.2
Total phenolic (µg GAE/g DW)	136 ± 8.33	126 ± 3.51	130.13 ± 0.56	141 ± 6.80	155 ± 3.68
Total flavonoids (µg QE/g DW)	8.13 ± 0.08	6.32 ± 0.14	7.9 ± 1.23	7.33 ± 0.04	8.2 ± 0.5
Total flavonols (µg QE/g DW)	25 ± 2.7	31 ± 6.1	28 ± 2.4*	27 ± 2.94*	26 ± 1.2*
DPPH (%)	20.7 ± 1.22	3.30 ± 0.35	4.9 ± 0.11*	5.8 ± 0.76*	6.9 ± 0.25*
ABTS (%)	41.41 ± 0.35	26.3 ± 0.2	28.40 ± 0.36*	30.08 ± 0.00*	31.34 ± 0.37*

Mean ± standard deviation

The lupine flour shows higher levels of total phenolic and total flavonoids than the wheat flour. Conversely, wheat flour shows higher levels of total flavonols. These results confirmed by statistical analysis, which highly significant differences ($P<0.05$) were observed between the two type of flours. Total phenolic and total flavonoids increased with increasing amount of lupine flour added to be 130.13, 141, 155 (µg GAE/g DW) and 7.9, 7.33, 8.2 (µg QE/g DW) for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis. The contents of phenolic acids in lupine used in this study are comparable to levels reported previously **(Ricardo-da-Silva et al., 1993)**, especially in cultivars of L. albus grown in Portugal. Phenolic content of lupins were higher than those of bean cultivars grown in Manitoba (**Oomah et al., 2005**) probably as a result of relatively high flavonoid content.

The antioxidant effects of extracts of various wheat flour (WF), lupine flour (LF) and their blends at different concentration (5, 10 and 15 %) were measured. Since the active substances of flour extracts tested are different, the antioxidant activities of these extracts cannot be evaluated by only a single method. Therefore, two different models were used in this study **(Huang et al., 2005).**

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others **(Dorman et al., 2003)**. The DPPH radical has been widely used to evaluate the free radicals' scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids **(Da Porto et al., 2000)**. The effect of antioxidants on diphenyl-p-picryl hydrazyl (DPPH) radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The assay is based on the reduction of DPPH. Because of its odd electron, DPPH gives strong absorption maxima at 515 nm (purple color) by visible spectroscopy. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, i.e., a free radical scavenging antioxidant, the absorption intensity is decreased, and the resulting decolorization is stochiometric with respect to the number of electrons captured **(Yamaguchi et al., 2000).**

Table (3) shows that the scavenging activity of methanolic extracts against DPPH for wheat flour (WF), lupine flour (LF) and their blends. Significant ($p < 0.05$) differences between wheat and lupine flour extracts were observed. Results clearly indicate that lupine flour exhibited higher antioxidant activity with DPPH and ABTS than the wheat flour. The antioxidant activity increased with increasing amount of lupine flour added to be 4.9, 5.8, 6.9 in DPPH and 28.40, 30.08, 31.34 in ABTS respectively, for substituting wheat flour with lupine flour at 5, 10 and 15 %, respectively on dry weight basis.

This results disagreement with **Wang et al., (1998)** who found that some compounds which have ABTS+ scavenging activity did not show DPPH scavenging activity. In this study The ABTS•+ scavenging data suggests that the components within the extracts are capable of scavenging free radicals via a mechanism of electron/hydrogen donation and should be able to protect susceptible matrices from free radical-mediated oxidative degradation.

3.1.4.2. Lupine fiber, wheat flour and their mixtures

The hull constitutes a considerable part of the lupine seeds (ca. 20 %) with a high content of dietary fibre (50–54 %) of good functionality **(Gorecka et al., 2000**). Compared to other leguminous crops, lupine seeds have a large proportion of hulls, which can be a source of valuable health promoting ingredients, including those with antioxidant properties. Therefore lupine hulls were also estimated.

Table 4: Phenolic compounds and antioxidant capacity of lupine fiber (L-fiber), wheat flour (WF) and their mixtures

Analysis	L-fiber	WF	L-fiber level (%)		
			5	10	15
Yield Extract (%)	15.8[a]	13.4[e]	13.9[d]	14.8[c]	15.1[b]
Total Phenolic (µg GAE/g DW)	42.53[e]	125.53[a]	125.17[b]	113.27[c]	104.07[d]
Total Flavonoids (µg QE/g DW)	7.43[a]	6.13[e]	6.43[d]	6.80[c]	7.10[b]
Total Flavonols (µg QE/g DW)	29.52[b]	31.02[a]	29.56[b]	28.5[c]	27.50[d]
DPPH (%)	3.65[d *]	3.21[e]	3.88[c *]	4.05[b *]	4.66[a *]
ABTS (%)	26.44[c *]	25.6[d]	26.35[c *]	27.80[b *]	28.73[a *]

Mean scores with the same letter are not significantly differed at the 5% level according to **Duncan(1957)**

Table (4) shows that extract yield, total polyphenols content and antioxidant capacity of wheat flour (WF), lupine fiber (L-fiber) and their blends. It was noticed that lupine fiber had total phenolic, and flavonols lower than wheat flour or lupine flour. No significant difference between wheat flour and lupine fiber in flavonoids content. The same trend was observed with the antioxidant activity for lupine fiber in DPPH and ABTS tests. These results were similar with the results of **Lampart-Szczapa et al., (2003)** who studied the antioxidant properties of lupine flours and hulls using the rancimat and oxidograph tests and he found that lupine tannins contents in the flours were a few times higher than in the hulls. Antioxidant activity was found both in the flours and in the hulls.

3.2. Biological evaluation for lupine flour (LF) and lupine fiber (L-fiber)

3.2.1. Body weight and Food intake

As shows in Table (5) it could notice that gain in body weight was 61.9 g for negative control, while it was decreased for the positive diabetic one to be 32.3 g and the reduction in body weight was 47.8 %. Diabetic rats which fed on neither lupine flour nor fiber showed similar results as normal control, and there were significantly differed.

Table 5: Body weight, feed intake and feed efficiency ratio (FER) of healthy and diabetic rats fed on basal diet supplemented with different levels of lupine flour (LF) and lupine fiber (L-fiber). (Mean + SD)

Diets	Initial body weight g	Final body weight g	Feed intake g	Gain body weight g	Daily feed intake g	FER %
Control A*	110.4[a]	172.3[f]	461.0[f]	61.90 [g]	14.00[de]	13.4.0[c]
Control B*	112.8[b]	145.1[b]	317.5[a]	32.30[b]	9.90[a]	10.2[b]
Group 1	114.5[c]	182.4[g]	499.5[h]	67.90[h]	15.10[e]	13.6[c]
Group 2	112.0[b]	171.7[f]	471.5[g]	59.70[f]	14.30[de]	12.6[c]
Group 3	118.7[e]	168.3[e]	433.0[e]	49.60[e]	13.20[cd]	11.4[b]
Group 4	116.1[d]	166.0[d]	429.5[d]	49.90[d]	12.90[cd]	11.1[b]
Group 5	115.1[cd]	152.8[c]	401.5[c]	37.70[c]	12.10[bc]	8.8[a]
Group 6	111.7[b]	143.3[a]	363.0[b]	31.60[a]	11.0[b]	8.1[a]

Mean scores with the same letter are not significantly differed at the 5% level according to **Duncan(1957)**

Control A*: Normal rats fed on a basal diet

Control B*: Diabetic rats fed on basal diet

Group 1: Diabetic rats fed on basal diet + 5 % lupine flour

Group 2: Diabetic rats fed on basal diet + 10 % lupine flour

Group 3: Diabetic rats fed on basal diet + 15 % lupine flour

Group 4: Diabetic rats fed on basal diet + 5 % lupine fiber

Group 5: Diabetic rats fed on basal diet + 10 % lupine fiber

Group 6: Diabetic rats fed on basal diet + 15 % lupine fiber

Food intake\day decrease in alloxan diabetic fed on 5, 10 and 15 % lupine fiber (12.9, 12.1 and 11 g/day respectively, compared to negative control 14 g/day. Slightly decrease was found in diabetic group fed on 15 % lupine flour and 5 % lupine fiber.

Newairy et al., (2002) showed that diabetic rats which treated with lupine showed an increase of their body weight as compared with the diabetic group. But **Abdel-Salam and Abdel-Megeid, (1998)**, reported that alloxan injection caused a significant decrease in average body weight in rats and there was a decrease in body weight in groups treated with raw and blanched lupine. These results were in agreement with the present results.

3.2. Biochemical analysis

3.2.1. Glucose level

For serum glucose, the present study in figure (1) shows that the levels of serum glucose for alloxan diabetic group were increased approximately 2 fold (227.10) compared with normal control (96.94). Feeding of alloxan diabetic groups shows significantly reduction in glucose levels by: 26.53 %, 33.34 %, and 32.80 % for

lupine flour at 5, 10 and 15 % respectively, 29.34 %, 39.14 % and 48.78 % for lupine fiber at 5, 10 and 15 % respectively as compared to alloxan diabetic control.

The results were in agreement with finding of **Mansour et al., (2002)**, who found that treated diabetic rats with 75mg\day\100g body wt. of lupine for 4 weeks reduced the glucose levels by 59 % as compared to diabetic alloxan rats. **Abdel-Salam and Abdel-Megeid, (1998)**, found that raw and blanched lupine at 5 and 10 % have hypoglycemic effect and blanched lupine have more effect than raw as compared to diabetic control The hypoglycaemic effect of lupine flour and lupine fiber may be due to the active constituents such as alkaloids, flavonoids, tannins, quinovic acid and its glycocidic derivatives, saponins and triterpenoid saponins **(Pollmann et al., 1997**). Other phenomenon due to saponins effect have hypoglycaemic activity, which may be due to the inhibition of hepatic gluconogensis **(Kubo et al., 2000)**.The effect of lupine may be due to the increase levels of serum insulin **(Eskander and Won Jun, 1995)**, and also may be due to the enhancement of peripheral metabolism of glucose **(Skim et al., 1999)**. The effects of lupine fiber on the diabetic symptoms in streptozotocin induced diabetic rats showed a decreased glucose levels in urine and lowering plasma glucose **(Yamamoto et al., 2000)**.

3.2.2. Serum cholesterol and total lipids

In diabetes mellitus, hypercholesterolemia is a common complication, which is thought to be secondary to accumulation of triacylglycerol rich lipoproteins due to impaired activity of lipoprotein lipase **(Kingman, 1991)**.

Fig1 (1) and (2) shows that serum total cholesterols and lipids were significantly increased in positive control group by 116.64 % and 76.7 % respectively, which modulated in groups fed on lupine flour and lupine fiber. These results are in harmony with those obtained by others as **Newairy et al., (2002)** findings on diabetic rats treated with terms, which reduced the level of cholesterol and total lipids as compared to diabetic rats. There is confirmed opinion suggested that the soluble polysaccharides present in the hypoglycemic plants were fermented in the colon producing short chain fatty acids, notably, propionic acid, it has an inhibitory effect and reducing cholesterol synthesis (**Chen and Anderson, 1986**).

Fig1: Glucose and Total cholesterols contents (mg/100 ml) of healthy and diabetic rats fed on basal diet supplemented with different levels of lupine flour (LF) and lupine fiber (L-fiber)

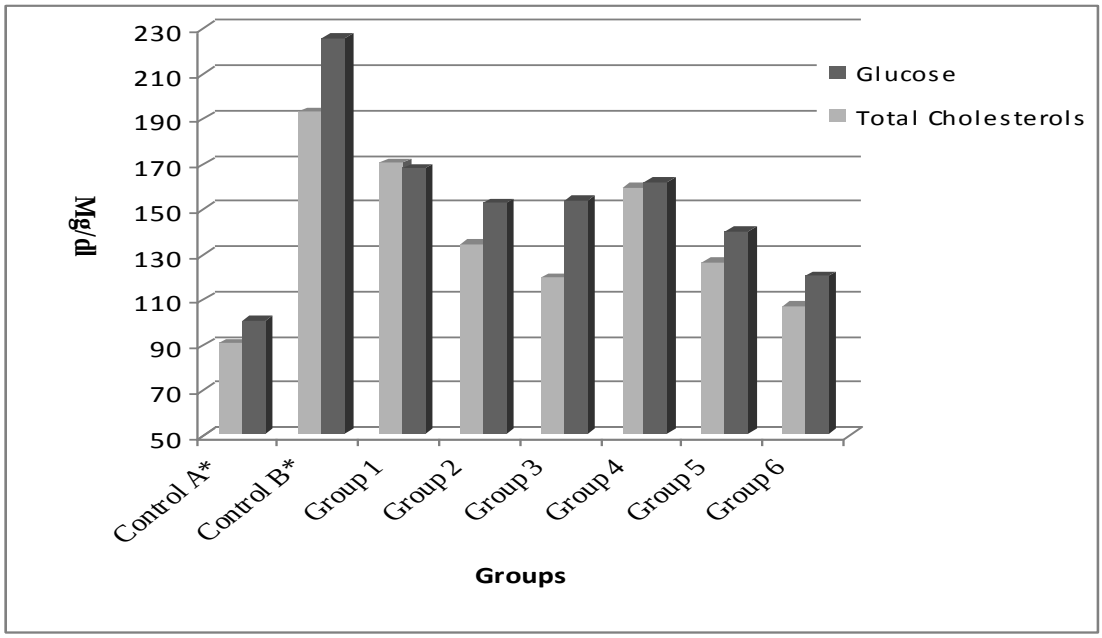
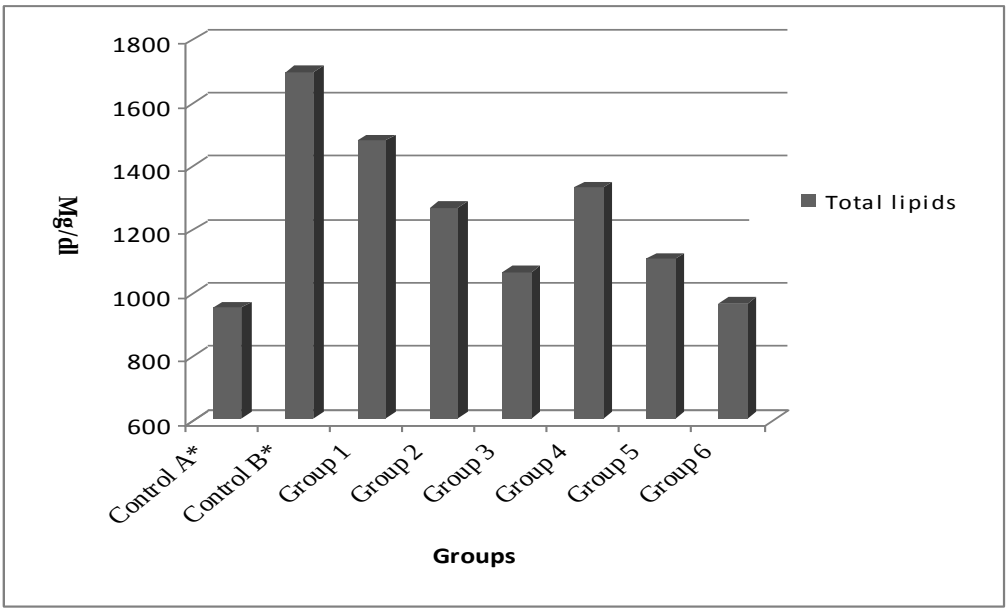


Fig 2: Total lipid contents (mg/100 ml) of healthy and diabetic rats fed on basal diet supplemented with different levels of lupine flour (LF) and lupine fiber (L-fiber).



Harisa and Alanazi, (2015) studied that the beneficial roles of lupineus luteus and lifestyle changes in management of metabolic syndrome and found that administration of lupine with lifestyle changes is good intervention for prevention and treatment of metabolic syndrome. Also, it has been shown that dietary propionate reduce total plasma cholesterol (**Bush and Milligan, 1971**). Other observation stated that some saponins lowered both total and LDL-cholesterol levels in the plasma hypercholesterolemic animals **(Sidhu et al., 1987)**, also increased the execration of cholesterol to 65 % **(Sim et al., 1984)**.This phenomenon regard to saponins stimulated lipoprotein lipase activity and it indicated that rats receiving germinated lupine might stimulate enzymes relating to the metabolism of lipids including cholesterol **(Sim et al., 1984)**. **Koo, (1983)** reported that saponins had been shown to prevent atheroscolerosis in experimental animals. **El Shewey, (2000)** indicated that rats receiving germinated lupine had significantly lowering levels of total cholesterol. It has been reported that lupin upregulates LDL receptors, and down regulates cholesterol biosynthesis genes **(Fontanari et al., 2012)**. The same author added that, lupine interferes with cholesterol enterohepatic circulation and decreases the accumulation of fat in the liver.

Dike et al., (2001), reported that fermented carob present a significant lowering levels of cholesterol. **Haber, (2002)** reported that carob pod fiber can significantly reduce cholesterol levels especially low density lipoprotein \ cholesterol in hypercholesterolemia people. **Zunft et al., (2001)**, reported that patients with hypercholesterolemia (total cholesterol (232-302 mg\dl) consuming 15 g of fiber\day as a supplement to their regular diet, after 4 weeks reduction of 7.1 % and 10 % in mean total cholesterol and LDL cholesterol and after 6 weeks showed 7.8 % and 12.2 % in both respectively.

IV. Conclusion

The lupine flour showed higher levels of moisture, crude protein, ash, crude fat and dietary fiber total phenolic and total flavonoids than the wheat flour. Conversely, wheat flour showed higher levels of starch and total flavonols. Results clearly indicate that lupine flour exhibited higher antioxidant activity with DPPH and ABTS than the wheat flour. Feeding diabetic rats on lupine flour and lupine fiber showed significant reduction in hyperglycaemia, Hypercholesterolemia and hyperlipidemia levels recorded. Finally, it could be conclude that lupine flour or fiber may be used successfully as hypoglycaemic agents in diabetic management.

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