



Improvement of Lipid Profile and Antioxidant of Hyperlipidemic Albino Rats By Functional *Plantago Psyllium* Cake

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Abstract

Plantago psyllium (PP) seed (black psyllium) is a valuable medicinal plant with plenty of biologically effective compounds. In this study, the dose-dependent effects of PP-based cakes on hyperlipidemia and oxidative damage in male Albino rats. The PP-based cakes 10% and 30% were administered with a hyperlipidemic diet for 28 days. The consumption of PP-based cakes by hyperlipidemic rat caused a reduction in total cholesterol and LDL-cholesterol levels and an increase in HDL-cholesterol. Utilization of PP-based cakes improved the antioxidant enzyme activities of the liver (GSH, SOD, GPx, and CAT) and caused a reduction in peroxidation of lipids. Additionally, there were no significant changes in liver enzymes levels (ALT and AST) in both the control and treatment groups. Beneficial effects of PP seeds resulted from their content of sterols, fibers, flavonoids and polyphenols. Overall, the results indicate that *Plantago psyllium* (PP) seed are promising sources of natural antioxidants and hyperlipidemic that can be used as additives in functional foods.



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Introduction


There are more than 200 species of the *Plantago* genus of which *Psyllium* is a common species. Mucilage-rich *Plantago* seeds are primarily acquired from *Plantago ovata* (blond *Psyllium*) and from *Plantago Psyllium* (PP) (black *Psyllium*).¹ Mucilage

in *Psyllium* consists of D-galacturonic acid, D-xylose, L-arabinose and other sugar traces.¹ Owing to its powerful capacity for forming a gelatinous material with water, *Psyllium* is classified as a mucilaginous fiber. It is a valuable medicinal plant with compounds such as phenols and flavonoids. *Plantago* plants have

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potent antioxidant activity due to their high content of phenolic compounds. *Psyllium* contains nutritious polyunsaturated fatty acids (PUFAs), as the essential fatty acids (Omega-3 and Omega-6), metabolites with bioactivities and, sulfur containing amino acids, making it a good choice for the development of functional food. *Psyllium* has hypolipidemic effect as it decreases the level of serum cholesterol due to attaching bile acids in intestine and decreasing coronary artery disease risk.²

Because of the nutritional and physicochemical characteristics of PP, and stability at various pH levels and temperatures, adding *Psyllium* to baked products have been studied extensively e.g. in supplementing wheat flour³ in mixtures free of gluten.⁴ Other studies' findings revealed increased absorption of water and enhanced dough workability with the incorporation of PP.⁵ Based upon the rate of hydration this inclusion also affected starch gelatinization and retro-gradation.⁶ The forming characteristics of *Psyllium* fiber; when it is well hydrated can affect the batter volume texture and the bread's crumb structure and thus help increase the dietary fiber concentration and give a lengthy shelf life to baked products.^{4,5}

Plantago products are becoming more and more important due to their nutritional and technical functions. This research was therefore intended to examine the hypolipidemic impact of *Psyllium* seeds and antioxidative activities in liver and blood when cake supplemented with *Psyllium* 10% and 30% -using whole commercial *Psyllium*- introduced to hyperlipidemic rats.

Materials and Methods

Plant Material and Preparation of Seeds

Plantago Psyllium seeds were purchased (October 2018) from a local market in Alexandria. To remove impurities, seeds were cleaned and then ground to fine powder and stored in an air-tight container till analysis.

Ingredients

Plantago Psyllium seeds powder and other cake ingredients (Listed in Table 1) were purchased from a local market in Alexandria, Egypt. For all formulations, *Psyllium* was ground and the particle fraction was between 0.5 and 1 mm.

Table 1: Recipe of cake supplemented with Psyllium seed powder (Grams based on the weight of cake flour)

| Ingredient | Blends | | |
|----------------------|--------|---------|---------|
| | C* | 10% PP* | 30% PP* |
| Flour | 1000 | 900 | 700 |
| Psyllium seed powder | 0 | 100 | 300 |
| Oil | 200gm | 200gm | 200gm |
| Refined sugar | 250gm | 250gm | 250gm |
| Eggs | 250gm | 250gm | 250gm |
| Baking powder | 25gm | 25gm | 25gm |
| Milk powder | 40gm | 40gm | 40gm |
| Vanilla | 2gm | 2gm | 2gm |
| Water | 150ml | 150ml | 150ml |

*C= 100% wheat flour; **PP = Psyllium seed powder.

Preparation of Cake

Cake formulations using wheat flour in different ratios are given in Table 1. Cake making was prepared according to Sudha, *et al.*,⁷ Wheat flour blends with of 0, 10 and 30 % of *Psyllium* seed powder were

used for making cake. Sugar and oil were mixed for 10 minutes to prepare the cake batter. Eggs were then added and blended. Thereafter, dry ingredients were added (milk powder, whey protein, baking powder, vanilla and flour). As a functional food

ingredient, *Psyllium* powder was introduced to the formulation. At the end, water and batter were added and blended for 5 min. All cakes have been manufactured on equal terms. An amount of 1500 g of cake batter were prepared. Then, cakes of 40 g batter were baked at 180-190°C for 20-25 minutes. Cakes were kept at room temperature in polyethylene packs.⁷

Proximate Analysis of *Plantago Psyllium* Seeds and their Supplemented Cake

In accordance with the AOAC methods 942.05 and 930.15,⁸ the ash content and moisture were determined. A Kjeldahl system was used for calculating the total nitrogen content. After Soxhlet extraction of dried specimens with hexane the raw fat content was measured gravimetrically. Triplicate measurements have been made. Difference method was used to calculate the total carbohydrate.

Extraction of *Plantago Psyllium* Seeds

Preparation of the Extracts

Twenty grams of powdered *Plantago Psyllium* seeds were extracted in 200 ml water by conventional extraction method at room temperature for 6 hours. For the removal of peel particles, the mixture was filtered through a Whatman No. 2 filter paper. The residues have been re-extracted twice, to make sure particles are fully recovered. The extract yield was lyophilized in a jar at -4°C before testing.

Determination of Antioxidant Activity of *Plantago Psyllium* Seeds Extract and their Cakes Products:

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging assay (Brand-Williams, *et al.*,⁹) was used. For antioxidant activity determination, 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was adopted (Re *et al.*,¹⁰).

Sensory Evaluation

Products were subjected to sensory evaluation test along with the control; by using a 9-point hedonic scale from 1 (extremely dislike) to 9 (extremely like).

The *In-Vivo* study

Experimental animals: A total of 28 Albino rats (180-200 g) were obtained from the Faculty of Pharmacy and Drug Manufacturing Animal House, Pharos University in Alexandria, Egypt. They were acclimated for 3 days before starting the experiment.

Induction of Dyslipidemia

High fat diet (HFD) (46% of calories from fat); 1 kg (standard laboratory diet), 1/2kg plant margarine rich in saturated fatty acids (SFA) from palm oil, and 1/4 kg lard.¹¹

All animals had standard cages (7 / cage), a 21-23°C of air conditioning, 60-65% relative humidity and a 12-hour light/12-hour dark period. Consuming water *ad libitum* until the experiment was completed. The animals received proper care according to the Scientific Research Committee of Pharos University, Manual for Care and Use of Laboratory Animals.

Four Groups Comprising 7 Rats in Each Group;

- Group: a) Rats fed on normal diet (Standard laboratory diet consists of: 60% Carbohydrates, 19% protein, 6% fats, 2.3% fibers and more than 5% vitamins and minerals, Energy: 3200 Kcal/kg). served as a negative control;
- Group: b) Rats fed on HFD for 4 weeks, and (control cake) (without added *Plantago Psyllium* powder); served as a positive control
- Group: c) Rats fed on HFD for 4 weeks, and PP cake 10% for 4 weeks;
- Group: d) Rats fed on HFD for 4 weeks, and PP cake 30% for 4 weeks.

Table 2: proximate composition of the standard diet and High fat diet

| Ingredient | Negative control(C) | Positive control(HFD) |
|-----------------------|---------------------|-----------------------|
| Carbohydrates | 60% | 60% |
| Protein | 19% | 19% |
| Fats | 6% | 46% |
| Fibers | 2.3% | 2.3% |
| Vitamins and minerals | 5% | 5% |

Measurements of Body Weight and Biochemical Blood Analysis

Body weights of all groups under investigation were assessed twice per week and at the end of experiment period, for 8 weeks the percentage of gain weight was expressed as (final weight-beginning weight)/ beginning weight) x 100.

After pentobarbital anesthesia, the blood samples were collected to obtain serum for assessment of aspartate aminotransferase (AST), and alanine aminotransferase (ALT) total protein, albumin content, total cholesterol and high-density lipoprotein (HDL) cholesterol, and triacylglycerol. Standard enzymatic spectrophotometry was performed for glucose and cholesterol using Bio-diagnostic kits (Giza - Egypt).

Antioxidant Enzyme Analysis

Reduced liver glutathione (GSH) was determined according to the method of Jollow.¹² Catalase (CAT; EC 1.11.1.6) activity was determined.¹³ Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured in different tissues according to

Misra and Fridovich.¹⁴ The activity of Glutathione peroxidase (GPX, EC. 1.1.1.9) was assayed according to Chiu.¹⁵ Liver thiobarbituric acid-reactive substances (TBARS) were measured at 532 nm by using 2-thiobarbituric (2,6-dihydroxy pyrimidine -2-thiol; TBA). An extinction coefficient of 156,000 M⁻¹ cm⁻¹ was used for calculation.¹⁶

Histopathology of Liver

Immediately after necropsy, the rats' livers were dissected out carefully and weighed. The rats' livers then were fixed in 10% formalin. Liver tissues were further embedded in paraffin blocks and sectioned into 5-6µm sections using Microtome (Leica, Italy) for serial specimens, mounted on glass slides and stained with hematoxylin eosin (HE) stain for histological light microscopic evaluation.¹⁷

Statistical Analysis

Mean and standard deviation were calculated for each studied variable. The interpretation of data obtained was done by analysis of variance (ANOVA) test. Level of significance was accepted at P<0.05.

Table 3: Chemical composition of *Plantago psyllium* seeds and supplemented cake (g/100 g dry weight)

| Determination (%) | PP seeds | PP Cake 10% | PP Cake 30% |
|-------------------|--------------|--------------|--------------|
| Protein | 9.34 ± 0.81 | 15.36 ± 0.70 | 11.70 ± 1.57 |
| Fat | 5.16 ± 0.85 | 5.48 ± 0.41 | 5.15 ± 0.80 |
| Carbohydrate | 59.26 ± 1.30 | 68.45 ± 1.55 | 70.33 ± 3.81 |
| Fiber | 23.83 ± 1.94 | 7.77 ± 1.19 | 9.43 ± 1.29 |
| Ash | 2.38 ± 0.70 | 2.92 ± 0.73 | 3.38 ± 0.74 |

Results are expressed as means ± standard deviation of three measurements.

Results and Discussion

Proximate Analysis of *Plantago Psyllium* Seeds and their Supplemented Cake

Plantago Psyllium seeds are known to increase the final product's nutritional value in wheatmeal flour.¹⁸ Table 3 shows the chemical composition of the cakes. The fiber content of *Plantago Psyllium* cake has been gradually increased with increasing *Plantago Psyllium* ratio; from 7.77 ± 1.19% in PP cake 10% to 9.43 ± 1.29% in PP cake 30%. Improved cake fiber content corresponds to the high *Plantago Psyllium* fiber content (23.83 ± 1.94%). Carbohydrate, ash,

and crude fiber contents of the PP cake mixes 30 percent, surpass PP cake 10% mixes and protein and fat content in *Plantago Psyllium* cupcake mixes 30% was lower than 10% (Table 3).

Compared with *Psyllium* cake samples, the seed sample had lower ash levels. A sufficient consumption of dietary fiber is well documented to reduce the risk of a variety of chronic diseases. The significant mineral content in the *Psyllium* sample (2.67 per cent w / w) was responsible for a rise in ash; similar results were found by Raymundo.¹⁹

Findings concerning *Plantago Psyllium's* cake were similar to the previous data stated by Beikzadeh¹⁸; that adding PP to wheat flour improves the nutritional value and gives cakes with higher carbohydrate, fiber and ash content than the control from wheat flour alone. On the other hand, Kaushal and Bhatia²⁰ showed that protein, fat, carbohydrate and ash contents of PP seeds ranged from 23 - 30 %, 2.9 - 3.5%, 50 - 60% and 3.0 - 3.5%, respectively. Romero-Baranzini²¹ cited that, PP contained crude

proteins (17.5%), crude fats (6.7%), ash (2.7%) and carbohydrates (49%). The amount of protein determines the effect of inclusion of *Psyllium* seed on the consistency of the cake. An increase in the amount of protein has been recorded to improve cookie firmness by Fustier.²² The total fiber was observed to be affected positively by increased content of PP. Arabinose, galactose, galacturonic acid, and rhamnose are the most common types of soluble fiber in *Psyllium* seed.²³

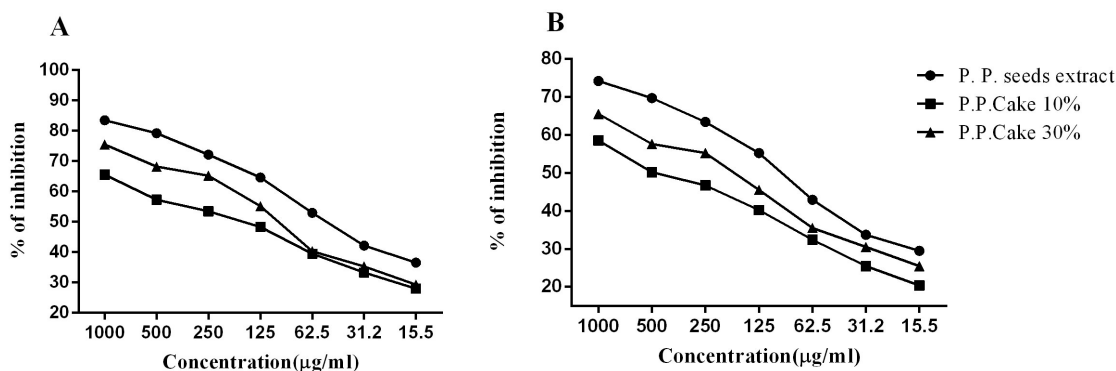


Fig. 1: Antioxidant activity of *Plantago psyllium* seeds extract and their supplemented cake. (A), ABTS; (B), DPPH; PP, *Plantago psyllium*

Antioxidant Activity of *Plantago Psyllium* Seeds Extract and their Supplemented Cake

Antioxidants play a key role in health promotion in the nutraceutical sector and inhibit functional food ingredient oxidation procedures. In addition, their activity can be measured with various mechanisms.²⁴ In the present study several antioxidant analyses, including radical scavenging of ABTS⁺ and DPPH, were conducted at several concentrations (15.62 to 1000 µgml⁻¹) to determine the antioxidant and various scavenging actions of *Plantago Psyllium* plants and their fortified cake (Fig. 1). The reducing capacity, antioxidant and scavenging activities of the extract of *Psyllium* seeds increased concomitantly with the concentration as well as increase supplementation of seeds in cake product (Fig. 1).

The capacity of ABTS radicals to scavenge (expressed as the percent inhibition) was used as an indicator for estimating total antioxidant activity.^{25,26} The maximum percent inhibition of *Plantago Psyllium* seeds was found to be 83.4% for 1000 µg/

ml concentration. In accordance, Pourmorad²⁷ found that the antioxidant activity of PP is 89.3% for 1000 µg/ml concentration. Similarly, in the present study 65.5% and 75.4 % inhibition were observed for PP cake 10% and PP cake 30%, respectively at 1000 µg/ml concentration (Fig. 1B). The DPPH radicals ' stability has been commonly used as a tool to estimate plant samples by free radical scavenging activities.²⁵ Similar to ABTS radicals, the highest DPPH scavenging activity for 1000 µg / ml was noted (Fig. 1). The maximum scavenging percentage 74.2% was found for *Plantago* seeds extract.

It was also observed that PP cake 30% extract showed significantly higher DPPH scavenging activity compared to PP cake 10% extract (Fig. 1A). The free-radical DPPH is more stable and can accept electron or hydrogen.²⁸ The findings showed that *Plantago* seeds extract could behave as a donor of hydrogen to scavenge DPPH free radicals.^{25, 26, 28} In this regard, Kobeasy²⁹ reported that, *Plantago* contained the following biologically active compounds: (vanylic acid), phenolics

(caffeic and ferulic acids) and flavonoids (luteolin). The findings of our study are consistent with Beara³⁰ who studied the antioxidant activities of *Plantago* species and stated that it could be viewed as a potential new natural antioxidant source.

Sensory Evaluation of *Plantago Psyllium*-Based Cake

The current trial explored the effect of *Plantago Psyllium* seed powder and its utilization into cake products (Fig 2). The sensory characteristics of the product were analyzed by 9 point hedonic scale. The PP products were desirable in term of color, taste and texture (Fig 3). The result of sensory evaluation showed that the incorporation of PP powder up

to 10% was found to be the most acceptable and achieved the highest score for cake products. Generally, PP incorporation makes the crumb more reddened, darker and less yellowish. The natural pigments in *Psyllium* seed are among the main reasons for color change.¹⁸ Meanwhile, Sudha³¹ noted that inclusion of fiber caused darkening of the resultant products.

(1) Dislike extremely; (2) dislike very much; (3) dislike moderately; (4) dislike slightly; (5) neither like nor dislike; (6) like slightly; (7) like moderately; (8) like very much and like extremely (9).

Values are mean +SE of thirty of panelists



Fig. 2: *plantago psyllium* seeds raw material (A), functional *plantago psyllium* cake 10% (B) and functional *plantago psyllium* cake 30 % (C)

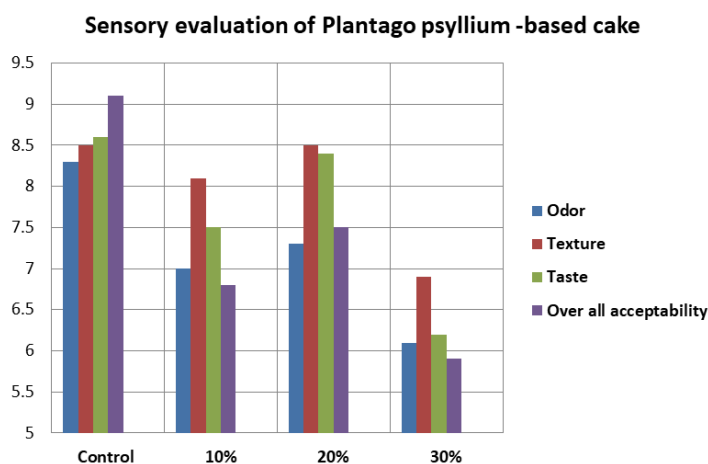


Fig. 3: Sensory evaluation of *Plantago psyllium*-based cake

Data of the present study indicated that among samples with 10 and 30 % PP, no significant differences in textural toughness were found. *Psyllium* seeds at lower concentrations are favorably improving

the gluten network, as found by Raymundo.¹⁹ An increase in cakes' fiber content; from 10 to 30 percent results in an increase in textural hardness. When approximately ten percent of *Psyllium* seeds

were added to cake in different studies, form, shape, crust, pores and taste have improved. However, the texture softness and hardness or chewiness were not significantly affected.¹⁸

In addition to difficulties in handling the product a negative effect on sensory tests was observed at higher *Psyllium* added concentrations in the

Park³² report. Similar effect has been shown by Raymundo.¹⁹ Moreover, the inclusion of 7.5 percent of *Psyllium* seeds can usually improve the overall sample quality.¹⁸

Effects of normal, high-fat diet and *Plantago Psyllium* cake diet supplementation on weight change, weight gain and feed intake of rats

Table4: Effects of normal, high-fat diet and *Plantago psyllium* cake diet supplementation on weight change, weight gain and feed intake of rats

| Parameters | Negative Control | (PP- control cake) | PP Cake 10% | PP Cake 30% |
|--------------------|--------------------------|--------------------------|-------------------------|-------------------------|
| Initial weight (g) | 181.5±5.3 ^a | 180.5±3.2 ^a | 177±3.2 ^d | 178±4.2 ^a |
| Final weight (g) | 240.3±5.3 ^a | 268.8±8.9 ^b | 233.4±2.1 ^c | 220.2±2.6 ^c |
| weight change(g) | 32.39 ±7.74 ^a | 32.84 ±5.61 ^b | 24.16±6.10 ^d | 19.16±3.62 ^d |
| Feed intake(g) | 22.5 ±0.7 ^a | 28.4 ±0.04 ^a | 24.7±0.7 ^a | 22 ±1.5 ^a |

Results are expressed as mean ± SD (n= 7). Means which are not significantly different have the same symbol; significance level at $P \leq 0.05$.

Table (4) reveals a significant increase in the body weight gain of the positive controls has also been found with the addition of HFD (cake free) in comparison to the negative control, a significant difference between body weight gain of the two PP cake 10% & 30% (24.16 and 19.16). There has

also been a positive link between the lower weight gain and the 30 percent PP cake consumption. This may be due to the presence of soluble gel-forming mucilage from the *Plantago Psyllium* seeds.³³ The development of viscous materials can help satiety and aid weight loss.

Table5: Effects of normal, high-fat diet and *Plantago psyllium* cake diet supplementation on serum lipid profile

| Treatments | TG (mg/dL) | TC (mg/dL) | LDL-C (mg/dL) | HDL-C (mg/dL) |
|--------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| Negative Control | 157.30±1.64 ^c | 121.73±2.02 ^c | 87.83±2.25 ^c | 33.90±2.27 ^c |
| (PP- control cake) | 204.37±2.27 ^a | 140.67±1.04 ^a | 107.90±1.47 ^a | 32.76±2.51 ^d |
| PP Cake 10% | 166.57±3.45 ^b | 132.10±1.32 ^b | 93.13±0.55 ^b | 38.96±1.30 ^c |
| PP Cake 30% | 154.67±1.50 ^d | 125.70±0.65 ^d | 81.33±1.46 ^d | 44.36±1.09 ^a |

TG, Triglycerides; TC, Total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol

Data are expressed as mean ± SD (n= 7). Means which are not significantly different have the same symbol; significance level at $P \leq 0.05$.

Serum Lipids

The current findings showed significantly increased levels of TC, TG and LDL-C in rats fed a high-fat diet (cake free) compared with the control group (Table 5). However, there has been a

reduction in HDL-C concentration in the HFD group ($P < 0.05$). Lard fat is abundant in saturated fatty acids, associated with boosting total and LDL-cholesterol, and is rich in monounsaturated fatty acids, that may increase serum triacylglycerol.^{34,35}

Moreover, *Plantago Psyllium* has shown a significant decrease in total cholesterol and LDL cholesterol when administered to humans with high cholesterol. It is also found effective in animals.³⁶ Increased serum triglyceride concentration may be ascribed to reduced triglyceride clearance secondary to reduced lipoprotein lipase (LPL) activity.³⁷ The elevated LDL-cholesterol concentration observed in hyperlipidemic rats can be ascribed to a reduction of saturated fatty acids and cholesterol included in the diet in LDL receptors.³⁸

Plantago Psyllium soluble fiber attaches to small intestine bile acids, forming a complex which prevents reabsorbing bile from the small intestine, thus increasing the production and secretion of the bile acids by drawing cholesterol to be used as a substitution for the missed acids³⁹; thereby lowering blood cholesterol levels.

Feeding the rats on HFD with PP cake 30% induced significant decreases of triglycerides (24.31%), total cholesterol (10.64 %) and LDL-cholesterol (24.62%) concentrations in comparison with the high-fat diet group. A significant decrease of four percent in total serum cholesterol and a 7 percent decrease in LDL cholesterol compared to the control diets were found by Anderson.⁴⁰ Cake 30 percent showed enhanced significant levels of HDL-cholesterol (26.14 %, compared with the PP-free cake group) due to PP seeds' ability to accelerate the decline of radical species produced during the administration of cholesterol.⁴¹ As a free radical scavenger, HDL-C prevents the peroxidation

of beta-lipoproteins.⁴² *Plantago Psyllium* seeds demonstrated enhanced anti-hyperlipidemic activity in rats but the extract regulation mechanism for TC, TG and LDL-C is not known. Meanwhile, PP can boost HDL cholesterol levels, which are still the main risk factors for coronary artery disease in the regular diet suggested by medical guides to fight against hypercholesterolemia.³⁹

Data presented here are in agreement with Xing³⁹ who recorded the PP extract and powder protection impact on hypercholesterolemic rats evidenced by a significant lower value of serum cholesterol, triglycerides, LDL level, cholesterol/ HDL, LDL / HDL. Furthermore, Anderson⁴⁰ reported that PP extract administered orally for four weeks to hypercholesterolemic and diabetic rats resulted in a superior lipid profile and a reduction in blood sugar level.² Similar observations were made by a number of workers, demonstrating a hypolipidemic effect of PP in experimental animals.

Liver Enzymes Activity, Total Protein and Albumin

The two main measures of liver damage and an evaluation toxicology test are the aspartate transaminase (AST) and the alanine transaminase (ALT).⁴³ The activities of serum AST and ALT in the present study were significantly high in the HFD compared to normal rats; these findings are in agreement with Sudhahar.⁴⁴ No significant differences were found in ALT & AST between the negative control group and PP cake 30%.

Table 6: Effects of normal, high-fat diet and *Plantago psyllium* cake diet supplementation on liver biomarkers

| Treatments | Total protein (mg/dL) | Albumin (mg/dL) | AST (U/L) | ALT (U/L) |
|-------------------------|------------------------|-------------------------|-------------------------|--------------------------|
| Negative Control | 8.50±0.26 ^a | 6.60±0.10 ^a | 50.66±1.41 ^c | 39.20±1.3 ^c |
| HFD + (PP-control cake) | 8.36±0.20 ^a | 5.95±0.33 ^{ab} | 66.33±1.04 ^a | 54.166±1.60 ^a |
| PP Cake 10% | 8.31±0.18 ^a | 5.76±0.50 ^b | 57.96±1.25 ^b | 45.43±0.70 ^b |
| PP Cake 30% | 8.44±0.15 ^a | 5.80±0.42 ^b | 52.13±1.46 ^c | 40.21±1.23 ^c |

Control: Normal diet; HFD: High Fat Diet; PP: *Plantago psyllium*

Consumption of HFD induced high levels of ALT and AST in hyperlipidemic rats compared to normal rats

(Table 6). Thus, if any toxic interaction is produced by the *Plantago Psyllium* cake diet, this could be noted

by increasing liver damage, the findings show that no toxicity or damage to the liver could be recorded. this result is consistent with those of Obatolu.⁴⁵ The total protein and albumin levels were not significantly different in all studied groups; they were within the normal range. As diets used in the present study were protein sufficient.

Activity of Antioxidant Enzymes

The unwanted effects of oxidative agents are affected by endogenous enzyme and non-enzyme antioxidants. Water-soluble antioxidants are SOD, GPx and CAT. Antioxidant enzymes like SOD, GPx, and CAT, may remove the reactive oxygen (ROS) and reactive nitrogen (RNS) species. The SOD is the first defensive line against oxidants, leading to oxygen disputes with H₂O₂. The GPx eliminates the need for glutathione (hydrogen donor and scavenger of H₂O₂, radical hydroxyl and chlorinated oxidants), to reduce organic peroxides H₂O₂ to H₂O and O₂. The H₂O₂ is reduced to water by CAT.^{46,47}

Enzyme complexes for free radical scavenging as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were tested to determine excessive oxidant stimulation (Table 7). The PP cake

30% was found to increase the activity of GSH, SOD, GPx, CAT enzymes by 64.80%, 32.07%, 49.96% and 36.37 %, respectively, in comparison to hyperlipidemic the control rats. Reduction in enzyme activity is linked to the aggregation of highly reactive free radicals which lead to adverse effects such as the loss of integrity and function of the cell membrane.⁴⁸ When free radicals are present in elevated levels, they can associate with the enzymes and inactivate them.⁴⁹ Moreover, Jung⁵⁰ observed an improvement in antioxidant potential in Sprague-Dawley (SD) rats on HFD supplemented with chitosan and *Psyllium* husk. Patel⁵¹ reported high antioxidant (*in vitro*) activity in *Psyllium*-extracted polysaccharide (*P. ovata*).

Pigeolet⁴⁹ revealed that PP has high concentration of phenolics which allow free radicals to break down, one of the key anti-oxidation mechanisms inhibiting lipid peroxidation chain reactions. The effect on polyphenol production was reported to be substantial, which makes possible that increased antioxidant activity to be related to increased production of polyphenols and flavonoids as well as improved hepatic antioxidant status, as measured by SOD and GPx activity when supplemented by PP.

Table 7: Effects of normal, high-fat diet and *Plantago Psyllium* cake diet supplementation on tissue antioxidant enzymes

| Treatments | TBARS (nmol/gm/ tissue) | GSH (IU/gm tissue) | SOD (IU/gm tissue) | GPx (IU/gm tissue) | CAT (IU/gm tissue) |
|-------------------------|-------------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| Negative Control | 30.63±1.15 ^b | 3.30±0.43 ^c | 66.23±1.70 ^b | 35.26±1.53 ^b | 34.46±1.45 ^c |
| HFD + (PP-control cake) | 45.23±1.64 ^a | 1.83±0.20 ^d | 50.47±1.45 ^c | 20.38±0.47 ^c | 26.06±0.88 ^d |
| PP Cake 10% | 32.86±1.34 ^b | 4.54±0.21 ^b | 67.73±0.68 ^b | 37.53±1.67 ^b | 37.36±0.66 ^b |
| PP Cake 30% | 31.96±0.95 ^b | 5.20±0.10 ^a | 74.30±1.85 ^a | 40.73±1.53 ^a | 40.96±1.68 ^a |

Data are expressed as mean ± SD (n= 7). Means which are not significantly different have the same symbol; significance level at P ≤ 0.05.

TBARS: Thiobarbituric acid reactive substances; GSH: Glutathione; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; CAT: Catalase.

In the present study hyperlipidemic rats showed elevation of hepatic TBARS concentration relative to negative control group (30.63±1.15 nmol/gm/ tissue) vs. treatment group (PP Cake 30%) (31.96±0.95 nmol/gm/ tissue) (Table7). A rise in TBARS levels has previously been recorded in HFD animals.⁵²

A positive correlation between total cholesterol and levels of triacylglycerol and generation of free radicals was demonstrated.⁵³ Furthermore, during the *in vitro* callus culture of this plant, the use of *Plantago Psyllium* seed additives demonstrated an increasing polyphenol and flavonoid contents.

Therefore, present study would be useful for the use of tissue cultivation in plant tissue production and their enhanced recovery from the tissue of the callus³¹. This can be linked to enhanced polyphenols accumulation during callus aging. The same kind of comment was made by Ozyigit.⁵⁴

The PP also contains high levels of polyphenolic antioxidants including flavonoids, ellagic, ferulic and gallic acids, pteridins, and catechins.

The polyphenols have important biological activity, including the inhibition of oxidation, free radical eradication and risk reduction of cardiovascular diseases. The anti-mutagenic and promising antioxidant activity of PP may be a responsibility of ellagitannins.⁵⁵ A product which improves the overall antioxidant status and reduces the oxidative stress markers of healthy individuals may use *Psyllium* as an additive.⁵¹

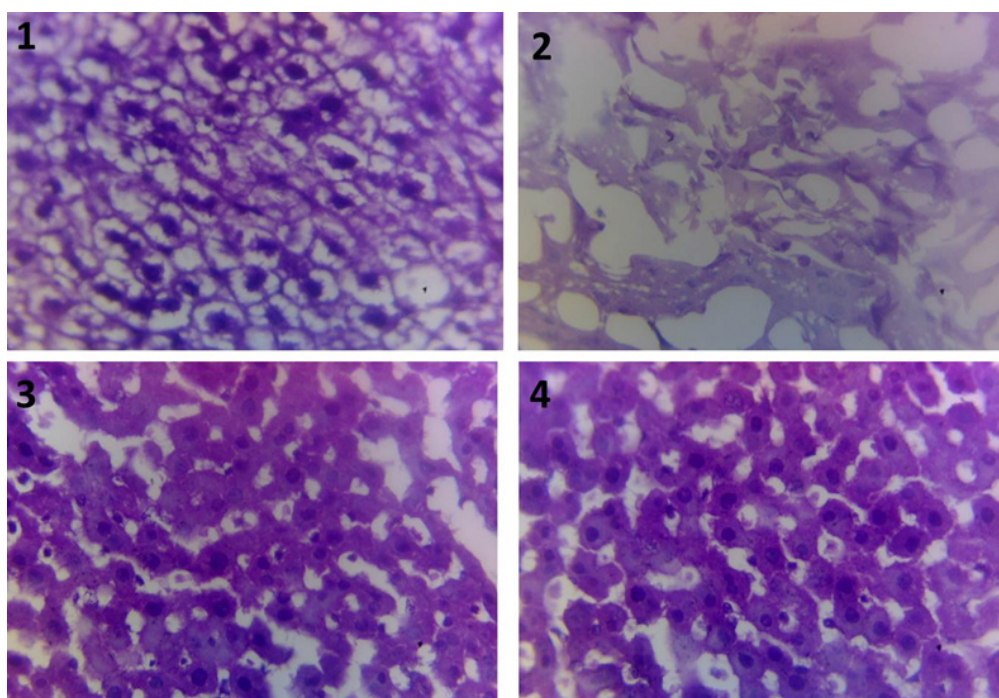


Fig. 4: Effects of *Plantago psyllium* cake on histology of liver tissues in rats fed a high fat diet (40×).

(1): rats on the normal diet (ND); (2): rats on the high fat diet (HFD); (3): rats on the high fat diet + *Plantago psyllium* cake 10%; (4): rats on the high fat diet + *Plantago psyllium* cake 30%

Liver Histology

The effects of *Plantago Psyllium* on liver histopathology are shown in Fig. 4. Hematoxylin and eosin staining revealed variations in the composition of the liver tissue and the lipid aggregation in rats with various dietary treatments.

The negative control group's livers had a structure that is well organized, but the HFD group revealed significant morphological changes. Their livers' structures showed a high degree of damage and

signs of necrosis and macro-vacuolization in hepatocytes. While the supplementation with PP visibly alleviated hepatocyte steatosis and lipid accumulation. In contrast with the negative control, the rats with HFD exhibited increased adipocyte size (Fig. 4). This was confirmed by the improvements in number of adipocytes per spot.

Higher levels of visceral fat in the liver often are accompanied by higher levels of serum lipid and are associated with insulin resistance, cardio-vascular

risk, and dyslipidemia.⁵⁶ Furthermore, excess fat accumulation in the liver can cause liver oxidative stress and liver damage.⁵⁷ Histological findings showed a significant decrease in lipid deposition in rats on *Plantago Psyllium*. *Plantago Psyllium* permits substantial reductions in liver and adipose tissue volume, which indicates that the administration of *Psyllium* may minimize hepatic steatosis and obesity caused by HFD.

Conclusions

Regarding to its high nutritional value, *Plantago Psyllium* seeds are promising sources of new biological molecules, and multi-functional polysaccharides and *Plantago Psyllium* products. The *Plantago Psyllium* seeds have a positive effect

as a prebiotic that can decrease the risk of various chronic conditions. To date, however, limited data have proven these health benefits. Further studies may be required to investigate the oxidative stress reduction mechanisms of this remarkable crop and its safety for humans or animals.

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Conflict of Interest

The authors declare no conflict of interest with any person or Organisation in publishing this article.

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