



Anti-Obesity and Anti-Inflammatory Effects of Banana Peel Extract Phytosome Dietary Supplement in High Fat Diet Induced Obesity in Male Sprague Dawley Rat Model

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ABSTRACT

The object is to examine the possible effect of banana peel extract phytosome dietary supplement (BPPS) on certain inflammatory biomarker complementary 3 (C3) and investigate the obesity related complication as measured by glycemic profile in the high fat diet- induced obesity in Sprague-Dawley rat, and also to determine phosphorus and poly phenol content of banana (*Musa cavendishii*) peel extract

Forty two male Sprague-Dawley rats (180-220 g of weight) were randomly divided into two main dietary groups: normal fat diet group (NFD; n=21, 9.5% fat) and HFD group (n=21, 45.0% fat) for 8 weeks. Seven rats of each group were sacrificed for baseline data analysis. The remaining rats (n=14) in the HFD group were further randomly sub-divided into two sub-groups for the second period of the study (8 weeks) when rats were fed their respective diets and subjected to the following interventions: (1) banana peel extract phytosome supplementation with NFD (NFD-BPPS; 350 mg banana peel extract)(2) NFD as control group (3) banana peel extract phytosome supplementation with HFD(HFD-BPPS; 350 mg banana peel extract)(4) HFD as control group. At the end of the study (16 weeks) all rats were sacrificed, blood samples were collected. Then, biochemical analysis including serum C3 and glycemic profile were undertaken

In conclusion, using banana peel extract as a model of high phosphorus and poly phenol extract in the form of phytosome dietary supplementation could have effects on inflammatory biomarkers complement 3 along with glycemic profile.

Keywords: Adipokines; Phytosome; Banana peel; Phosphorus; Complement

INTRODUCTION

The growing prevalence of either overweight and obesity has been described as a global pandemic, 30% of the global population, are overweight or obese and 5% of the deaths worldwide are attributable to obesity. It has now become clear that low-grade inflammation is a key player in the pathogenesis of obesity and its related to non-communicable diseases including diabetes type 2 and Hypertension.

Sterile inflammation induced by hypertrophic white adipose tissue observed in obese people while classically is identified by increased cytokine expression that affect the differentiation of immune cells and regulation of immune response also pro- and anti-inflammatory adipokines which are playing key roles in oxidative stress activation and reduce antioxidant sources [1].

MATERIALS AND METHODS

The outcomes of this study showed that acetone extract of banana peel (*Musa cavendishii*) exhibited high phenolic content (1175 ± 0.55 mg GAE/100 g), Furthermore, banana peel showed high phosphorus content (390 mg/100gm). While accompanied BPPS to HFD in this study showed significant effect on weight gain (20.00 ± 6.28 gm $p \leq 0.05$) compared to HFD (57.00 ± 4.39 gm $p \leq 0.05$), additionally the improvement of glycemic profile of HFD-BPPS fasting glucose level (87.25 ± 5.51) serum insulin level (206.75 ± 12.37 $p \leq 0.05$) and insulin resistance, estimated by HOMA (1.03 ± 0.03). Significantly as compared to HFD control group (113.75 ± 1.25), (312.25 ± 18.24) (2.01 ± 0.06) respectively; $p \leq 0.05$). Complement 3 level showed significant decrease in HFD-BPPS group (266.25 ± 2.65) compared to HFD group (488.25 ± 3.51 $p \leq 0.05$).

Based on the findings from previous studies, banana peel is a potential waste product that could be used for its nutraceutical properties directly utilized as functional compounds in human nutrition. It is rich in mineral content especially Phosphorus, also it contains high amount of phenolic compounds which are capable to protect against oxidative destruction through various mechanisms. Many reports demonstrated that the peel contains an effect for antioxidant, antimicrobial, hypolipidemic and antihypertensive properties.

The old and conservative systems of drug processing that suffered from several disadvantages, like processing problems, inaccurate dose and delivery, high cost, time consuming, etc., are resolved by advanced delivery nanosystems, which has been developed by incorporating standardized plant extract containing active compound or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes called phytosomes which creates a small cell where the specific components of botanical extracts are sheltered from destruction. Nano carriers applying to herbal medicines will carry optimal amount of the drug to their intended site avoiding all the barriers such as acidic pH of stomach and increase the sustained circulation of the drug into the blood due to their small size. The continuous development of nanomedicines has the possible to provide plentiful benefits, including better efficacy, bioavailability, dose-response, directing ability, personalization, and safety compared to conventional medicines which has major limitations of biocompatibility and colloidal size. Pharmacological interventions for weight loss still limited, with long range of side-effects often compensating effectiveness. Remarkably, significant early weight loss was associated with sustained use of the drugs The cost efficiency of long-term pharmacotherapy of obesity is still an unanswered question. Banana peel phytosome dietary supplement can be cost-effective type of supplement to treat obesity

Therefore, the research problem of this study is to investigate the effect of banana peel extract phytosome dietary supplement, as a high phosphorus and poly phenol extract model on obesity by measuring changes in weight status, glycemic profiles as well as certain inflammatory biomarkers including complementary system(C3), in Sprague-Dawley rats fed the high fat diet-induced obesity [2].

Experimental Animals and Diets

Forty two male Sprague-Dawley rats (180-220 gm of weight) were obtained from the central animal house unit at the Jordan University of Science and Technology (Irbid, Jordan). Then animals were transferred and kept at the Animal Unit, School of Agriculture, University of Jordan. Animals were housed separately in a single

metabolically-ventilated plastic cage under standard condition ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 45% to 55% relative humidity, with a 12:12 h light/dark cycle), ad libitum water and standard laboratory chow for one week of acclimatization.

The rats (n=42) were randomly divided by using simple random sampling method into two diet groups; Group (1), rats were fed high fat diet (HFD ; n=21) , Group (2) rats were fed normal fat diet (NFD; n=21). The compositions of these diets according to Reeves (1996). An American Institute of Nutrition (AIN) standard chow for nutritional and toxicological research was modified to replace casein with egg white solids (AIN-93M-EGG) as a protein source combined with modification in the composition of vitamins and minerals.

Following eight weeks, 7 rats from each group were sacrificed for baseline data. Each group were further subdivided randomly into (2) sub groups each sub group has (7) rats with (1) Banana Peelextract Phytosome Supplement (BPPS), (2) control group for 8 weeks.

Experimental Termination and Sample Collection

At the end of the study, all rats were sacrificed after 12 hours of fasting under carbon dioxide anesthesia and blood samples were collected by orbital sinus and centrifuged immediately at 3000 rpm for 10 minutes. Serum and plasma sample were kept at -20°C until used. It is worth to mention that three rats died throughout the experiment .Groups distribution and schematic diagram are shown in Table 1, Figure 1; respectively. Rats were weighed once a week. Fasting Blood Glucose (FBG) levels were measured weekly as well using glucometer (GlucoLabTM, United Kingdom). Insulin resistance was evaluated by the homeostasis model assessment for insulin resistance method [3].

Phytosome Preparation

Banana peel extract phytosome was prepared after the extraction of banana peel with acetone solvent, and then determined the polyphenol content in the extract to use it as a reference to set the amount of phosphatidyl choline PC used in phytosome preparation. The phytosome are prepared by reacting of 2 mole PC with 1 mole of polyphenols in the extracted banana peel. In brief, 2 moles of PC was dissolved in a solution containing 200 ml of acetone, and then one mole of polyphenolic compounds of banana peel acetone extract was added. The mixture was mixed on a rotary shaker for two hours at 180 rpm at room temperature. After that, ultrasonic bath was used to enhance the formation of the phytosome structure at 37°C for 15 minutes. The solvent was evaporated under vacuum using a rotary evaporator at 38°C and 120 rpm. Finally, 50 ml of acetone was added to each mixture, and then left in the dark at room temperature to precipitate phytosome structure.

Characterization and Evaluation of Phytosomes by Visualization

Microscope digital camera was used to confirm the formation of phytosome complex between plant extracts and the phospholipids.

Doses of Banana Peelextract Phytosome

Each rat in both NFD and HFD group received daily dose of 350 mg of banana peel extract in the form of phytosome .it was mixed with soybean milk and provided to rats by oral gavage (2 ml/day)(Horigome, et al., 1992).

Biochemical Analysis

Blood samples were collected by orbital sinus and centrifuged immediately at 3000 rpm for 10 minutes. Serum and plasma were kept at -20°C , until used. Insulin, and C3, were measured in duplicate by Enzyme-Linked

Immunosorbent Assay (ELISA). All the biochemical tests were examined in Mega Lab clinical laboratory Amman/Jordan by the researcher.

Brief method, dilution and preparation of samples, buffers, controls and blanks were done as recommended procedure, sample dilution was (1:10), then 100 μ L of standard or sample were added to each well and incubate for 90 minutes at 37°C. After that, the plates were washed 2 times with 100 μ L Biotin-labeled antibody working solution were added to each well and incubate for 60 minutes at 37°C. Followed by washing for 3 times and 100 μ L SABC Working Solution were added into each well and incubate for 30 minutes at 37°C then 90 μ L TMB Substrate were added. then incubated 15 to 30 minutes at 37°C finally 50 μ L of Stop Solution were added, the results were read at wave length λ 450 nm and the concentration were calculated in pg/ml.

Statistical Analysis

The normality of continuous variables were determined using a Kolmogorov-Smirnov test. All results were presented as mean \pm SEM and were evaluated by analysis of covariance (ANCOVA) to adjust the weight effect for histochemistry. $P < 0.05$ were accepted as significant for all tests. Differences among treatment means were separated using the Tukey test at $p < 0.05$. All statistical analyses were performed using for Windows.

RESULTS

In vitro experimentation

- Banana Peel Extract Analysis
- Phosphorus Determination for Banana Peel Extract
- Total phosphorus content in banana peel extract was 390 mg/100gm
- Total Phenolic Compounds Content of Banana Peel Extract
- Total phenolic compounds values of banana peel extract were expressed as mg GAE/100gm. Total phenolic compounds content in banana peel was 1175 mg/100gm
- Evaluation of Phytosomes by Visualization

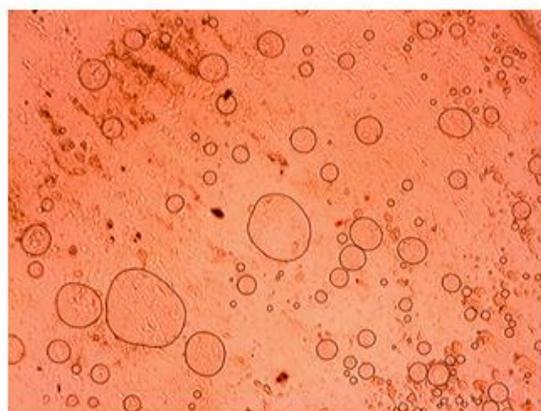


Figure 1: Represented the microscope digital camera of phytosome complex between plant extracts and the phospholipids.

In vivo experimentation

It is clear from Table 2 that the HFD group demonstrate significantly higher serum concentrations of C3 compared to the NFD group ($p<0.05$). Moreover, serum concentrations of C3 in the High Fat Diet- banana peel extract phytosome supplement was significantly low compared to the HFD group ($p<0.05$) [4].

Table 1: The Effect of the High Fat Diet Combined with Supplementation of Banana Peel Extract Phytosome for the last 8 Weeks on C3 Serum Levels.

Sub-groups	Initial weight (g)	Final weight (g)	Weight gain (g)	% of weight gain	FE
NFD (n=7)	12.29±285.50a	24.46±331.75a	44.16±6.05 a	+15.4a	0.61±0.02 a
NFD-BPPS (n=7)	9.40±283.66a	16.25±317.00b	6.88±33.33b	+11.7b	0.47±0.04 a
HFD (n=7)	15.47±312.80b	13.78±369.80c	57.00±4.39 c	+18.2c	0.69±0.06 a
HFD-BPPS(n=7)	5.81±333.00b	7.88±353.00b	6.28±20.00b	+6.0d	0.26±0.03 a

Table 2: The effect of the high fat diet combined with supplementation of banana peel extract phytosome for the last 8 weeks on final fasting blood glucose, fasting insulin and homeostatic model assessment for insulin-resistance.

Variables*	Initial FBG (mg/dl)	Final FBG (mg/dl)	Fasting insulin (pg/ml)	HOMR-IR
NFD(n=7)	4.40±107.57c	3.69±109.14b	273.00±15.61 a	1.70±0.07 a
NFD-BPPS (n=7)	6.17±112.00a	0.91±100.00a	236.25±10.47 b ^d	1.34±0.02 c
HFD (n=7)	6.61±113.50a b	1.25±113.75b	312.25±18.24 a c	2.01±0.06 a
HFD-BPPS(n=7)	7.55±110.16c	5.51±87.25c	206.75±12.37 ^d	1.03±0.03 ^d

NFD: Normal fat diet ; HFD: High fat diet ; HFD-BPPS: High fat diet with banana peel extract phytosome supplementation , NFD-BPPS: normal fat diet with banana peel extract phytosome supplementation ; FBG: Fasting blood glucose, HOMA-IR: homeostatic model assessment for insulin-resistance.

DISCUSSION

An abnormal inflammatory response in an obese state can be a response of enhanced metabolic complications. Actually, adipocytes from obese individuals show a transformed adipokines profile with upregulated expression and secretion of pro-inflammatory cytokines such as interleukin (IL-6), tumor necrosis factor alpha (TNF- α), as well as the complement system. Likewise, the plasma phosphorus status was reported to be related to body weight and cytokines secretion.

Both phosphorus and polyphenol provided robust anti-inflammatory capabilities in various experimental models. Banana peel extract is rich in phosphorus and polyphenol content using phytosome can enhance the bioavailability and absorption of plant extract. Therefore, the main objective is to determine the phosphorus content of banana peel extract which was chosen in a current study as a model for phosphorus-rich plant extracts, examine the possible effects of banana peel phytosome dietary supplements on inflammatory biomarkers complementary (C3) and investigate obesity related complications as measured by glycemic levels in a high fat, diet-induced obesity in a Sprague-Dawley rat model.

Banana peel is composed of different kinds of polyphenols that provide their anti-inflammatory antimicrobial and anti-angiogenic capacity, and our findings are consistent with the results that banana peel extracted through acetone has a high polyphenol content.

Our results also show that the phosphorus content of banana peel extract is near the same as the results declared that the phosphorus content of the peels of several different species of banana is about 18-23% of the daily requirement. These variations may be explained by cultivation, soil nutrition and region.

The microscoping scanning of the produced phytosomes displayed a cell-like structure that was in agreement with earlier studies. The cell like structure of phytosomes is caused by the binding of the phytoconstituent of plant extract with PC and consuming it to produce a microsphere unit called the phytosome which is much the same as the cell membrane conformation that accelerate absorption of plant compounds into blood circulation [5].

This study found a significant difference in body weight between the HFD group when compared to the HFD-BPPS the weight lessening effect of BPPS due to the rich content of phosphorus in banana peel extract. This agrees with many studies that mentioned the anti-obesity effect of phosphorus supplementation through contribution in ATP formation to be available for thermogenesis, inflammatory cytokines production and hemostasis, affect food intake and appetite through neural afferents to the central nervous system, while above studies used P supplement from inorganic sources with high doses compared to banana peel extract phytosome that contain natural source of phosphorus. Although many studies state the active role of polyphenols from different plants in maintaining the inflammatory response to reduce body weight and others demonstrate the anti-inflammatory effect of banana peel, but no observation had found the direct weight management ability of polyphenols in banana peel; therefore, our results could be related to phosphorus supplementation regard

Our experiment's findings show a significant decrease in blood glucose level and HOMR-IR test for the groups which took banana peel extract phytosome in both different diet types compared to control groups. Furthermore, our findings present a significantly lower difference in insulin levels in HFD-BPPS but not in NFD-BPPS that may be

explained by the different calorie density of NFD compared to HFD and the possible competition for phosphorus requirements to enhance phosphorylation and insulin secretion.

In addition, banana peel extract phytosome contains high levels of polyphenols which have a significant influence on blood glucose at different levels, and may also interfere with the insulin signaling pathway by reducing oxidative stress.

CONCLUSION

The banana peel extract phytosome supplement showed a significant effect on C3 concentration compared to the HFD group which is demonstrated by the highest C3 level due to the inflammation induced by high fat diet that trigger the complement system activation. The outcome of BPPS is similar to studies which mentioned that maintenance of the blood phosphorus concentration can lead to ATP production, homeostasis inflammatory response and inhibit TNF- α secretion which is stimulate C3. Other studies have shown a decrease C3 level after polyphenol supplementation according to polyphenols anti-inflammatory properties these finding are compensating with ours. Although this study is the first one that studied the effect of banana peel extract phytosome on body weight status it still had some limitation like we did not measure serum phosphorus level before and after the treatment.

Based on findings of this study, banana peel extract was high in polyphenols, and phosphorus content. While feeding animals high fat diet for 8weeks induced weight gain, insulin resistance ,high insulin level . Furthermore, feeding high fat diet induced significant increase in Complement 3 levels. While, providing banana peel extract phytosome along with the high fat diet for 8 weeks had significantly reduced weight gain, glycemic profile and C3 serum levels as compared to the high fat diet group

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