COMPARATIVE STUDY BETWEEN BEE POLLENS, LECITHIN AND PHENYLALANINE ON WEIGHT REDUCTION OF OBESE RATS

Journal

J. Biol. Chem. Environ. Sci., 2007, Vol. 2(2): 103-117 www.acepsag.org

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ABSTRACT

The risk of death from all causes, cardiovascular disease, cancer or other diseases increases in overweight men and women in all age groups. While regular exercise and proper diet may be the closest thing to treat obesity, there are supplements that can help appetite satisfaction and weight control. The present study was performed to evaluate the effects of bee pollens, lecithin and phenylalanine on reduction of body weight of obese rats. The effects on lipid profiles, thyroid hormones activity and cortisol concentration of obese rats and the correlations between these variables were studied.

A total of 24 Sprague - Dawley female rats (260-270g) were divided into 4 groups and administered bee pollens, lecithin and phenylalanine before meals. Feeding these materials caused significant reduction in body weight of obese rats which the highest reduction achieved by using bee pollens followed by phenylalanine and lecithin compared with control obese rats. The treatment caused significant decrease in serum total cholesterol levels, but increased the levels of total lipids, HDL and phospholipids. Thyroid hormones were significantly increased due to bee pollens fed obese rats compared with control rats, while cortisol production was significantly decreased in studied groups compared with control obese rats.

From the present results it could be observed that bee pollens fed obese rats were much more effective than lecithin and phenylalanine in reducing body weight of obese rats and have good effects on lipid profiles and thyroid hormones activity which led to weight loss. On the other hand, phenylalanine fed obese rats was more effective than lecithin in reducing body weight because it primary has an appetite suppressant effect. We recommend that bee pollens granules should be a part of daily food intake before meals to achieve a good weight loss effect.

Key Words: Bee pollens; Lecithin; Phenylalanine; Weight reduction; T_3 and T_4 hormones; Cortisol concentration.

INTRODUCTION

Bee pollen is a natural way to improve metabolism, help control and take weight off. It is noticed that bee pollen improves metabolism by correcting a chemical imbalance and by supplying the missing factors that can not supply by other foods. Bee pollen supposedly speeds the process of converting sugar into energy providing oxygen that fat can use to speed their conversion into energy. Bee pollen is used to reduce cravings and hunger. For weight reduction, bee pollen is taken 30 minutes before meals with a glass of water to find that come meal times appetite is reduced and portion size will be considerably less (Wade, 1992).

Effects of bee pollen diet on the growth of male and female Sprague Dawley rats were studied by Liebelt and Calcagnett (1999). The control animals were fed purina lablox laboratory chow and the experimental animals were fed natural bee pollen granules. Both the male and female control animals gained significantly greater body weights than the bee pollen fed animals. The bee pollen fed males had significantly less fat content in both fat depots as compared to the control males.

Bee pollen is not a drug, it is a food. Nature's most perfect food and it's becoming recognized as nature's true weight-loss superfood. Bee pollen contains phenylalanine which is a natural amino acid that the body requires for appetite suppression, by acting on appestat, the control center of the body that signals fullness and hunger. Phenylalanine works naturally within the body, exerting an appetite suppressant effect if human is overweight, maintaining the weight if person is just right and actually increasing the appetite if anybody need to gain weight. Phenylalanine as a precursor to neurotransmitters has potentially impacting appetite regulation (Fernstrom and Fernstorm, 1995).

A double-blind, placebo-controlled trial that enrolled 158 moderately overweight volunteers tested a mixture of chromium, cayenne, inulin (a non digestable carbohydrate), and phenylalanine (an amino acid) for weight reduction. All participants lost weight over the 4-week trial. Those using the supplement lost a bit more weight. However, positive news came from close examination of results. Among those taking the supplement, a significantly higher percentage of the weight loss came from fat instead of muscle. (Hoeger *et al.* 1998).

Bee pollen offers 15% lecithin by volume, which is a nutrient that assists the metabolism in dissolving and flushing fat from the body's most vital internal organs. Lecithin is said to be lipotropic supplement, meaning that it help body metabolize fat or slowdown the rate at which fat is stored. Diet pills use lecithin to promote weight loss by keeping fat soluble in water. It is a substance called a phospholipid and produce daily by the liver if the diet is adequate. Primary component of lecithin is choline which was found to reduce body weight and fat pad mass in rats with a combination of caffeine and carnitine as stated by Hongu and Sachan (2000). Lecithin increases the speed at which calories are burned and stabilizes poor metabolism, for this reason bee pollen is used in some weight loss supplements.

Numerous studies have demonstrated that endocrine (glandular) disorders are usually a result of obesity and not the primary causes for people becoming overweight. Apparently, the only true endocrine disorder which causes obesity is hyperadrenocorticism, although hypothyroidism (low thyroid function) can clearly lower the metabolism enough to cause weight gain and prevent weight loss. An under active thyroid can cause weight gain. The reduction of body weight which observed in overweight people may have been due to increases in a thyroid hormone (T_3 and T_4) that plays a major role in determining a person's metabolic rate, although the levels of T_3 did not exceed the normal range (Colker *et al.* 1999 and Antonio *et al.* 1999).

The occurrence of obesity and central distribution of fat with hypercortisolemia have been studied. Some of these studies have shown an association of increased cortisol production rats with increased body weight or fat mass. Cortisol excretion rate did not correlate with blood pressure but correlated strongly with body mass index (positive) and HDL-cholesterol (negative). Obesity syndromes are characterized by the occurrence of many hormonometabolic alterations, including some degree of hypercorticism and/or responsiveness of the hypothalamo - pituitary - adrenal axis, particularly in those with abdominal obesity. Cortisol production rate significantly increased with increasing body mass index and percentage of body fat. Cortisol production rate and free cortisol levels were, however, significantly higher in men than women. The increase in hypothalamic- pituitary- adrenal axis activity may play a role in the alterations in body composition and central fat distribution in men and women (Andrew *et al.* 1998, Robert *et al.* 1999, Björntorp and Rosmond, 2000 and Jonathan *et al.* 2004).

The aim of the present study is to evaluate the effects of bee pollens and its main components, i.e., lecithin and phenylalanine as a natural food supplements on body weight and lipid profiles (total cholesterol, HDL-cholesterol, total lipids and phospholipids) levels in serum of obese rats. Thyroid hormones and cortisol concentration in obese rats were also studied.

MATERIALS AND METHODS

Bee pollens: Mixed bee pollens were purchased from the agriculture extension station Fayoum Governorate, Egypt. The mixed bee pollens (a mixture of pollens from different plants) were cleaned from dust, dried, and finely ground and kept in a refrigerator at (-5°C) until used.

Lecithin: Lecithin was obtained from soybean pilot plant, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. It was used as it is, after dissolving in corn oil.

Phenylalanine: Phenylalanine was purchased from El-Gomhoria Company, Cairo, Egypt, as a fine powder and dissolved in distilled water.

Basal diet: The basal diet composed of casein (12%), cellulose (5%), vitamin mixture (1%), salt mixture (4%), corn oil (5%) and corn starch (73%). The ingredients of basal diet formulation were performed according to A.O.A.C. (2000).

Animals: A total of 24 Sprague-Dawley female rats were purchased from the animal house of Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. The animals housed in plastic cages and fed on basal diet and water for one week as an adeptation period. The initial weights of rats were in the range from 260 to 270g.

Biological experiments: The rats were divided into 4 groups, each group contained 6 rats. The first group was fed on the basal diet and considered as a control group. The second group was fed on basal diet supplemented with ground bee pollens after suspended in water (0.12 g bee pollens). Lecithin (0.175 g) was dissolved in 17.5 ml of corn oil and given to the third group of rats orally by stomach tube. While, phenylalanine administered to the fourth group at dose of 0.015 g after dissolving in water using stomach tube. All groups were fed the supplements early in the morning before meals.

Blood sampling: Blood samples of rats were obtained from orbital plexus venous by means of fine capillary glass tubes at the end of the experiment. The blood samples were allowed to clot for one min., at 37°C, centrifuged at 1500xg for 10min., and the separated serum was kept frozen at (-20°C) until analysis.

Biochemical measurements: Serum total cholesterol, high-density lipoprotein-cholesterol and total lipids were measured according to the methods of Roeschlau *et al.* (1974); Assmann, (1979) and Frings and Dunn (1970), respectively. Fat content in feces was determined according to A.O.A.C. (2000), while phospholipids were performed by modified method of Holman (1943). The thickness of femur bone was measured by using Caliper device. The thyroid hormones (T_3 and T_4) were measured according to the methods of Papanastasiou - Diamandi and Khosravi, (1991) and Chopra *et al.* (1971), respectively. Serum cortisol concentration was determined by the method of Check (1995). Blood hemoglobin and hematocrit concentrations were measured according to Dacie and Lewis (1984).

Statistical analysis: The standard analysis of variance procedure in a completely randomized design was applied for the present data according to Gomez and Gomez (1984). Least significant difference (LSD) test and Duncan's test were done to compare a pair of group means. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Data presented in Table (1) show the effects of bee pollens, lecithin and phenylalanine on body weight of obese rats from zero time to the end of the experiment (30 days). Body weight of control obese rats for all groups at zero time was not significantly different. Obese rats fed on bee pollens lost significantly higher body weight compared with the other two groups (lecithin and phenylalanine). The reduction in body weight was 18.37% for bee pollens fed rats, 15.68% for lecithin and 17.19% for phenylalanine fed rats, which indicates that bee pollens were much effective for weight loss than lecithin and phenylalanine, perhaps because of the interaction between lecithin and phenylalanine containing bee pollens. The body weight was gradually decreased using bee pollens, lecithin and phenylalanine during the whole experiment period from zero time till the end of the experiment, which indicates that the reduction in body weight of obese rats is not occurring suddenly by using studied materials, but it takes some time to achieve the desired results.

Table (1): Effects of bee pollens, lecithin and phenylalanine on body weight of obese rats

Time / Groups	Body weight (g).			
	zero time	15 day	21 day	30 day
Control	269.3 A	268.7 AB	267.0 A	266.7 A
Bee pollens	268.3 A	243.0 B	231.0 C	219.0 D
Lecithin	266.0 A	254.7 B	240.7 C	224.3 D
Phenylalanine	265.3 A	244.3 B	232.7 C	219.7 D
L.S.D.	22.76	45.50	23.87	24.47

L.S.D. for interaction between time and treatments = 10.82.

Table (2) shows the effects of bee pollens, lecithin and phenylalanine on thickness of femur bone and fat content in feces of obese rats. The thickness of femur bone is used as a measure of degree of weight loss. Bee pollens, lecithin and phenylalanine caused a significant reduction in the thickness of femur bone compared with control obese rats. Bee pollens achieved the highest reduction followed by phenylalanine and lecithin. The low fat content in feces of obese rats indicates the greater body weight loss. From Table (2), the fat content was significantly low for the three groups compared with control obese rats and it was 0.220%, 1.063% and 1.023% for bee pollens, lecithin and phenylalanine, respectively. Bee pollens achieved a significantly lower percentage of fat content in feces compared with phenylalanine and lecithin.

Table (2): Effects of bee pollens, lecithin and phenylalanine on thickness of femur bone and fat content in feces of obese rats.

Groups	Thickness of femur bone (cm)	Fat content in feces (%)
Control	1.357 A	2.730 A
Bee pollens	0.880 B	0.220 F
Lecithin	1.030 B	1.063 D
Phenylalanine	0.927 B	1.023 D
L.S.D.	0.248	0.124

Too much body fat may result in a poor lipid profiles (Total cholesterol, HDL and LDL) levels, which in turn may lead to plaque buildup and hardening of the arteries. Table (3) represents the effects of bee pollens, lecithin and phenylalanine on total cholesterol, HDL-cholesterol, total lipids and phospholipids levels in serum of obese rats. Serum total cholesterol level was significantly decreased in bee pollens fed rats as compared to control obese rats. The highest reduction in serum cholesterol level was achieved by using bee pollens (86.0mg/dl) compared with (98.0mg/dl) for control obese rats followed by lecithin, while phenylalanine was not significantly different compared with lecithin fed rats. HDL-cholesterol level in obese rats fed bee pollens was significantly higher than control obese rats, but the other two groups (lecithin and phenylalanine) did not achieved significant difference compared with control obese rats. These data indicate higher lipids-lowering properties of bee pollens.

Bee pollens fed animals and humans caused pronounced reduction in total cholesterol content by 67%, while the serum HDL-cholesterol level was increased by 19% in rabbits fed pollens.The serum cholesterol level in the bee pollens fed male rats were 50% lower as compared to control. An increase in HDL-cholesterol concentration was reported after greater weight loss (Wojcicki *et al.* 1986; Liebelt and Calcagnett, 1999 and Baba *et al.* 1999). The systolic blood pressure, triglycerides and fasting insulin were significantly higher and HDL-cholesterol was significantly lower in the heavy adolescents. However, a reduction of body weight reverses these interactions as noticed by Alan *et al.* (2005).

Table (3): Effects of bee pollens, lecithin and phenylalanine on lipid profiles of obese rats.

Groups	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Total lipids (mg/dl)	Phospholipids (mg/dl)
Control	98.00 C	27.33 BC	374.3 D	95.33 B
Bee pollens	86.00 B	37.00 A	505.0 A	103.00 A
Lecithin	95.67 C	28.33 B	399.0 B	97.00 B
Phenylalanine	97.33 C	26.00 C	381.0 C	84.00 C
L.S.D.	4.382	2.034	5.596	4.831

Data tabulated in Table (3) indicate that bee pollens caused a significant increase in total lipids followed by lecithin and phenylalanine as compared to control obese rats. Phospholipids concentrations were also increased by using bee pollens in comparison with control obese rats, while this increase was not significantly different for lecithin compared with control rats. These data were in agreement with previous findings by Ficek and Stankiewicz (1987) who found that rats fed bee pollens contained optimum contents of phospholipids and non-specific esterases, which indicates benefical effect of feeding bee pollens.

Table (4) shows the effects of bee pollens, lecithin and phenylalanine on hemoglobin concentrations, white blood cell counts and platelets counts of obese rats.Bee pollens caused a significant increase in hemoglobin concentrations, white blood cell counts and platelets counts as compared to control obese rats. Lecithin and phenylalanine caused an increase in platelets counts which were significantly different compared with control rats. Hemoglobin concentrations and white blood cell counts were not significantly different for lecithin and phenylalanine compared with control obese rats.

Table (4): Effects of bee pollens, lecithin and phenylalanine on hemoglobin concentrations, white blood cell counts and platelets counts of obese rats.

Groups	Hemoglobin concentrations	white blood cell counts	platelets counts
Control	10.833 B	6400.00 B	541.33 D
Bee pollens	15.733 A	6800.00 A	750.33 A
Lecithin	11.900 B	6516.66 B	589.76 C
Phenylalanine	12.400 B	6633.33 AB	645.00 B
L.S.D.	1.985	292.7	15.87

When bee pollen is given to anemic patients, their levels of hemoglobin increase considerably. Bee pollen improves the digestive utilization of iron and the regeneration efficiency of hemoglobin. The white blood cell counts, hematocrit, hemoglobin concentrations and mean corpuscular volume of the red blood cells were significantly increased by adding bee pollen to the diet of rats (Liebelt and Calcagnett, 1999 and Haro *et al.* 2000).

Data presented in Table (5) show the effects of bee pollens, lecithin and phenylalanine on serum T_3, T_4 and cortisol concentrations of obese rats. T_3 and T_4 hormones (thyroid hormones) concentrations were significantly increased in bee pollens fed obese rats compared with control obese rats. The highest increase in T_3 and T_4 hormones was achieved by using bee pollens followed by phenylalanine and lecithin. The increase in thyroid hormones was significantly different as compared with control obese rats for all studied groups. Starvation for protein (hunger) increases serum T_3 concentration in the rat. Increased blood levels of thyroid hormones are proposed to be responsible for increasing expression of branched-chain (alpha)-keto acid dehydrogenase complex in animals starved for protein as a result of hunger (Robert *et al.* 2001).

From Table (5) it could be observed that cortisol concentration was significantly decreased in bee pollens fed obese rats compared with control obese rats. Cortisol production was 0.600 µg/dl for bee pollens fed obese rats, 0.650 µg/dl for phenylalanine and 0.750 µg/dl for lecithin fed obese rats as compared with 0.850 µg/dl for control obese rats and all the three studied groups were significantly different compared with control obese rats. Splanchnic cortisol uptake was greater in the obese diabetic than lean non diabetic subjects (25 ± 2.9 vs.15.3 ± 2.5 µg/dl; $p < 0.05$) and cortisol production was correlated with total body weight but not with visceral fat (Basu *et al.* 2005).

Table (5): Effects of bee pollens, lecithin and phenylalanine on serum T_3, T_4 and cortisol concentrations of obese rats.

Groups	T_3 (ng/ml)	T_4 (µg/dl)	Cortisol (µg/dl)
Control	0.300 D	2.150 D	0.850 D
Bee pollens	0.420 A	2.797 A	0.600 A
Lecithin	0.370 B	2.493 B	0.750 C
Phenylalanine	0.393 C	2.663 C	0.650 B
L.S.D.	0.0188	0.0595	0.0376

Table (6) shows the correlation coefficient between the different parameters studied at the end of the experiment. There is a strong negative correlation between HDL- cholesterol and total lipids with total cholesterol. There is also a strong positive correlation between total lipids and phospholipids with HDL- cholesterol. Thyroid hormones T_3 and T_4 are strongly correlated (positive) with total lipids, while T_3 hormone is strongly positively correlated with T_4 hormone. Thyroid hormones (T_3 and T_4) did not correlate with cortisol. Cortisol excretion rate is negatively correlated with HDL-cholesterol, total lipids and phospholipids and this was in agreement with the results obtained by Robert *et al.* (1999). Cortisol is strongly correlated with white blood cells and platelets counts (positive) and this is may be due to the excretion of cortisol is mainly occurred in the blood.

The inverse relationship between cortisol and HDL- cholesterol may affect peripheral cholesterol metabolism to alter HDL-cholesterol formation and this is associated with cardiovascular risk, a small long- term excess of cortisol may explain in part the risk associated with obesity (Anderson *et al.* 1991).

Table (6): Correlation coefficient between variables at the end of the experiment.

	Total cholesterol	HDL-cholesterol	Total lipids	Phospholipids	T_3	T_4	Cortisol	Hemoglobin	White blood cells	Platelets
Total cholesterol	------	-0.874**	-0.925**	-0.664*	-0.656*	-0.683*	0.224	0.431	0.098	0.224
HDL-cholesterol		------	0.964**	0.733**	0.610*	0.647*	-0.420*	-0.643*	-0.219	-0.409
Tota l lipids			------	0.687*	0.717**	0.749**	-0.287*	-0.579*	-0.168	-0.265
Phospholipids				------	0.355	0.383	-0.676*	-0.814**	-0.641*	-0.722**
T_3					------	0.967**	-0.334	-0.256	0.216	0.319
T_4						------	-0.312	-0.338	0.240	0.271
Cortisol							------	0. 660*	0.802**	0.957**
Hemoglobin								------	0.475	0.720**
White blood cells									------	0.753**
Platelets										------

** Significant at ($p \leq 0.01$)

* Significant at ($p \leq 0.05$)

The present study was performed in order to evaluate the effects of bee pollens as a natural food supplement on weight loss in obese rats and comparing the effects of its main components lecithin and phenylalanine on weight loss. From the present data it could be observed that bee pollens fed obese rats were much more effective than lecithin and phenylalanine in reducing body weight of obese rats and have good effects on lipid profiles and thyroid hormones activity which led to weight loss. On the other hand, phenylalanine fed obese rats was more effective than lecithin in reducing body weight because it primary has an appetite suppressant effect. We recommend that bee pollens granules should be a part of daily food intake before meals to achieve a good weight loss effect.

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دراسة مقارنة بين حبوب اللقاح، الليسيسين والفينيل ألانين علي خفض الوزن في الفئران المصابة بالسمنة

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إن خطر الوفاة من أمراض القلب والسرطان والأمراض الأخري كنتيجة لزيادة الوزن تزداد في الرجال والنساء من مختلف الأعمار وبينما نجد أن ممارسة الرياضة بإنتظام والحرص علي الغذاء المتوازن قد تكون الحل الأمثل للسمنة فإنة توجد بعض المساعدات الغذائية التي تؤدي ألي ضبط الشهية والتحكم في الوزن وقد تم إجراء هذة الدراسة لتقدير مدي تإثير حبوب اللقاح والليسيسين والفينيل ألانين علي خفض الوزن في الفئران المصابة بالسمنة وكذلك التأثير علي مستوي الدهون ونشاط هرمونات الغدة الدرقية وتركيز الكورتيزون في الفئران المصابة بالسمنة ومدي العلاقة بين هذة العوامل.

تم في هذا البحث استخدام 24 فأر زائد الوزن وقسمت ألي أربع مجموعات تم تغذيتها علي حبوب اللقاح ، الليسيسين والفينيل ألانين قبل الوجبات وظهر من استخدام هذة المواد حدوث إنخفاض ملحوظ في وزن هذة الفئران مع ظهور أكبر إنخفاض بإستخدام حبوب اللقاح يتبعها الفينيل ألانين ثم الليسيسين بالمقارنة بالكنترول . واستخدام هذة المواد الطبيعية سبب أيضا نقص ملحوظ في الكوليستيرول الكلي ولكن سبب زيادة في مستويات الدهون الكلية ، والليبوبروتينات عالية الكثافة والفسفوليبيدات . إرتفعت معدلات هرمونات الغدة الدرقية بإستخدام حبوب اللقاح بالمقارنة مع الكنترول بينما إنخفضت معدلات إنتاج الكورتيزون في المجموعات تحت الدراسة .

ظهر من هذا البحث أن حبوب اللقاح كانت أكثر كفاءة من الفينيل ألانين والليسيسين في خفض الوزن للفئران المصابة بالسمنة ولها تأثيرات جيدة علي مستوي الدهون ونشاط هرمونات الغدة الدرقية والتي تساعد علي خفض الوزن ونوصي بإدراج حبوب اللقاح كجزء أساسي في الغذاء المتناول يوميا للحصول علي نتائج جيدة في خفض الوزن .