

BIOLOGICAL EFFECT OF MUSHROOM CONSUMPTION ON OBESE MALE RATS

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ABSTRACT

Current research aims to study the biological effect of mushroom consumption on body weight gain, internal organ weights and biochemical analysis of obese male rats. Forty male albino rats were randomly divided into two main groups. The first main group (8 rats) was considered as negative control group (healthy rats) fed on basal diet while the second main group (32 rats) were induced with obesity by feeding rats on high animal fat diet, the second main group consists of 4 subgroups each of (8 rats). One of these groups was chosen as a positive control. The rats in the positive control continued feeding on high animal fat diet. The three remaining groups of rats received high animal fat diets enriched with different levels of dried oyster mushroom "*Pleurotus ostruatus*" (6%, 9% and 12% mushroom) for 6 weeks. The results revealed that all obese groups which feed with 6%, 9% and 12% mushroom resulted a decrease in body weight gain, on the other hand internal organ weights data declared that there are significant differences ($p < 0.05$) between the positive control group and obese group which treated with 12% mushroom in liver and kidney weights, whereas the results showed that there are significant differences ($p < 0.05$) between the positive control group and obese groups treated with 9%, 12% mushroom for heart weights. The results indicated that all obese groups which treated with 6%, 9% and 12% mushroom resulted in significant decrease ($p < 0.05$) in the values of serum cholesterol, TG, LDL-c and VLDL-c but showed a significant increase ($p < 0.05$) in the values of serum HDL-c comparing with control positive group. For liver enzymes, results showed decrease in GOT, GPT and ALP in obese groups treated with 6%, 9% and 12% mushroom. The results also showed that there were no significant differences between the positive control group and obese groups which treated with 6%, 9% and 12% mushroom with respect to kidney functions (urea, uric acid and creatinin). The study recommended that the addition of mushroom to diet to overcome the problem of obesity beside improving the health of infants and adults.

INTRODUCTION

Obesity is a chronic health problem. It is predicted that by 2015 approximately 2.3 billion adults will become overweight, and more than 700 million will be obese (WHO, 2006). The World Health Organization (WHO) predicts that overweight and obesity may soon replace more traditional public health concerns such as under nutrition and infectious diseases as the most significant cause of poor health (Loscalzo *et al.*, 2008). It is leading preventable cause of death worldwide, with increasing prevalence in adults and children, and authorities view and it as one of the most serious public health problems of the 21 century (Barnes *et al.*, 2007).

It causes many complications including dyslipidaemia, diabetes, hypertension and heart diseases. Recently, obesity has also been associated with increasing incidence of many cancers (Huang and Chen, 2009).

In many studies, dietary fiber induced greater satiety comparing with digestible polysaccharides and simple sugars (Howarth *et al.*, 2001).

Dietary fiber may also prolong meal duration and result in increased mastication with possible cephalic and peripheral influences on satiety. (Sakata, 1995). It may affect palatability of food, possibly reducing energy intake (Drewnowski, 1998). and containing meals have a lower energy density (Pereira and Ludwig, 2001). Getting enough fiber has also been linked to a lower Body Mass Index, an indicator of obesity, as well as being a potential factor in weight loss and maintenance body weight (Maskarinec *et al.*, 2006).

Fungi are good source of digestible proteins and fiber, are low in fat and energy and make a useful contribution to vitamin and mineral intake (Roman *et al.*, 2006).

Fresh mushrooms contain both soluble and insoluble fiber. The soluble fiber is mainly beta-glucans and chitosans, which are components of the cell walls (Sadler, 2003). Soluble fiber has been shown to help prevent and manage cardiovascular disease by lowering total cholesterol and LDL cholesterol levels. It also helps regulating blood glucose levels (Chandalia *et al.*, 2000).

Mushroom is a manifestation of a common saying, 'Medicines and foods have a common origin', in constituting both a nutritionally functional food and a source of physiologically beneficial medicine. Many centuries ago, medicinal properties of mushroom has been recognized in China, Korea and Japan. Although from ancient times, mushroom have been treated as a special kind of nutraceutical. Major medicinal properties attributed to mushrooms include anticancer activity, antibiotic activity, antiviral activity, immune response-stimulating effects, anti-hypersensitive and blood lipid lowering effects (Wasser and Wise, 1999; Kaul, 2001 and Yang *et al.*, 2002).

Mushroom is known to have high amounts of protein which is better than many legume sources like soybeans and peanuts, and protein-yielding vegetable foods (Chang and Buswell, 1996; Chang and Mshigeni, 2001 and Bárbara *et al.*, 2008). Moreover, mushroom proteins contain all the essential amino acids needed in the human diet and are especially rich in lysine and leucine which are lacking in most staple cereal foods (Chang and Buswell, 1996; Sadler, 2003). Mushrooms are low in total fat content and have a high proportion of polyunsaturated fatty acids (72 to 85%) relative to total fat content, mainly due to linoleic acid. The high content of linoleic acids is one of the reasons why mushrooms are considered a health food (Chang and Mshigeni, 2001; Sadler, 2003). Furthermore, mushroom had significant levels of vitamins, thiamine, riboflavin, ascorbic acid and vitamin D, as well as minerals (Mattila *et al.*, 2000).

The substances present in mushroom with immunomodulatory activity are mainly polysaccharides (in particular β -D-glucans), polysaccharopeptides, and polysaccharide proteins (Lull *et al.*, 2005).

The present study aimed to investigate the effect of mushroom consumption at three levels 6,9 and 12% on obese male rats.

MATERIALS AND METHODS

Materials:

Dried mushroom

The dried oyster mushroom "*Pleurotus ostruatus*" used in this investigation were purchased from Fungi company , 8 Kura Ben Sureek , Mourad st.,Giza .

Chemicals

Vitamins, minerals, cellulose ,bile salts and choline chloride were purchased from EL-Gomhoria Company, Cairo

Kits

Kits were purchased from Gama Trade Company for chemicals, Cairo, Egypt.

Methods:

Biological Studies:

Experimental animals

Forty male albino rats (Sprague Dawley strain) weighing 165.35 ± 3.69 gm were obtained from (Food Technology Research Institute, Agriculture Research Center, Giza) .All rats were fed on basal diet for one week, after one week period , the rats were divided into two main groups. The first main group (n=8 rats) was fed only on the basal diet as a control negative group (C-).The basal diet consists of protein (10%) , corn oil (10%) , choline chloride (0.2%) cellulose (5%), vitamin mixture (1%) (AIN , 1993) , salt mixture (4%) (Hegested *et al.*, 1941) and corn starch (up to 100%). The second main group (n=32 rats) was fed on the basal diet plus high animal fat (Danni fat) . After obese induced the second main group of rats was divided into 4 subgroups continued feeding for 6 week as follows:

Group 1 (C+) : 8 rats :Control positive group, was fed on positive diet (basal diet plus animal fat (20% Danni fat)).

Group 2 (C1) : 8 rats fed on positive diet +6% mushroom.

Group 3 (C2) : 8 rats fed on positive diet +9% mushroom .

Group 4 (C3) : 8 rats fed on positive diet +12% mushroom .

Blood sampling

At the end of experimental period(6 weeks) rats were fasted over night before sacrificing .Blood was collected and centrifuged (3000rpm), serum was separated for analysis .Serum was carefully aspirate, transferred in to clean cuvet tubes and stored frozen at -20°C for analysis. Organs were taken, washed with saline (10%NaCl) and dried with filter paper ,then weighed .Organs weight (Liver, kidney and heart) was recorded. Body weight gain % was calculated by following formula :

$$BWG\% = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Chemical analysis :

For each group analyses included the following:

Total cholesterol (TC) was determined according to Allen, (1974).The determination of serum triglycerides(TG) was done according to Fassati and Prencipe, (1982). While high density lipoprotein-cholesterol (HDL-c) was

determined according to Lopez , (1977) , whereas Low density lipoprotein-cholesterol (LDL-c) were determined according to Friedewable *et al.*, (1972) .

$$\text{LDL-c} = \text{TC} - [\text{HDL-c} + (\text{TG}/5)]$$

$$\text{VLDL-c} = \text{TG}/5$$

Determination of GOT (AST) and GPT (ALT) were determined according to Reitman and Frankel,(1957), while determination of serum alkaline phosphates (ALP) was carried out according to Belfield and Goldberg, (1971). Urea was determined according to Pattn and Crouch, (1977), while Uric acid was determined according to Schultz, (1984), the determination of Creatinin was determined according to Henry, (1947). And determination of Glucose oxidase was carried out according to Tietz , (1976).

Statistical Analysis :

Statistical analysis were performed by using computer of statistical package for social science (SPSS version 11.0). The results are presented as means \pm S E , means \pm S D . One way analysis of variance (ANOVA) was used to test the differences between groups (SPSS,1999).

RESULTS AND DISCUSSION

Effect of mushroom on body weight gain(BWG%)of the experimental rat groups:

Data in Figure (1) showed that the highest body weight gain was observed for positive control group C(+), whereas the lowest body weight gain was observed for negative control group C(-). However , the obese rats treated with (6%,9%,12% mushroom) demonstrated lower values of body weight gain as compared to positive control group C(+). Increasing the level of mushroom addition showed a lower value of body weight gain .

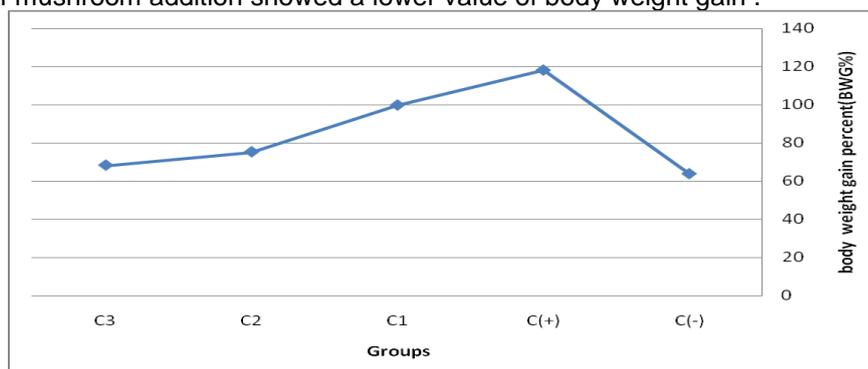


Figure (1): Effect of mushroom on body weight gain(BWG%)of the experimental rat groups.

C(-)=negative control group , C(+)=positive control group, C1,C2, C3= obese groups treated with 6%,9%,12% mushroom.

These data agree with those obtained by Handayani *et al.*,(2011) who revealed that the existence of negative correlations between the amount of Shiitake mushroom supplementation and body weight gain. In this respect,

Jeon *et al.*, (2005) investigated how mushroom yogurt supplemented as dietary fiber (10 and 20%) reduced the body weights in rats as compared with the control rats. This is also in accordance with Alam *et al.*, (2011) who found that feeding a diet containing a 5% powder of *Pleurotus ostreatus* mushroom to hypercholesterolemic rats also significantly reduced body weight in hypercholesterolemic rats.

Effect of mushroom on the weights of internal organs of the experimental rat groups:

Results in table (1) showed that liver weight has appeared significant differences ($p < 0.05$) between positive control group C(+) and obese group treated with (12% mushroom), while there are non significant differences ($p < 0.05$) between negative control group C(-) and obese groups which treated with (9%,12% mushroom). For kidney weight there are significant differences ($p < 0.05$) between positive control group C(+) and obese group treated with (12% mushroom), while heart weight showed a significant differences ($p < 0.05$) between positive control group C(+) and obese groups treated with (9%,12% mushroom) but there are non significant differences ($p < 0.05$) between negative control group C(-) and obese groups treated with 6%,9%,12% mushroom.

Table (1): Effect of mushroom on weights of internal organs of the experimental rat groups.

Groups	Liver Weight (g)	Kidney Weight (g)	Heart Weight (g)
C(-)	6.85±0.33 ^b	1.33±0.68 ^d	0.73±0.05 ^b
C(+)	9.59±1.26 ^a	1.88±0.30 ^a	1.11± 0.28 ^a
C1	9.08±0.91 ^a	1.72±0.22 ^{ab}	0.92± 0.11 ^{ab}
C2	8.12±1.52 ^{ab}	1.64 ±0.15 ^{ac}	0.87±0.07 ^b
C3	7.34±1.40 ^b	1.63±0.12 ^{bc}	0.80±0.09 ^b

C(-) =negative control group , C(+) =positive control group,C1,C2,C3= obese groups treated with 6%,9%,12% mushroom.

Different letters on same column represent statistically significant($P < 0.05$) difference between means, Values are means ± SD for 8 rats.

Effect of mushroom on serum lipid profile of the experimental rat groups:

Data presented in table (2) showed that all treated rat groups showed a significant decrease ($p < 0.05$) in the values of serum cholesterol , TG , LDL-c and VLDL-c but showed a significant increase ($p < 0.05$) in the values of serum HDL-c comparing with control positive group .

These results agree with Khatun *et al.*, (2007) and Handayani *et al.*, (2011) who demonstrated that mushroom significantly reduced TG and cholesterol of diabetic subjects without any deleterious effect on liver and kidney. This is in accordance with those of Jeon *et al.*, (2005) and Alam *et al.*, (2011) who found that feeding a diet containing 5% powder of *Pleurotus ostreatus* fruiting bodies to hypercholesterolemic rats reduced plasma total cholesterol, triglyceride, low-density lipoprotein (LDL).

Table (2): Effect of mushroom on serum lipid profile of the experimental rat groups.

Groups	cholesterol (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
C(-)	109.25±3.73 ^e	69.50± 4.86 ^d	51.50±1.19 ^a	43.85±1.90 ^d	13.90±0.97 ^d
C(+)	272.50±4.14 ^a	113.25±8.36 ^a	30.50±4.44 ^d	219.35±8.70 ^a	22.65±1.67 ^a
C1	145.50±1.77 ^b	93.00±2.00 ^b	40.75±4.43 ^b	86.15±5.50 ^b	18.60±0.40 ^b
C2	136.50±1.92 ^c	82.00±2.72 ^c	36.25±2.86 ^c	83.85±0.71 ^b	16.40±0.54 ^c
C3	122.50±1.60 ^d	81.50±4.62 ^c	34.75±3.32 ^c	71.45±1.75 ^c	16.30±0.92 ^c

C(-) =negative control group , C(+)=positive control group,C1,C2,C3 =obese groups treated with 6%,9%,12% mushroom.

Different letters on same column represent statistically significant (P<0.05) difference between means, Values are means ± SD for 8 rats.

Effect of mushroom on blood glucose of the experimental rat groups:

Data in Figure (2) cleared that the highest blood glucose value was observed for positive control group C(+), whereas the lowest blood glucose value was observed for negative control group C(-). On the other hand the obese rats treated with 6%,9%,12% mushroom demonstrated a lower values of blood glucose as compared to positive control group C(+). Very slight differences were noticed among groups which fed on diets with 6%,9%,12% mushroom. Increasing the level of mushroom addition showed a lower value of blood glucose. These results are in the same line with Khatun *et al.*, (2007) and Handayani *et al.*, (2011) who demonstrated that mushroom significantly reduced blood glucose in diabetic subjects.

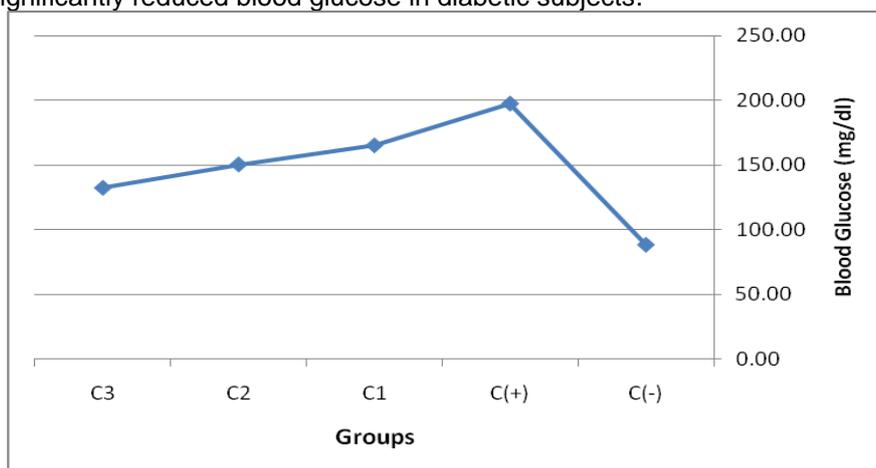


Figure (2): Effect of mushroom on blood glucose (mg/dl) of the experimental rat groups.

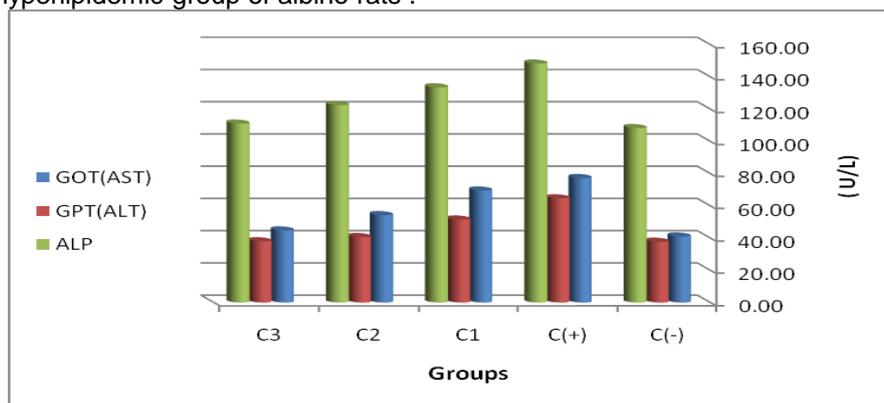
C(-) =negative control group , C(+)=positive control group,C1,C2,C3= obese groups treated with 6%,9%,12% mushroom.

Effect of mushroom on liver functions of the experimental rat groups:

Data in Figure (3) showed that the highest liver enzymes values (GOT, GPT, ALP) were observed for positive control group C(+), whereas the lowest liver enzymes values (GOT, GPT, ALP) were observed for negative

control group C(-).For obese groups which treated with 6%,9%,12% mushroom demonstrated a lower values of liver enzymes comparing to positive control group C(+).Very slight differences were noticed among groups fed on diets with 6%,9%,12% mushroom. Increasing the level of mushroom addition showed a lower value of liver enzymes.

These results are in the same line with those of Mishral and Singh (2010); Priya and Chellaram(2011) they found that methonalic extract of mushroom reduced the enzymes of Alanine Transaminase (ALT) , Aspartate Transaminase (AST) and the alkaline phosphates (ALP) enzyme in hyperlipidemic group of albino rats .



Figure(3): Effect of mushroom on liver functions of the experimental rat groups

C(-) =negative control group , C(+)=positive control group,C1,C2,C3= obese groups treated with 6%,9%,12% mushroom.

Effect of mushroom on kidney functions of the experimental rat groups:

Table (3) showed that there are non significant differences ($p < 0.05$) between positive control group C(+) and obese groups which treated with 6%,9%,12% mushroom(C1,C2,C3 groups) in kidney functions (urea ,uric acid, Creatinin).

In this respect, Alam *et al.*, (2011) found that feeding a diet containing a 5% powder of *Pleurotus ostreatus* fruiting bodies to hypercholesterolemic rats had no effects on creatinin, blood urea nitrogen, uric acid.

Table (3): Effect of mushroom on Kidney functions of the experimental rat groups.

Groups	Urea(mg/dl)	Uric acid(mg/dl)	Creatinin(mg/dl)
C(-)	28.25+6.48 ^{bc}	3.20+0.82 ^b	0.62 +0.07 ^b
C(+)	38.00+3.38 ^a	3.93+0.44 ^a	0.83+0.07 ^a
C1	37.00+ 3.85 ^a	3.80+0.46 ^a	0.81 +0.09 ^a
C2	35.00±1.31 ^{ab}	3.78 ±0.48 ^a	0.78 ±0.07 ^a
C3	34.75+3.32 ^{ac}	3.76+0.47 ^{ab}	0.75 + 0.09 ^a

C(-) =negative control group , C(+)=positive control group,C1,C2,C3= obese groups treated with 6%,9%,12% mushroom.

Different letters on same column represent statistically significant ($P < 0.05$) difference between means, Values are means ± SD for 8 rats.

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التأثير البيولوجي لتناول عيش الغراب على ذكور الفئران المصابة بالسمنة
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يهدف البحث الحالي إلى دراسة التأثير البيولوجي لتناول عيش الغراب على ذكور الفئران المصابة بالسمنة . أجريت الدراسة على ٤٠ فأر من ذكور الألبينو و تم تقسيمهم إلى مجموعتين رئيسيتين، المجموعة الرئيسية الأولى (٨ فئران) تم تغذيتها علي الغذاء الأساسي فقط (المجموعة الضابطة غير المصابة) ، أما المجموعة الرئيسية الثانية (٣٢ فأر) فقد تم إصابتهم بالسمنة من خلال تغذيتهم على غذاء مرتفع في محتواه من الدهون، وقد تم تقسيم تلك المجموعة إلى أربع مجموعات فرعية متساوية العدد(٨ فئران) ، واستخدمت إحداهما كمجموعة ضابطة موجبة، أما المجموعات الفرعية الثلاثة الباقية (مجموعة ٢، ٣ ، ٤) فقد أضيف إلي غذائها عيش الغراب المحاري بنسب ٦% ، ٩% ، و ١٢% علي التوالي لمدة ٦ أسابيع . وقد أوضحت نتائج الدراسة أن الفئران المصابة بالسمنة والتي تغذت على الوجبة المضاف إليها ٦%، ٩%، و ١٢% عيش غراب قد صاحبها انخفاض معنوي عند مستوي معنوية ٠,٠٥ في كل من مستوى الكولسترول الكلي ، الجليسيريدات الثلاثية ، كولسترول البروتينات الدهنية المنخفضة الكثافة (LDL-c) ، (VLDL-c) ، كما أظهرت ارتفاع معنوي في كولسترول البروتينات الدهنية العالية الكثافة (HDL-c). ولقد أوضحت النتائج أيضاً عدم وجود فروق معنوية عند مستوي معنوية ٠,٠٥ بين المجموعة الضابطة الموجبة ومجموعات الفئران الفرعية (٢ ، ٣ ، ٤) في وظائف الكلي(اليوريا ، حمض البوليك والكرياتنين). بينما ظهر انخفاض في الأوزان المكتسبة ، مستوي جلوكوز الدم ، ووظائف الكبد (GOT, GPT, ALP) بإضافة فطر عيش الغراب المحاري إلي الوجبة بنسبة ٦% ، ٩% ، و ١٢%. وقد أوصت الدراسة بإضافة فطر عيش الغراب المحاري إلي وجبات الأطفال والبالغين بهدف تحسين الصحة وحل مشكلة السمنة.

قام بتحكيم البحث

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