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Evaluation Of Dietary White Button Mushroom(Agaricus Bisporus) Against Hyperlipidiemia In Rat Model

BIOLOGY

KEYWORDS : Agaricus bisporus, chitosan, Hypercholesterolemic rats, Hypolipidemic, Hyperlipidemia ,Obesity, Lee-Obesity Index.

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ABSTRACT

To achieve weight loss and cholesterol reduction, dietary restriction remains a cornerstone of therapy. The primary objective was to assess the effect of the dietary Mushroom powder (Agaricus bisporus) and mushroom chitosan towards obesity. Hence the present study proposes mushroom dietary inclusions is suitable and cost effective way for the deriving a potential obesity control strategy. This study has created evidence on the effectiveness of edible mushroom and mushroom derived chitosan in a weight loss treatment. Six-week old male Wistar rats were divided into three groups of 10 rats each. Feeding a diet containing a 10% powder of Agaricus bisporus fruiting bodies to hyper-cholesterolemic rats reduced plasma total cholesterol by30.18% and dietary inclusion of chitosan also was effective in reducing it by 32-35%. Feeding mushroom fruiting body and chitosan also significantly reduced body weight in hypercholesterolemic rats. However, it had no adverse effects on plasma albumin,total bilirubin, direct bilirubin, creatinin, blood urea nitrogen, uric acid, glucose, total protein, calcium, sodium, potassium,chloride, inorganic phosphate, magnesium, or enzyme profiles(data not included). There was positive correlation(r= 0.95821; p= 0.0005< 0.05) found between body weight and total cholesterol of the animals. The obesity induction model was successful in inducing obesity. From the "Homogeneity of variance test" of ANOVA results we observed equal variance. During the inclusion of mushroom and chitosan in the diets,the weight reduction was analysed by one way ANOVA and was significant(p=0.00053) @0.05 level of significance and through mean comparisons of Tukey's test the significant weight reduction in the obese rats by dietary supplementation of mushroom fruiting body and chitosan was validated.

Introduction

Mushrooms have been widely used as a food since 17th century. The use of mushrooms as a food items is probably as old as civilization. More than 2000 species of mushrooms exist in nature; however, less than 25 species are widely accepted as food and only a few have attained the level of an item of commerce (Lindequist et.al.,2005; Smith,1972). They were preferred only for their culinary characteristics while the nutritive values of mushrooms were recognized much later(Crisan,1978; Khanna & Garcha,1981). Mushrooms are also a source of some minerals, including selenium, potassium and phosphorus (Mattila et.al.,2002).

A. bisporus contains a high level of beta glucans (Polysaccharides), compounds known for stimulating the immune system. Mushrooms had long been used for medicinal and food purposes since decades. It is now increasingly recognized that correct diet, controls and modulates many functions of human body and consequently participates in the maintenance of state of good health, necessary to reduce the risk of many diseases. Modern pharmacological research confirms large parts of traditional knowledge regarding the medicinal effects of mushrooms due to their antifungal, antibacterial, antioxidant and antiviral properties, besides being used as functional foods. *A. bisporus* has been shown to lower blood glucose and cholesterol levels by Jeong et.al.,(2010). *A. bisporus* have been found effective against cancer, cholesterol reduction, stress, insomnia, asthma, allergies and diabetes (Bahl,1983). The work of Bilal Ahmad Wani et.al.,(2010) sums up diverse beneficial health effects of mushrooms to humans, in the form of proteins, carbohydrates, fats, vitamins, minerals, food and drugs, and medicines.

Human population studies indicate that hypocholesterolemia is an significant risk factor for coronary heart disease and obesity (Pekkanen et.al.,1990) and that increased dietary fibre intake is suggested to decrease plasma cholesterol concentration(Brown et.al.,2001; Fernandez,2001). Animal studies have shown that chitosan feeding may inhibit dietary fat digestion (Kanauchi et.al.,1995; Deuchi et.al.,1995) and decrease plasma cholesterol concentration. Increased fecal cholesterol accompanied with or without bile acid excretion by interfering intestinal micelle formation is believed to be responsible for its hypocholesterolemic properties (Sugano et.al.,1980; Gallaher et.al.,2001).

A hypolipidemic activity study is pertinent because the hypolipidemic activity of *Agaricus bisporus* essential for its antiatherosclerotic function. Moreover, it has the potential to serve as an effective therapeutic agent for hyperlipidemic diseases, especially cardiovascular disease.Despite the medicinal importance and its therapeutic potential, no detailed studies on the biochemical function of hypercholesterolemia have been performed, and comprehensive studies on the anti-hyperlepidemic effects of this mushroom are not available. Therefore we examined the potential hypolipidemic activity of *Agaricus.sp* and chitosan isolated from mushroom to generate awareness of its health benefit. The present study in obesity induced rats was to test the hypothesis that the oral feed of *Agaricus bisporus* powder and Mushroom chitosan for a period of 3 Weeks could have possible effect on reduction in body weight and Plasma total cholesterol in turn a hypolipidemic activity.

Experimental methods

Commercial collection of *Agaricus bisporus* powder:*Agaricus bisporus* powder was purchased from Johnsun Mushroom Bio-Technology Co., Ltd, (Manufacturer and Supplier), Hong Kong .

Experimental design(Phase-I): Male Wistar rat (initially body weight of 23 to 45g) purchased from the National Center for Laboratory animal house (Hyderabad) were housed individually in stainless steel mesh cages in a room kept at 23 ± 1°C and 60 ± 5% relative humidity with a 12-hr light and dark cycle (lighting from 8:00 a.m. to 8:00 p.m.), and were allowed free access to a commercial standard chow and water for 2 days to allow adaptation of the environment. Rats were housed in plastic cages (12×14cm) 5 animals per cage and given food and water *ad libitium*. All these experimental procedures conducted in the University of Madras,Chennai,Tamilnadu,India. The Ethical Committee for Conduction of Animal Studies at the University of Madras approved the experimental protocol and all animals were cared for in accordance with the principles and guidelines of the Indian Council on Animal Care as outlined in the Guide to the Care and Use of Experimental Animals.

Rats were fed a standard chow diet for control group (standard chow 100%) and study group1 and group 2 were fed on standard chow with addition of high calorie diet for 4 weeks respectively. For diet induced obesity the obesity-induction model suggested by Naderali et.al.,(2005) proposed by Vinicius Von Diemen was adopted where in the composition of the high caloric diet 93G

diet was as follows (33% standard chow, 33% Nestlé® condensed milk, 7% cheese and 27% water). Food intake was measured daily, and body weight was measured every week and the average body weights was assessed and calibrated with Lee obesity index (Bracco et.al.,1983) .

Experimental design (Phase-II): The animals were randomly divided into three groups with ten animals in each group (group 1, group 2, Control group). While control rats were maintained on standard chow, the study group 1 & 2 were fed with the addition of high caloric diet as specially prepared. Each animal's weight was measured every 5 days in order to determine any weight gain. The animals were treated by diethyl ether suffocation, weighed; their nasoanal lengths determined and Lee Index calculated using the formula by Bracco et.al., (1983). The normal range of Lee obesity index was 310 to 320 kg/m^2, thus the status of animals as to being obese or not, was confirmed. During this same period, rats fed pure chow weighed an average of 50 grams.

$$\text{Lee obesity index (LOI)} = \frac{\text{weight } (g^{0.33})}{\text{nasoanal length (mm).}} \times 1000$$

Estimation of total cholesterol: The tail vein blood sample was collected. Blood was placed in a centrifuge tube and allowed to clot to obtain the serum. Serum was separated by centrifugation at 1400g for 10min.Three sterile test tubes were labelled blank (B), standard (S) and test (T). The cholesterol reagent (1000µl) and cholesterol standard (conc200mg/dl) (10µl) was taken in the standard, serum/plasma (10µl) and cholesterol reagent (1000µl) was taken in test, and cholesterol reagent (1000µl) was taken as a blank. The tube contents were mixed well and incubated at 37°C for 10 minutes or at room temperature (15 – 30°C) for 30 min.The analyser was programmed as per assay parameters. Absorbance of coloured dye was measured at 505 nm and is proportional to amount of total cholesterol concentration in the sample(Nader et.al.,1994;Naderali et.al.,2004). The results were calculated as per given formula for estimating total cholesterol

$$\text{Total Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Evaluation of Mushroom powder and chitosan against hyperlipidiemia (Phase-III): Group 1(Obesity induced rats) were feed with standard chow and mushroom powder (10%) and group 2(obesity induced rats) were feed with standard chow and Chitosan isolated from mushroom sources (10%) for a period of 21 days. Each individual animal body weight and length was measured every 5 days in order to determine weight loss and correlated with Lee obesity index and then the obesity status of animal was identified. The blood sample was collected and total cholesterol was estimated by following standard method proposed by(Crescenzio,1981; Herber,1994).

The results are presented as means ± standard deviations. Significance of difference was tested using analysis of variance in conjunction with Tukey's test. A probability of 0.05 was chosen as the significant level. Correlations analyses were determined by two tailed test of significance in the Pearson's linear correlation test (Curi,1987).OriginPro8SRO v8.0724 (Origin Lab Corporation. Northampton, U.S.A) a data analysis and graphing software copyrighted © 1991-2007, Origin lab corporation was used and a $p \leq 0.05$ was considered statistically significant.

Results

Obesity induced by high caloric diet: The body weight of study group 1 & 2 were significantly higher than those fed standard chow in the control group(Fig.1). The body weight and total cholesterol were measured and correlated with Lee obesity index (Table 1). Each animal's body weight and nasoanal length were recorded for indirect computation of body composition via the Lee obesity index method to being obese or not, were confirmed. Consequently, the Lee obesity index, reflecting percent body fat, continued to increased body weight than the control group. The result of obesity bodyweight was correlated with Lee Obesity Index of normal range 310-320 kg/m^2 and it was identified as in the obese range (Table 2). These confirms that high caloric diet inducing obesity model were successful in the study group 1 & 2.

Estimation of total cholesterol: The progressive increase in body weight and nasoanal length, and total cholesterol during the course of the experiment, was due to the high caloric diet rich feeding with different durations (Fig.2). There was positive correlation($r= 0.95821$; $p= 0.0005$) determined by two tailed test of significance in the Pearson's linear correlation test between body weight and total cholesterol of the animals during the three phases of study.

Effect of Mushroom powder and chitosan towards hyperlipidiemia: From the obesity induced study group 1 (n:10) were fed with the dietary components of Mushroom powder with the addition of standard chow in the range of 10% for 21 days. Each individual animal weight was measured every 5 days in order to determine any weight loss. The blood sample was collected from the treated group 1 rats for the estimation of total cholesterol. The body weight and nasoanal length was measured from the treated group1 rats and used for Lee obesity index correlation to assess the obesity status .Lee obesity index range was found to be within the normal range(lee obesity index is 310 to 325kg/m^{2}indicates non-obese range). These result from Lee obesity index suggest a normal non obese range(Table.3).Similarly, obesity induced study group 2 rats were fed with modified diet containing mushroom chitosan and standard chow in the range of 10% for the duration of 21 days. Each individual animal weight was measured every 7 days in order to determine any weight loss. The blood sample was collected from the treated group 2 rat and total cholesterol was estimated. The body weight and nasoanal length was measured from the treated group1 rats and used for Lee obesity index correlation to assess the obesity status. Lee obesity index range was found to be within the normal range. These results from Lee obesity index of normal non obese range are included in table 4.

. The obesity induction model was successful in inducing obesity. From the "Homogeneity of variance test" of ANOVA results we observe equal variance , $p=0.21431 > 0.05$.During the inclusion of mushroom and chitosan in the diets of Group 1 and Group-2,the weight reduction was analysed by one way ANOVA and was significant($p=0.00053$) @0.05 level of significance and through mean comparisons of Tukey's test, a significance value =1 indicates the difference is significant.Therefore,the data analysis validates the significant weight reduction in the obese rats by dietary supplementation of mushroom(*Agaricus.sp*) and chitosan isolated from mushroom.

Discussion

The present study indicates that the rat fed with modified diet containing mushroom powder and mushroom chitosan resulted in the moderate depression of plasma cholesterol and body weight. The reduced rate of food intake, body weight, and total cholesterol were significantly lower than those fed with high caloric diet. The Lee Obesity index method was correlated and which indicates that the study group was none obese.

This study was designed to investigate the effect of mushroom powder and mushroom chitosan on the parameters of body

weight and total cholesterol. The rats fed a diet containing mushroom powder with the addition of standard chow for 3 weeks had significantly lowered body weight and total cholesterol, when compared with those fed with the standard chow in the control group. Results from this study was correlated with the other experimental finding, suggest that the dietary inclusion of edible mushroom components has effective role in cholesterol lowering activity against hyperlipidiemia. The *A. bisporus* powder is proved to be a good sourcce of Beta-glucan used in dietary supplements and therefore represent a valuable source of biologically active compounds (Chen & Huang ,2009) .

Gruen and Wong indicated that edible mushrooms were highly nutritional and compared favourably with meat, egg and milk food(Gruen & Wong,1982). *A. bisporus* contains Ca (0.04 g), Mg (0.16), P (0.75 g), Fe (7.8 g), Cu (9.4 mg), Mn (0.833 mg) and Zn (8.6 mg) per kilogram fresh weight (Varo et.al.,1980). Pharmaceutical substances with potent and unique health enhancing properties have been isolated from mushrooms(Wasser &Weis,1999). Fresh mushrooms are known to contain both soluble and insoluble fibres; the soluble fibre is mainly beta-glucans polysaccharides and chitosan which are components of the cell walls (Sadler,2003). Soluble fibre present in mushrooms prevents and manages cardiovascular diseases (Chandalia et.al.,2000).

Similarly *Agaricus bisporus* has been shown to lower blood glucose and cholesterol levels in streptozotocin (STZ)-induced diabetic and rats fed a hypercholesterolemic diet(Jeong et.al.,2010). The STZ induced diabetic male Sprague-Dawley rats fed *Agaricus bisporus* powder (200 mg/kg of bodyweight) for 3 weeks had significantly reduced plasma glucose and triglyceride concentrations (Fukushima et.al.,2000). In this present study, the result demonstrated from the hypercholesterolemic rats, oral feeding of the *Agaricus bisporus* powder for 3 weeks resulted in a significant decrease in total cholesterol and body weight respectively.

Several studies have shown the cholesterol-lowering effect of chitosan related to its dietary levels and particle size. On feeding a high cholesterol diet for 20 days, addition of 2 to 5% chitosan resulted in a significant reduction, by 25% to 30%, of plasma cholesterol without influencing food intake and growth. The concentration of liver cholesterol and triglyceride also decreased significantly. Chitosan at the 10% level further reduced plasma cholesterol, but depressed growth. In this study, dietary cholesterol and saturated fat increased plasma total cholesterol while chitosan moderated this cholesterol-induced increase and no adverse effects were evident at gross anatomical examination when experiments were conducted for up to 12 weeks.

Similarly, Chitosan acts as a weak anion exchange resin and exhibits a substantial viscosity in vitro. Either of these properties of chitosan could mediate its hypocholesterolemic effect. However, chitosan preparations of different in vitro viscosities all demonstrated equivalent hypocholesterolemic effects, arguing against a role for viscosity, resulted in excreting relatively more cholesterol and less coprostanol in rats treated with chitosan(Sugano et.al.,1980) . The anion exchange property of chitosan would seem to be favoured as an explanation for its hypocholesterolemic properties. An equal mixture of chitosan and glucomannan, fed with 7.5% of the diet, reduced cholesterol absorption and increased bile acid excretion in rats fed with a cellulose-based diet(Gallaher et.al.,2001). Increased bile acid excretion could reduce cholesterol concentrations because plasma or liver cholesterol would be utilized to maintain the bile acid pool. In the study, we also found a strong trend to a decrease in body weight and total cholesterol, when compared to control group indicates the finding was successful.

The present study in obesity induced rats, was to test the hypothesis set that the oral feed *A. bisporus* and mushroom derived chitosan for a period of 3 week could have positive effect on plasma Total Cholesterol in turn a hypolipidiemic activity. Therefore a dietary inclusion of mushroom can regulate the cholesterol level and counter act obesity.

We may conclude about the diverse benefits of mushrooms towards humans by the words of the father of medicine that is, Hippocrates "Let food be your medicine and medicine be your food". This saying aptly suits mushrooms, as they have tremendous medicinal food, drug and mineral values; hence they are valuable asset for the welfare of humans.

Table 1 : Body weight of Wistar male rat

Experiment	Groups (No. of rats)	Dietary manipulations	Body weight (gm)					
			Phase I		Phase II		Phase III	
			Mean	S.D	Mean	S.D	Mean	S.D
1	10	Control (Standard chow)	21.8	1.78	38.2	2.16	43.0	1.58
2	10	Group 1 (Mushroom powder)	35.4	3.81	92.2	2.28	86.8	2.86
3	10	Group 2 (Mushroom chitosan)	37.6	2.96	115.6	3.78	107.4	5.59

Phase1- Normal feed ,Phase II- High caloric feed & Phase III- 10%Mushroom& chitosan in feed.
(Value = Mean ± S.D)

Figure 1. Estimation of total cholesterol

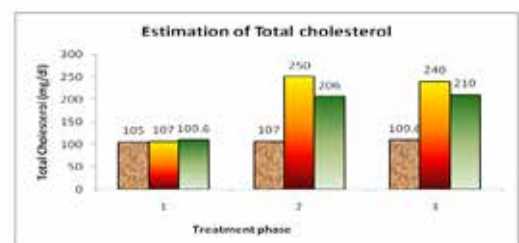

Table 2: Body weight and Lee obesity index of rat fed with mushroom fruiting bodies

Experiment	Group 1 (Mushroom powder)	Body weight (gm)		Lee Obesity index (kg/m^2)	
		Obese weight	Reduced weight	Obese	Reduced
1	10	90	81	426.80	317.25
2	10	95	83	414.81	313
3	10	91	85	418.82	315
4	10	98	89	404.42	314
5	10	97	86	389.38	315
Mean ±SD	10±0.0	94.20±3.56	84.80±3.03	410.85±14.46	314.85±1.58

*Mean of 10 animals

Table 3. Body weight and Lee obesity index of rat fed with chitosan supplementation.

Experiment	Group 2 (Mushroom chitosan)	Body weight (gm)		Lee Obesity index (kg/m^2)	
		Obese weight	Reduced weight	Obese	Reduced
1	10	120	102	365.36	315.23
2	10	110	95	424.01	319.52
3	10	115	101	405.24	317
4	10	118	103	386.21	312
5	10	115	104	429.03	319.15
Mean ±SD	10±0.0	115.60±3.78	101.00±3.54	401.97±26.54	316.58±3.09

*Mean of 10 animals

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