Agaricus bisporous effect on Sugar, Cholesterol and the Bactericidal power of human serum

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Abstract

This research was carried out to study the effect of *Agaricus bisporous* mushroom on reducing the sugar and cholesterol in blood and determine its activity on improving the bactericidal power of serum collected from a group of healthy volunteers. So, about fifty healthy volunteers from both sexes ranging in age from (20-30 years) and of a weight between (65-100 kg) were included in this study.

Results of nourishing (150g) of *Agaricus bisporus* mushroom as a diet to about (40) healthy volunteers from both sexes twice a week for two months did not show any change in body weight mass (BW) compared with the controls after two months of mushroom administration. However, a significant difference (P <0.05) in serum glucose and cholesterol concentrations in both (male and female volunteers of the study) was detected. In addition, the effect of *Agaricus bisporus* mushroom administration on the bactericidal activity of normal serum was also studied and a significant difference (P <0.05) in the bacterial colony counts especially in the colony counts of the bacterium *Staphylococcus aureus* was recorded. Moreover, the ethanolic extract of the *Agaricus bisporous* mushroom showed a significant (P <0.05) antibacterial activity against *Staphylococcus aureus* strains only.

تأثير *Agaricus bisporous* على سكر، كولسترول والقوة المضادة للبكتريا في مصل الانسان

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الخلاصة

تم اجراء هذا البحث لغرض دراسة تأثير فطر *Agaricus bisporous* على تخفيض السكر وكولسترول الدم وتحديد فعاليته في تعزيز القوة القاتلة للبكتريا في مصل الدم لمجموعة من المتطوعين الاصحاء. لذا تم اخذ نحو خمسين متطوعا من كلا الجنسين لهذه الدراسة تراوحت اعمارهم بين (٢٠-٣٠) سنة واوزانهم بين (٦٥-١٠٠) كغم. وأظهرت نتائج التغذية لأربعين متطوعا بما يقارب (١٥٠)غم من فطر *Agaricus bisporous* مرتين في الاسبوع ولمدة شهرين عدم حدوث اي تغيير في الوزن مقارنة مع مجموعة السيطرة واظهرت نتائج فحص الدم لمتناولي الفطر فرقا معنويا (P <0.05) في تراكيز السكر والكولسترول في الاناث والذكور من المتطوعين للدراسة مقارنة مع مجموعة السيطرة. بالإضافة الى ذلك ، فقد تم دراسة تأثير فطر *Agaricus bisporous* على تعزيز قدرة المصل القاتلة للبكتريا وتم تسجيل فرقا معنويا (P <0.05) في عدد المستعمرات البكتيرية وخاصة في مستعمرات بكتريا *Staphylococcus aureus*. أيضا، فقد أظهر المستخلص الإيثانولي للفطر تأثيرا معنويا (P <0.05) مضادا لسلالات بكتريا *Staphylococcus aureus* فقط.

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Introduction

There is a significant interest in the use of mushrooms and/or mushroom extracts as dietary supplements based on theories that they enhance immune function and promote health (1,2,3,4). To some extent, *Agaricus bisporus* mushrooms have been shown to produce stimulatory action on immune responsiveness, particularly when studied *in vitro* (2,3,4,5).

In addition, there have been a number of studies that have shown that dietary supplementation with *Agaricus bisporus* mushrooms can provide benefits, such as cholesterol and sugar reduction (5,6,7) and since the cardiovascular diseases and diabetes are the major causes of morbidity and mortality in the whole world (8). Epidemiological studies suggested that the risk factors for cardiovascular disease and diabetes are largely influenced by diet supplementation especially *Agaricus bisporus* mushrooms (8,9).

However, despite their widespread use for potential health benefits, there was a surprising rarity of epidemiologic and experimental studies that mentioned the biologic activities of mushrooms after oral administration to humans especially the type *Agaricus bisporus* that is very popular in our markets today(9).

Therefore, this research was designed to study the effect of *Agaricus bisporous* mushroom on reducing blood sugar and cholesterol and to study its activity on increasing the bactericidal power of serum collected from healthy volunteers.

Key words: *Agaricus bisporous*, mushroom, sugar &cholesterol, bactericidal power of human serum.

Materials &methods:

1-Plant of this study :

The *Agaricus bisporous* mushroom used in this study was freshly obtained from the markets of Kirkuk city under different names of (Qarch Dizful- Qarch Yehktah and Qarch- Shehraire / Iran) .

2- Volunteers of this study:

A number individuals from the community of Kirkuk city were volunteered randomly to be included within this study. They were subjected to a medical history inquiry about their past diseases and any medications and they performed a number of medical tests included (general urine analysis, Erythrocyte sedimentation time (ESR), complete blood count and liver function tests).The volunteers that showed normal values remained in this study, about (50) healthy volunteers including (25 females and 25 males) were selected ranging in age from (20-30 years) and weighed about (65-100 Kg). Ten of them (5 females and 5 males) were considered as controls and were deprived from mushroom consumption during the period of study while, the rest of them (20 males and 20 females)were subjected to the study protocol of mushroom administration.

3-Mushroom administration

This method of mushroom administration was specifically designed in this study as follows:

1. One hundred and fifty grams of *Agaricus bisporus* (white button mushroom) was processed with mild heating and administrated to volunteers in (two days / a week) for two months.

2. Five milliliters of blood were collected from the study volunteers (control and mushroom fed individuals) using the venipuncture method at the end of each week and serum was obtained according to standard procedures (10).

4- Determination of sugar and cholesterol:

In vitro random glucose and total cholesterol levels were measured using commercial Kits for glucose(GLUC CAL-RANDOX-UK- LOT Number 1895GL) and for cholesterol (CHOD-PAP- BIOLABO-France- CODE CNQ-E6) at the end of each week for two months.

5- The Bactericidal test(11):

1. Bacterial isolates provided from the (Medical Laboratory Techniques Department) which included (*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella oxytoca* and *E.coli* were inoculated in nutrient medium (HIMEDIA LABS Ltd. / Mumbai, India) broth.
2. One milliliter of serum samples collected at the end of each week from volunteers of the study were mixed with the four types of the bacterial broth and incubated at 37℃ for one hour.
3. One milliliter of each mixture was poured into nutrient agar plates and spread using a spreader. Then, the nutrient agar plates incubated at 37℃/48 hours.
4. Bacterial growth was recorded and compared with the control plates by measuring the growth zones with ruler.

6- The Antibacterial activity test of mushroom extract *in vitro*:

This procedure was followed after the methodology mentioned in (10) as follows

1. One hundred and fifty grams of *Agaricus bisporus* mushroom was dried in oven at 40 ℃
2. The dried mushroom was mixed with 500 ml of ethanol (BDH/ UK) and stirring at 30 ℃/150 rpm for 1 hour
3. Filtration was followed through Wattman filter paper No.(1)
4. Freeze dried at -10℃ with (Freeze dryer/ Christ/ Germany) .The powder was stored at 4℃ for further use.
5. Bacterial isolates were inoculated in nutrient broth and about (1ml) of each bacterial isolate was poured into nutrient agar plates and spread using a spreader. Then, wells were made in the nutrient agar plates with the wide end of a pipette.
6. The powder of mushroom ethanol extract was dissolved in distilled water to a final concentration of (20%) and sterilized by filtration through (0.22µm) membrane filter unit. Then, about (100µl) of mushroom extract was filled into the wells of the cultivated nutrient agar plates and were incubated at 37℃/48 hours.
7. Studies were performed in duplicate and the inhibition zones were compared to those of reference antibiotic discs (Ampicillin, Gentamycin, Tetracycline and penicillin/ 10 µg/ BDH –UK)(10).

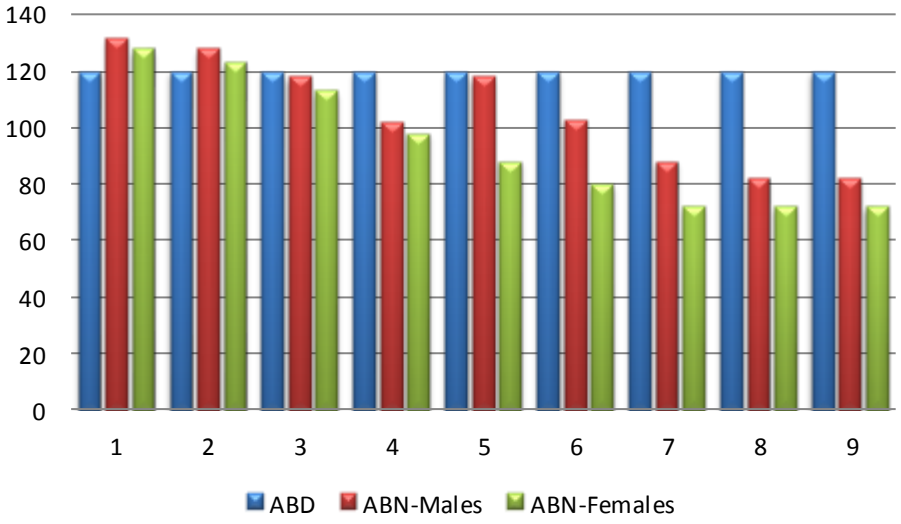
7- Statistical Analysis:

Data from the study were analyzed using T- test by using SPSS program Ver.10 for Windows. A *P* value of <0.05 was considered indicative of a statistically significant difference .

Results

Results of nourishing (150g) of *Agaricus bisporus* mushroom as a diet to about (40) healthy volunteers from both sexes for two months did not show any body weight (BW) gain and the body weight of the volunteers from both *Agaricus bisporus* – nourished group (ABN) and the controls (*Agaricus bisporus* – deprived group (ABD) appeared the same after two months of mushroom administration.

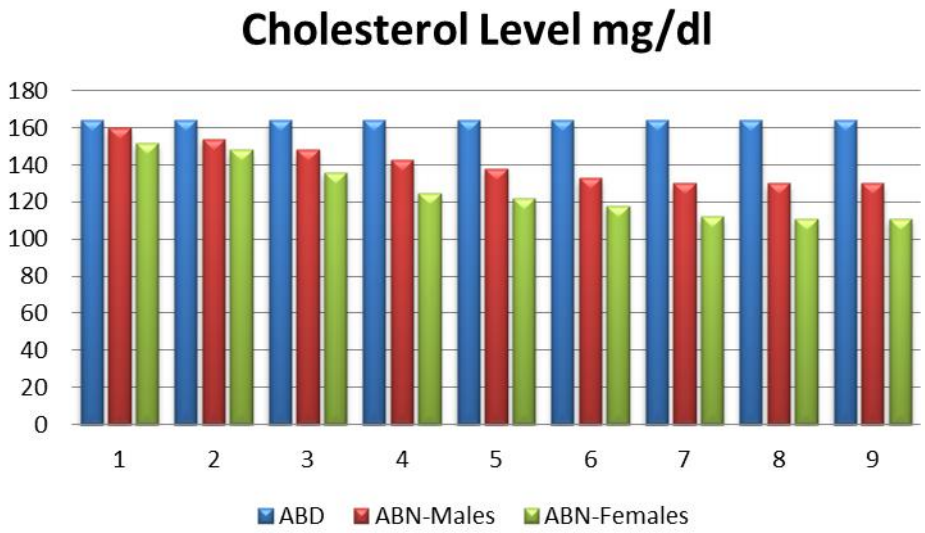
However, a significant difference (P <0.05) in serum glucose levels in both (male and female volunteers of the study) was detected during the period of mushroom administration between the (ABN- group) compared to that serum glucose levels of the (ABD - group) shown in figure (1).



Figure(1): Glucose level mg/dl

***ABD = control group deprived from eating mushroom for two months**
***ABN-males = *Agaricus bisporus* – males nourished group**
***ABN- females = *Agaricus bisporus* – females nourished group**
***means ± SEM of (20 ABN- females and 20 ABN- males) and (10 control ABD).**

Moreover, a significant reduction (P <0.05) in serum cholesterol levels in both (male and female volunteers of the study was also been detected during the period of mushroom administration between *Agaricus bisporus* – nourished group (ABN) compared with the serum cholesterol levels of the deprived group (ABD) as shown in figure (2).



Figure(2): Cholesterol level mg/dl

***ABD = control group deprived from eating mushroom for two months**
***ABN-males = *Agaricus bisporus* – males nourished group**
***ABN- females = *Agaricus bisporus* – females nourished group**
***means ± SEM of (20 ABN- females and 20 ABN- males) and (10 control ABD).**

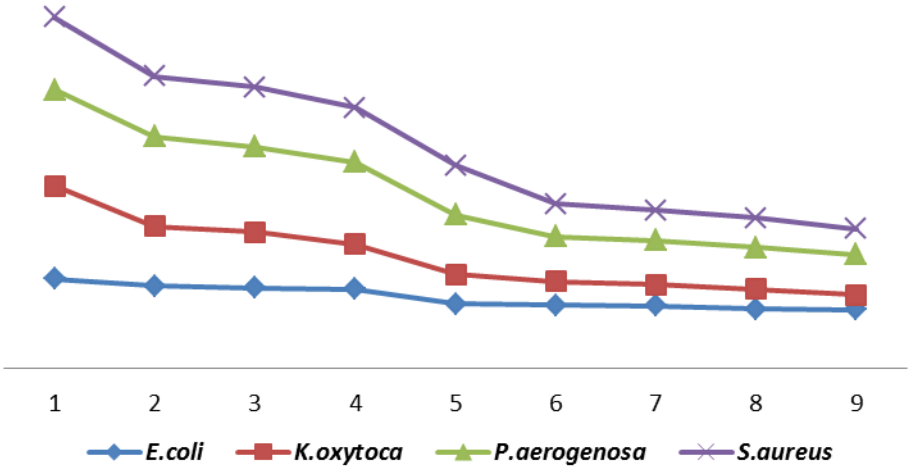
Moreover, a comparison in the reduction levels of the glucose and cholesterol between ABN-males and females showed that, male volunteers showed more significant decrease (P <0.05) in their glucose and cholesterol levels than female volunteers table (1).

Table(1): Reduction of glucose and cholesterol concentration in blood recorded between *Agaricus bisporous* nourished males (ABN-males), *Agaricus bisporus* – nourished females group (ABN-Females) and *Agaricus bisporous*-deprived group (ABD).

Study Groups	Reduction of Glucose Concentration in (mg/dl)	Reduction of Glucose Concentration in blood %	Reduction of Cholesterol Concentration in (mg/dl)	Reduction of Cholesterol Concentration in blood %
ABN-Male	50 mg/dl	62.12%	30mg/dl	81.25%
ABN-Female	56mg/dl	56.25%	41 mg/dl	73.03%
ABD	0mg/dl	0%	0mg/dl	0%

In addition, the effect of *Agaricus bisporus* mushroom administration on the bactericidal activity of normal serum was also studied and the serum collected from both *Agaricus bisporus* – nourished males and females groups (ABN) along with the controls (*Agaricus bisporus* – deprived group (ABD) were tested against four bacterial strains and the

bactericidal activity was recorded in colony counts per week of mushroom administration) as shown in figure (3).



Figure(3): ***Agaricus bisporus*** **effect on the bactericidal activity of normal serum (cfu/ml)**

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In addition, the antibacterial activity of ethanol extract from *Agaricus bisporous* mushroom was also tested with the same bacterial strains and results were shown in table (2).

Table (2): Antibacterial activity of ethanol extract of *Agaricus bisporus* and the antibiotic sensitivity of the tested bacteria (zone sizes, mm).

Tested bacteria	Bacterial Growth Inhibition Zone (mm)				
	Agaricus bisporus (100µl/well)	Ampicillin (10 µg/disc)	Penicillin (10 µg/disc)	Gentamycin (10 µg/disc)	Tetracycline (10 µg/disc)
Escherichia coli	Resistant	4mm	6mm	15mm	Resistant
Staphylococcus aureus	12mm	7mm	5mm	18mm	4mm
Klebsiella species	Resistant	Resistant	Resistant	2mm	Resistant
Pseudomonas aerogenosa	Resistant	Resistant	Resistant	11mm	Resistant

Discussion

Results of the study showed that, there were no body weight gain in the group of volunteers that were nourished with the *Agaricus bisporus* mushroom as a diet. These results agreed with the results obtained by (12 and13) no body weight was gained in the mushroom nourished individuals due to normal daily food and water intake which resembled to control group.

on the other hand, it was found that, ingestion of *Agaricus bisporus* twice in week for two months had significantly reduced (P <0.05) blood glucose concentration in about (81.25%) for male and (56.25%) for female . In addition, ingestion of *Agaricus bisporus* had also significantly reduced (P <0.05) blood cholesterol concentration in about (62.12%) for male and (73.03%) for female. The same results were obtained by (14-16) where they showed that, the high dietary fiber content and possibly other carbohydrate components in the *Agaricus bisporus* mushroom may account for its glucose-lowering effect in a way similar to other high fiber and carbohydrate-containing foods so that, these dietary carbohydrate stimulate the increased secretion of insulin that is correlated with a marked low glucose response. In addition, it was found that, the *Agaricus bisporus* mushroom contains a lectin-like molecule that has been shown to stimulate insulin and glucagon release from islet cells which is a key metabolic pathway involved in regulating glucose response (17). As well as, the high fiber content of the *Agaricus bisporus* mushroom may act as a barrier to digestive enzyme action, thereby, contributing to lower blood glucose response(1, 3 and 6).

Another possible explanation for the glucose-lowering effect of the *Agaricus bisporus* mushroom is that, bacterial fermentation of the *Agaricus bisporus* mushroom fiber, which is rich in polysaccharide and oligosaccharide, in the colon may lead to the production of short-chain fatty acids such as acetate, propionate, and butyrate (17 and 18).

The results of the bactericidal test showed that, the administration of *Agaricus bisporus* mushroom to healthy volunteers during the period of two months came with positive effects on the bactericidal activity of their serum clarified by the reduction in the colony counts (cfu/ml) of the tested bacteria so that, *Agaricus bisporus* mushroom produced a reduction of about (29.5%) in the (cfu/ml) of *E.coli*; (77.24%) in the (cfu/ml) of *Staphylococcus aureus*; (53.4%) in the (cfu/ml) of *Klebsiella oxytoca* and (55.33%) in the (cfu/ml) of *Pseudomonas aerogenosa* (cfu/ml) respectively. These results came in agreement with the results of (19) whose mentioned that, mushrooms were appreciated for their active biological compounds of medicinal value and they accumulate a variety of secondary metabolites, including phenolic compounds, terpenes and steroids that provide active effects to the body against different microorganisms (19, 20,21,22 and 23).

The antibacterial activity of the ethanol extract of the *Agaricus bisporus* mushroom detected against *Staphylococcus aureus* in table (2) this result agreed with the studies of (23,24 and 25) where they revealed antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Micrococcus flavus*, *Staphylococcus aureus* with little or no activity against Gram negative bacteria probably due to the presence of a number of compounds that have been described as active against Gram-positive bacteria such compounds as terpenes, confluentin, grifolin and neogrifolin.

Conclusions

The regular ingestion of the *Agaricus bisporus* mushroom as a diet might be helpful in reducing glucose and cholesterol concentrations in blood without affecting the body weight probably due to the presence of high levels of dietary fibers and antioxidants ; besides its ethanol extract that produced a significant effect on *Staphylococcus aureus* which could be employed for the treatment of certain infections.

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