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# STUDY OF THE EFFECT OF CHITOSAN EXTRACTED FROM THE MUSHROOM ON THE EXPERIMENTALLY INDUCED HYPERLIPIDEMIA IN MALE RABBITS

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## ABSTRACT

**The present study aimed to identify the therapeutic evaluation of chitosan extracted from the fungus cushroom and pure chitosan on glucose and lipid profile in the blood of 35 male rabbits with hyperlipidemia induced experimentally by cholesterol. The tests included estimation of glucose levels, total cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, and very low-density lipoproteins. hyperlipidemia was induced in the male rabbits used in the study which was administered orally with cholesterol 150mg/kg body weight for a week. rabbits were divided into seven groups: control, cholesterol, pure chitosan, mushroom chitosan, cholesterol and pure chitosan, cholesterol and mushroom chitosan and cholesterol and simvastatin. The results of the study showed, the hyperlipidemia induced experimentally resulted a significant increase (P<0.05) in TC, TG, LDL, and VLDL, while no significant difference in HDL compared with control group, on the otherwise the glucose level significantly increase than control. Also, groups of animals treatment with pure chitosan and mushroom chitosan showed a significant decrease (P<0.05) in glucose, TC, TG, LDL, and VLDL, and no significant difference in HDL compared with control group. While, the groups showed treatment with cholesterol and pure chitosan, cholesterol and mushroom chitosan, cholesterol and simvastatin a significant decrease (P<0.05) in glucose, TC, TG, LDL, and VLDL, and a significant increase (P<0.05) in HDL compared with the cholesterol group. The research study revealed that chitosan extracted from mushroom can control the levels of fat concentrations and their complications, in addition to its important role in biochemical variables, and treatment of most disease cases, especially cardiovascular disease.**

**Keywords:** Chitosan, Mushroom, Hyperlipidemia.

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# دراسة تأثير الكايتوسان المستخلص من فطر العرهون على فرط الدهون المستحث تجريبا في ذكور الأرانب

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**الخلاصة**

**هدفت الدراسة الحالية الى التعرف على التقييم العلاجي للكايتوسان المستخلص من فطر العرهون والكايتوسان النقي على سكر الكلوكوز والدهون في مصل الدم لذكور الارانب المحلية المصابة بفرط الدهون المستحث فيها تجريبياً بواسطة الكوليسترول وتضمنت الفحوصات الكيموحيوية تقدير مستويات الكلوكوز والكوليسترول الكلي والدهون الثلاثية والبروتينات الدهنية عالية الكثافة والبروتينات الدهنية واطئة الكثافة والبروتينات الدهنية واطئة الكثافة جداً، تم استحداث فرط الدهون في ذكور الارانب المستخدمة في الدراسة التي تم تجريعها فمويا بالكوليسترول بتركيز 150 mg/kg من وزن جسم الحيوان، حيث قسمت ذكور الارانب الى سبعة مجاميع: السيطرة، الكوليسترول، الكايتوسان النقي، كايتوسان العرهون، الكوليسترول والكايتوسان النقي، الكوليسترول وكايتوسان العرهون، الكوليسترول وعقار السمفستاتين، وقد أظهرت نتائج الدراسة، أن استحداث فرط الدهون التجريبي أدى إلى ارتفاع معنوي (P<0.05) في تراكيز الكلوكوز، TC، TG، LDL، VLDL، فيما لم يظهر فرق معنوي في HDL، مقارنة مع مجموعة السيطرة، كما اظهرت مجاميع الحيوانات المعاملة بالكايتوسان النقي وكايتوسان العرهون انخفاضاً معنويا (P<0.05) في تراكيز مستويات الكلوكوز والكوليسترول الكلي والكليسيريدات الثلاثية وLDL وVLDL وعدم فرق معنوي في تركيز HDL مقارنة مع مجموعة السيطرة، بينما اظهرت المجاميع المعاملة بالكوليسترول والكايتوسان النقي، الكوليسترول وكايتوسان العرهون، الكوليسترول وعقار السمفستاتين انخفاضا معنوياً (P<0.05) في تراكيز مستويات الكلوكوز والكوليسترول الكلي والكليسيريدات الثلاثية وLDL وVLDL وارتفاعا معنويا في تركيز HDL مقارنة مع مجموعة الكوليسترول، وتبين ان الكايتوسان المستخلص من العرهون بامكانه السيطرة على مستويات تراكيز الدهون ومضاعفاتها فضلا عن دوره المهم في المتغيرات الكيموحيوية دون تاثيرات اخرى، وعلاج اغلب الحالات المرضية خصوصا امراض القلب الوعائية.**

**الكلمات المفتاحية:** الكايتوسان، العرهون، فرط الدهون.

## INTRODUCTION

Nutritional fungi, including mushroom, are rich in carbohydrates that include dietary fibers and polysaccharides such as gluconate, glycogen, monosaccharides and disaccharides, and it are rich in protein content because it contains most of amino acids, and it also contain low levels of fats, and they are in the form of sterols. And unsaturated fatty acids, as well as containing low levels of sodium and high levels of potassium, iron and selenium, in addition to containing vitamins and antioxidants **(Ulziijargal & Mau 2001; Kruzselyi & Vette 2013)**. Studies also indicated that It contains heart-enhancing chemical compounds, anti-cholesterol, liver-protective, blood-sugar-level, anti-cancer, bacteria, viruses **(Jua *et al.*, 2010; Ramirez-Anguiano *et al.*, 2007)**. Hyperlipidemia is a disease caused by a metabolic disorder that leads to disturbances in the transport of lipoproteins in the blood plasma (high levels of low-density lipoproteins and very low-density proteins containing high cholesterol) and this in turn has a great impact on the incidence of atherosclerosis **(Brown & Goldstein 1987)**. Chitosan is known as a natural multiple sugar consisting of amino-2-deoxy-D-glucopyranose units linked to gether by β-(14)glycosidic bonds, and with the formula $(C_6H_{11}O_4N)$ n, it is one of the most important derivatives of chitin and can be obtained from the removal process Deacetylation of a group of acetylated carbon 2 atom in chitin using concentrated base conditions **(Wang &**

**Chen 2014)**. Chitosan contains the active hydroxyl groups at the site of carbon atom 6 and 3, and contains the amine group at the site of carbon 2, as in (Figure 1).

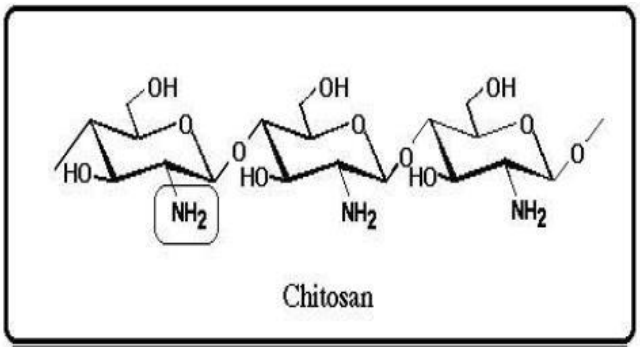


**Figure (1):** The chemical composition of chitosan.

Chitosan has the ability to reduce heart disease in general through its work to reduce cholesterol levels in the blood, leading to a reduction in fat and weight by reducing the absorption of fats in the small intestine **(Zhang *et al*., 2008)**. Therefore, it counteracts some metabolic disorders and changes and reduces obesity caused by diet **(Dhamodharan & Mirunalini 2012)**.

Chitin and chitosan have received great interest as vital functional substances in a wide range of different applications in the fields of agriculture, medicine, pharmacy, cosmetics, paper-making and water purification, chitin and chitosan are produced from crustaceans such as shrimp and crabs. Interest has increased in recent times to search for alternative. For the production of chitosan **(Cheng *et al*., 2014)**, as many studies have indicated the possibility of producing chitosan from food fungi as an alternative source, including truffles, as these fungi are a successful alternative to the production of chitosan **(Yen & Mau 2007)**.

In view of the importance of chitosan from a medical and preventive point of view, this study aimed at the therapeutic evaluation of chitosan extracted from Mushroom against hyperlipidemia induced in rabbitsand compare its effect with simvastatin.

## MATERIALS AND METHODS

### Collection and preparation of Sample

Samples of mushrooms were collected from Al-Wadq Farm, Baghdad, Iraq. The samples were washed well with running tap water several times to get rid of soluble organic matter, adherent proteins and other impurities. Then they were dried for 4 consecutive days by exposing them to the heat of the sun, then grinded to obtain a homogeneous fine powder. Keep it in closed plastic containers at room temperature until use.

### Extraction of Chitosan

Chitosan was extract according to the method used by **(Kamil *et al*., 2002)** with some modifications made to it.

### Diagnosis of chitosan

The chitosan was diagnosed using a Fourier Transform InfraRed Spectrophotometer (FT-IR) Shimadzu company/ Japan affiliated to the laboratories of the Department of Chemistry, College of Science, University of Tikrit **(Vaingankar & Juvekar 2014)**. And a person was also diagnosed by dissolving it in 5% acetic acid, because the dissolution process is in itself one of the evidence for the formation of chitosan.

**Initialization of laboratory animals**

The experiment was using 35 rabbits male were getting from the local market with weights ranging 1.5-2.0 kg and ages 5-8 months, the animals were divided and distributed uniformly of weight and placed in tight wooden cages with dimensions of 50 × 60 × 150cm. The animals were subjected to laboratory conditions with a light cycle divided into 12 hours of light and 12 hr of darkness.

**Experience Design**

The experimental animals were divided and distributed uniformly in terms of weight into Seven groups, 5 animals for each group. The animals were treated once daily for 21 days. Hyperlipidemia was induced in 4 groups of experimental animals using cholesterol by oral injection 150 mg/ kg dissolved in soybean oil a period of two weeks, and this was confirmed by conducting lipid tests for the animal group, then the animals were left for 48 hour, the treatment stage began during the period of 21 days, according to the following groups:

(1): (Control) was given the standard feed and water.
(2): (Cholesterol) were given 150 mg/ kg cholesterol.
(3): (Pure chitosan) were given 30 mg/ kg pure chitosan.
(4): (Mushroom chitosan) were given 75 mg/ kg mushroom chitosan.
(5): (Cholesterol and pure chitosan): the hyperlipidemia animals group were given 30 mg/ kg of pure chitosan.
(6): (Cholesterol and mushroom chitosan): the hyperlipidemia animals group were given 75 mg/ kg mushroom chitosan.
(7): (Cholesterol and simvastatin): the hyperlipidemia animals group were given 1 mg/ kg simvastatin.

**Collection of blood samples**

After the end of experiment, the animals were anesthetized with chloroform to collect blood samples from the jugular vein and placed in gel tube, were centrifuged (4000 rotation/min) for 15 min, to obtain the serum was stored in plan tube a temperature of -20°C until the analysis was conducted.

**Biochemical of blood tests**

The device was chemical analyzer USA(Smart-150) to estimate all the tests glucose, total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). According to the manufacturer of the solutions.

**Statistical Analysis**

The data of results in the present study were analyzed by using the ANOVA analysis, utilized the general linear model of the Statistically Analysis System. Also, significant differences were evaluated by using Duncan's multiple-range test **(Duncan, 1955)**, and significance level is based on level of probability ($P<0.05$).

## RESULTS AND DISCUSSION

Chitosan was obtained from mushroom as in (Figure 2).

**Figure (2):** Extracted of chitosan from mushroom**.**

### FTIR spectroscopy

The FTIR spectrum of the chitosan model extracted from mushroom,(Figure 3) shows the amino group ($NH_2$) is the most important active group, that absorption peak appeared at the frequency 1490 $cm^{-1}$, where the appearance of this group on the carbon 2 site of glucose amine is an evidence of the presence of chitosan, and these results are close to what he found **(Kumirska *et al.*, 2010)**.While the active group, which represents the bundle of N - H groups, its absorption peak appeared at frequencies 3265 $cm^{-1}$. While the active group, which represents the hydroxyl stretching band, its absorption peak appeared at frequencies 3366 $cm^{-1}$, as this group appears in chitosan and chitin because it is not affected by the removal process Acetyl groups or the hydrolysis process is therefore a reference to ensure the presence of chitin and chitosan **(Ebrahimzadeh *et al.*, 2013)**.The beams at frequencies, 1628 $cm^{-1}$ refer to the group C = O in the primary group (Amide I), and their intensity depends on the degree of removal of the acetyl group **(Arantes *et al.*, 2014)**. The absorption bands that appeared at the frequencies, 2879 $cm^{-1}$ which belong to the stretches of the C - H group, this result agreed with study **(Al-Abbasy *et al.*, 2018)**. The glycosidic bond of the β-anomer of chitosan, its absorption peak appeared at frequencies 895 $cm^{-1}$, this result is in agreement with what was mentioned **(Wu *et al.*, 2019)**.

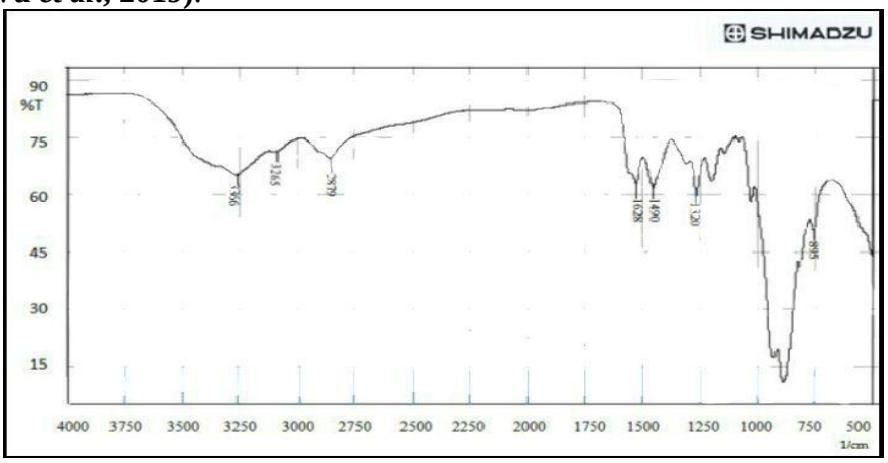


**Figure (3):** FTIR spectrum model of a chitosan extracted from mushroom.

### Biochemical tests of blood

The results of the current study showed in (Table 1) that the development of hyperlipidemia in cholesterol led to a significant increase ($P<0.05$) in the concentration of

glucose level in the blood of male rabbits with new hypercholesterolemia compared with the control group.

**Table (1):** Effect of extracted chitosan on glucose and lipid profile in serum of male rabbits induced hyperlipidemia.

| Parameter / groups | Glucose | cholesterol | Triglycerides | HDL | LDL | VLDL |
|---|---|---|---|---|---|---|
| | Mg/dL | | | | | |
| Control | 134.0±0.10 cdef | 200.0±1.20 E | 100.0±1.34 cde | 25.0±0.09 bc | 155.9±0.83 de | 20.0±1.23 bcd |
| Cholesterol | 143.0±0.07 a | 305.0±0.90 A | 155.0±1.76 a | 23.0±1.00 c | 251.4±1.90 a | 31.0±1.00 a |
| Pure Chitosan | 136.0±0.21 abcde | 121.0±1.30 L | 63.0±1.09 h | 29.0±1.07 a | 79.6±1.45 i | 12.4±0.94 g |
| Mushroom Chitosan | 130.0±0.12 f | 177.0±1.08 F | 68.0±1.50 gh | 28.0±1.20 b | 135.4±1.23 ef | 13.6±1.30 fg |
| Cholesterol + Pure Chitosan | 138.0±0.20 abc | 205.0±1.50 D | 97.0±1.78 bcde | 30.0±1.42 a | 155.6±1.08 de | 19.4±0.69 bcde |
| Cholesterol +Mushroom Chitosan | 130.0±0.16 f | 160.0±1.02 f | 98.0±1.45 bcd | 30.0±1.93 a | 140.4±1.54 ef | 19.6±1.75 bcde |
| Cholesterol +Simvastatin | 139.0±0.12 abc | 179.0±1.13 gh | 85.0±1.13 defg | 26.0±1.75 bc | 146.8±1.00 e | 17.0±1.11 cdefg |

It also showed a significant decrease ($P<0.05$) in the group treated with (MushroomChitosan), and there was no significant difference in the group treated with (Pure Chitosan) compared with the control group. While there was a significant decrease ($P<0.05$) in the groups treated animals with (cholesterol and pure chitosan, cholesterol and MushroomChitosan, cholesterol andsimvastatin) compared with the cholesterol group.

There is a relationship between high glucose and high blood lipid levels, and this is reflected in a group of mechanisms, including weak insulin signal in muscles with other tissues, according to the increase and accumulation of extracellular fats **(Kopelman 2000)**.

The reason for the high blood sugar concentration may be attributed to the increase in the generation of free radicals that destroy beta cells in the pancreas and work to stop their work and destroy them, as free radicals stimulate the process of lipid peroxidation and breakdown of the RNA and inhibit the synthesis of the primary insulin, the blood sugar concentration increases, thus halting the degradation of blood sugar and stimulating the processes of blood sugar formation and glycogenolysis **(Ayala*et al*, 2014)**. After eating a meal, the blood sugar level will increase, and is preceded by inflammation and endoplasmic reticulum stress, which leads to an increase in insulin resistance and weakening of insulin secretion **(Bender *et al*., 2014)**. Polyunsaturated fatty acids suppress liver fat synthesis **(Albert *et al*., 2014)**. The fatty acids reduce the reproduction of genes encoded for the breakdown of liver fats or glycolytic enzymes.

The presence of a significant decrease in the glucose concentration for groups of animals treated with Pure Chitosan and MushroomChitosan may be due to the fact that chitosan stimulates the secretion of insulin from the pancreatic islets directly, leading to facilitating the entry of calcium ions into the pancreatic beta cells, which is the key to the metabolic pathway in regulating the glucose response **(Zhang & Lin 2004)**.

The results of the present study showed in (Table 1) that the development of hyperlipidemia in cholesterol led to a significant increase ($P<0.05$) in the concentrations of levels of total cholesterol, triglycerides, low-density lipoproteins, and very low-density

lipoproteins, and no significant difference in the concentration of high-density lipoproteins in Serum of male rabbits infected with induced hypercholesterolemia compared with the control group.

Also, the groups of animals treated with (pure Chitosan, MushroomChitosan) showed a significant decrease (P<0.05) in the concentrations of levels of TC, TG, LDL and VLDL, and no significant difference in HDL concentration compared with the control group.

While the groups treated with (cholesterol and pure chitosan, cholesterol and mushroomchitosan, cholesterol and simvastatin) showed a significant decrease (P<0.05) in the concentrations of total cholesterol, triglycerides, LDL and VLDL levels, and a significant increase in the HDL concentration compared with the cholesterol group.

The significant increase in the concentration levels of (TC), (TG), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL), in the blood serum of male rabbits infected with new hypercholesterolemia is a natural result as a result of feeding animals with cholesterol, which may be due to the increase in the resulting cholesterol esters This confirms the ability of cholesterol to raise the concentration of triglycerides, or it may be due to changes in the absorption process and excretion of steroids or a decrease in the level of bile salts, or the increase in cholesterol may be due to the presence of a disease affecting the liver, which leads to its inability to Benefit from cholesterol for conversion into HDL and LDL **(Abdelhalim & Al-Ayed 2008)**.

HDL-C plays a cholesterol-lowering role as it picks up excess cholesterol from the blood and peripheral tissues to the liver, where it is converted into bile acids, and plays an important role in inhibiting the formation of atherosclerotic plaques in the arteries and for this reason it is known as good cholesterol **(Kim *et al.*, 2008)**, and it is possible to increase HDL-C, with treatment, is due to the activation and increase of the activity of the enzyme Lecithin-Cholesteryl acyl transferase (LCAT), which is the enzyme responsible for combining cholesterol with HDL, and inhibiting the enzyme Hepatic triglycerides lipase (HTL), which leads to rapid lipid catabolism through the suprahepatic tissue **(Anila & Vijayalakshmi 2002)**.

LDL-C is responsible for transporting cholesterol to the cells of the body, it transports about 60-70% of total cholesterol, so the increase in TC levels is followed by an increase in LDL-C that cannot be eliminated in the fat metabolism process, and it is more likely that it will enter into the distance.Subepithelialis as a prelude to oxidation, and LDL-C carries oxidation by inhibitors of macrophages, and large fat-laden macrophages will leave pulp rich or full of fat and cholesterol after storage and then atherosclerosis begins **(Beckman *et al.*, 2002)**.

The significant decrease in the levels of cholesterol, triglycerides, LDL-C and VLDL-C levels, and an increase in HDL-C levels in the groups treated with chitosan may be due to the effect of chitosan that reduces the absorption of fat and cholesterol, and that chitosan is degraded into multiple sugars in the intestine, which causes an increase in Viscosity and reduced absorption of fat and cholesterol **(Panith *et al.*, 2016)**. In addition, the decomposition of chitosan into glucose amine reduces the formation of triglycerides in the liver **(Kobayashi *et al.*, 2006)**. Chitosan is also considered a dietary fiber, and its action is to surround fat drops and prevent the digestive enzymes from reaching the fat, which leads to its excretion outside the body **(Biskup *et al.*, 2007)**.

Studies indicate that the positive charge carried by the chitosan molecule (amino groups) makes it associated with negatively charged substances such as fats, cholesterol and lipoproteins. Chitosan mixes with the fat in the food in the stomach and then interferes with the fat droplets to form a lipid-chitosan complex. In the small intestine, thus preventing lipolysis and thus the secretion of undigested fats, including cholesterol **(Santas *et al.*, 2012)**. The results of the current study are consistent with what was reported by **(Bahijri *et al.*, 2017)**

through their study when giving rats a food containing chitosan for a week, which led to a decrease in the average values of cholesterol, triglycerides and LDL **(Bahijri *et al.*, 2017)**. It also agreed with the study **(Zhang *et al.*, 2013)**.

Simvastatin showed a significant decrease in the levels of TC, TG, LDL and VLDL, and an increase in the levels of HDL, due to the effect of statins on the body's ability to manufacture cholesterol by targeting hepatocytes and inhibiting the enzyme HMG-CoA-reductase that converts HMG CoA into mevalonic acid. The raw material for the manufacture of cholesterol, and statins are similar to HMG CoA in concentration and take its place in the enzyme and reduce the rate of production of mevalonate and this affects because most of the circulating and transported cholesterol is made in the liver, and when the liver is unable to produce larger quantities of cholesterol, this will consequently reduce its level **(Stancu & Sima, 2001)**. Simvastatin also plays a role in increasing the effectiveness of lipoprotein enzyme lipase and thus reducing triglyceride levels, as well as its ability to raise HDL concentrations **(Trapani *et al.*, 2013)**.

## CONCLUSIONS

It was found that the presence of chitosan in the Arhun in the following packages: group (NH2) at frequency 1490 $cm^{-1}$, group N – H at frequency 3265 $cm^{-1}$, extension package (OH) at frequency 3366 $cm^{-1}$, the bond (Amide I) at frequency 1628 $cm^{-1}$, The glycosidic bond of chitosan at frequency 895 $cm^{-1}$.

Also, the creation of excess fat in animals has caused disturbances in the biochemical variables under study that varied between increase and decrease, which leads to the disruption of many metabolic activities in the body such as the metabolism of fats, carbohydrates and proteins, which leads to cases of high glucose concentration and defects in the functions of body organs.

The treatment with mushroom and chitosan extracted from it also led to the reversal of the disturbances of all the values of biochemical variables resulting from the development of hyperlipidemia towards the normal values or close to them, which makes it possible to use it alone or in synergy with other substances to reduce the level of sugar or fat or to prevent it.

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