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The Therapeutic Evaluation of Extracted Chitosan from Local Fungus Truffle Against Hyperlipidemia Induced In Male Rabbits

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ABSTRACT

The present study aimed to identify the therapeutic evaluation of chitosan extracted from the fungus truffle and pure chitosan on glucose and lipid profile in the blood of 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. Male rabbits were divided into eight groups: control, Cholesterol, Pure chitosan, Brown truffle chitosan, White truffle chitosan, Cholesterol and pure chitosan, Cholesterol and brown truffle chitosan and Cholesterol and white truffle chitosan. The results of the study showed, The hyperlipidemia induced experimentally resulted a significant increase ($P<0.05$) in the concentrations of glucose, TC, TG, LDL, and VLDL, while no significant difference in HDL compared with control group. The treatment of male rabbits with pure chitosan resulted in no significant differences in glucose, HDL. While there was a significant decrease ($P<0.05$) in TC, TG, LDL, and VLDL compared with control group. The treatment of animals with cholesterol and pure chitosan led to a significant increase ($P<0.05$) in HDL, while a significant decrease ($P<0.05$) in glucose, TC, TG, LDL, and VLDL, compared with cholesterol group. When the rabbits were treated with brown truffles chitosan, there was no significant difference in glucose, HDL, while there was a significant decrease ($P<0.05$) in TC, TG, LDL, and VLDL compared with the control group. The treatment with cholesterol and white truffle chitosan, showed increase ($P<0.05$) significant in HDL, while there was a significant decrease ($P<0.05$) in TC, TG, LDL, and VLDL, compared with the cholesterol group. When rabbits were the treated with cholesterol and brown truffles chitosan, led to a significant increase ($P<0.05$) in HDL, significant decrease ($P<0.05$) in TC, TG, LDL, and VLDL, while there was no significant difference in glucose compared with the cholesterol group. The treatment with cholesterol and white truffle chitosan, showed increase ($P<0.05$) significant in HDL, while there was a significant decrease ($P<0.05$) in TC, TG, LDL, and VLDL, compared with the cholesterol group. It is evident from the current study that the extracted chitosan has an important role to reduce the high levels of fats and their complications.

Key word: chitosan, truffle, hyperlipidemia

Introduction

Chitosan is known as a natural multiple sugar consisting of amino-2-deoxy-D-glucopyranose units linked together by β-(1 → 4) glycoside bonds, and form $(C_6H_{11}O_4N)n$, chitosan has received great interest as a vital functional material in a range of applications, as agriculture, medicine, cosmetics and water purification[(1)], Chitosan is produced from crustaceans such as shrimp and crabs. Interest has increased in recent times to search for alternative sources for chitosan production [(2)], as many studies have indicated the possibility of producing chitosan from fungi as alternative source, including truffles, mushroom [(3)]. Truffles are considered a large food fungus, if the weight of the truffle ranges from 30 to 300 grams. Regular soft flesh has a smooth or tuberous surface and varies in color from white to black [(4)]. Truffles are also rich in chitosan and other carbohydrates that include dietary fibers and polysaccharides such as gluconate, glycogen, monosaccharides and disaccharides, and it is rich in proteins because it contains all the essential amino acids, It also contains low levels of sodium and high levels of potassium, iron and selenium, in addition to containing vitamins and antioxidants [(5)]. The chemical composition and nutritional value of *Terfezia claveryi* and *Tirmania nivea* were studied, which showed the presence of protein, fats, dietary fiber, ash, ascorbic acid, essential amino acids and minerals (potassium,

phosphorous, iron, copper, zinc, and manganese) [6]. Hyperlipidemia is a disease caused by a metabolic disorder that leads to disturbances in the transport of lipoproteins in the blood plasma, and this in turn has a significant impact on Atherosclerosis [7]. It was found that in a previous study in mice, chitosan reduced levels of total and LDL cholesterol in plasma and triglycerides in the liver and helped increase the excretion of bile acids in the stool without a clear change in the level of HDL and cholesterol [8]. Chitosan surrounds fat droplets in the stomach when taken together, which reduces the effect of the lipase enzyme [9], and thus prevents the digestion of fat in the small intestine [10]. Also, oral administration of chitosan has the ability to reduce plasma lipids in healthy men [11].

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 Truffles against hyperlipidemia induced in rabbits.

Materials and methods:

Collection and preparation of Sample:

Collected of samples brown truffles (*Terfezia claveryi*) and white truffles (*Tirmania nivea*). The sample collection process in the period from January –April,2019 from the northwestern regions of the Shirqat city /Salah al-Din – Iraq.

These species were classified according to the type and shape of the truffles, in addition to the meanders on their surface[6].

Samples were washed well with water several times, then dried by the sun's heat, then grinded homogeneous to obtained powder and kept in closed plastic containers at room temperature until use [12].

Extraction of Chitosan:

Chitosan was extract according to the method used by (Kamil and others) with some modifications made to it [13].

Diagnosis of chitosan using FTIR :

The chitosan was diagnosed using a Fourier Transform Infra Red Spectrophotometer (FT-IR) Shimadzu company/ Japan affiliated to the laboratories of the Department of Chemistry / University of Tikrit - College of Science [14].

Initialization of laboratory animals:

The experiment was using (40) rabbits male were getting from the local market with weights ranging (1.5-2.0) kg and ages (8-5) months, the animals were divided and distributed uniformly of weight and placed in tight wooden cages with dimensions of (125 x 60 x 50) cm. The animals were subjected to laboratory conditions with a light cycle divided into 12 hours of light and 12 hours of darkness.

Experience Design :

The experimental animals were divided and distributed uniformly in terms of weight into eight 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 (150mg / 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) were given the standard feed and water.
(2): (Cholesterol) were given 150mg / kg cholesterol.
(3): (Pure chitosan) were given 30 mg / kg pure chitosan.
(4): (Brown truffle chitosan) were given 75 mg / kg brown truffle chitosan.
(5): (White truffle chitosan) were given 75 mg / kg of white truffle chitosan.
(6): (Cholesterol and pure chitosan): the hyperlipidemia animals group were given 30 mg / kg of pure chitosan.
(7): (Cholesterol and brown truffles chitosan): the hyperlipidemia animals group were given 75 mg / kg brown truffle chitosan.
(8): (Cholesterol and white truffles chitosan): the hyperlipidemia animals group were given 75 mg / kg white truffle chitosan.

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 (3000 rotation/min) for 15 minutes, 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 ($P<0.05$) [15].

Results and discussion:

Chitosan was obtained from (brown and white truffles) figure (1).

Figure: (1) Extracted of chitosan (a-brown truffles, b-white truffles)

FTIR spectroscopy:

Figures (2) and (3) shows the FTIR spectrum of the chitosan model extracted from brown truffles, and (3) the FTIR spectrum of the chitosan model extracted from white truffles. The amino group (NH2) is the most important active group, whose absorption peak appeared at the frequency 1592 cm-1 of this spectrum for both the chitosan model extracted from brown and white truffles. The bundles of the amine group showed their absorption at the frequencies 1592 cm-1 and 1520 cm-1 for each of the brown truffle chitosan and the white truffle chitosan respectively, 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 (Bilbao and others) [16]. While the active group, which represents the bundle of N - H groups, its absorption peak appeared at frequencies 3290 cm-1 and 3288 cm-1 for Chitosan and Chitosan brown truffles and Chitosan white truffles respectively. While the active group, which represents the hydroxyl stretching band, its absorption peak appeared at frequencies 3362 cm-1 and 3363 cm-1 for each of Chitosan brown truffles and Chitosan white truffles respectively, 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 [17]. The beams at frequencies, 1664 cm-1 and 1648 cm-1 refer to the group C = O in the primary group (Amide I) for each of chitosan, brown truffles and white truffles, respectively, and their intensity depends on the degree of removal of the acetyl group [18].

The absorption bands that appeared at the frequencies, 2876 cm-1 and 2882 cm-1 which belong to the stretches of the C - H group of both Chitosan brown truffles and Chitosan white truffles, these results agreed with (Hind) in her study [19]. The glycosidic bond of the anomer-of chitosan, its absorption peak appeared at frequencies 892 cm-1 and 890 cm-1 for each of the chitosan extracted brown truffles and white truffles respectively, this result is in agreement with what was mentioned [16].

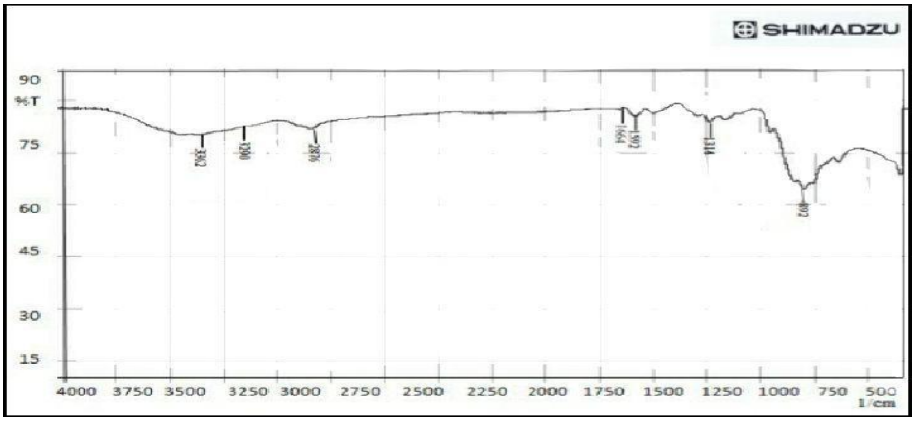

Figure (2) FTIR spectrum model of a chitosan extracted from brown truffles

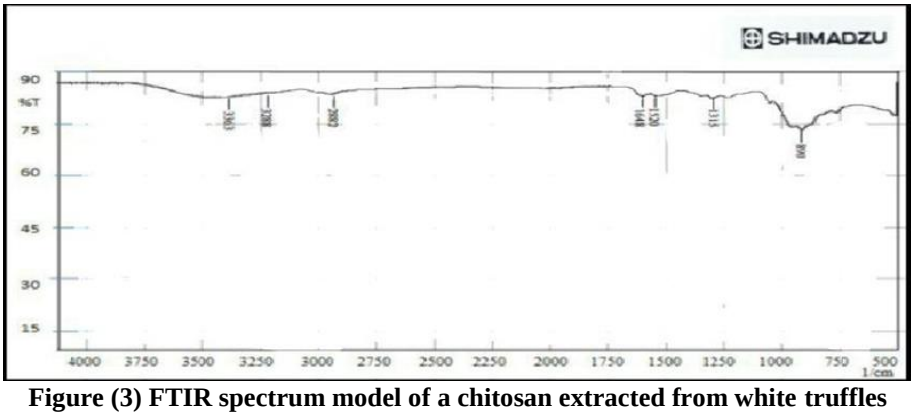

Figure (3) FTIR spectrum model of a chitosan extracted from white truffles

Biochemical tests of blood:

The results of the current study showed in Table (1) that the induced of hyperlipidemia in cholesterol led to a significant increase ($P<0.05$) in the glucose level of male rabbits with 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 cd	155.9±0.83 de	20.0±1.23 bcd
Cholesterol	141.0±0.07 a	305.0±0.90 a	155.0±1.76 b	23.0±1.00 d	251.4±1.90 a	31.0±1.00 a
Pure Chitosan	136.0±0.21 abcde	121.0±1.30 l	64.0±1.09 i	29.0±1.07 bc	79.6±1.45 i	12.4±0.94 g
Brown Truffle Chitosan	133.0±0.12 def	171.0±1.08 i	75.0±1.50 ghi	29.0±1.20 bc	127.0±1.23 f	15.0±1.30 efg
White Truffle Chitosan	136.0±0.11 abcde	144.0±1.31 k	70.0±1.36 hi	27.0±1.12 cd	103.0±1.22 h	15.0±1.86 efg
Cholesterol + Pure Chitosan	139.0±0.20 abc	205.0±1.50 d	97.0±1.78 cdef	30.0±1.42 bc	155.6±1.08 de	14.0±0.69 fg
Cholesterol + Brown Truffle Chitosan	141.0±0.16 a	207.0±1.02 d	106.0±1.45 cd	30.0±1.93 bc	155.8±1.54 d	21.2±1.75 bc
Cholesterol + White Truffle Chitosan	133.0±0.12 def	198.0±1.13 e	111.0±1.13 c	29.0±1.75 bc	146.8±1.00 e	22.2±1.11 d

*****Different of letters mean significant difference at the level of significance (P<0.05).

Also, there was a significant decrease (P<0.05) in the groups treated with (pure Chitosan, brown truffle Chitosan, cholesterol and white truffle chitosan compared with cholesterol group, while there were no significant differences in the groups of animals treated with (pure Chitosan, brown truffle Chitosan, white truffle Chitosan) compared to with control group, the group treated with (cholesterol and chitosan brown truffle) did not significant difference compared with 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 [(20)].

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, thus inhibiting the secretion of insulin Consequently, the blood sugar concentration increases [(21)], thus halting the degradation of blood sugar and stimulating the processes of blood sugar formation and glycogenolysis. 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 [(22)]. Polyunsaturated fatty acids suppress liver fat synthesis [(23)]. The fatty acids reduce the reproduction of genes encoded for the breakdown of liver fats or glycolytic enzymes.

The results of the present study showed in Table (1).The groups of animals treated with (pure chitosan, brown truffle chitosan, white truffle chitosan) showed a significant decrease (P<0.05) in the concentration of cholesterol compared with control group, and the groups treated with (cholesterol and pure chitosan, cholesterol and brown truffle chitosan, cholesterol and white truffle chitosan) had a significant decrease (P<0.05) compared with cholesterol group.

The results of the present study also showed that there were significant differences (P<0.05) in the concentration of HDL level in the blood of male rabbits infected with the hypercholesterolemia compared with control group. the results also showed that there were no significant differences with the groups treated with (pure chitosan, brown truffle chitosan, white truffle chitosan) compared with control group, as well as the absence of significant differences in the groups treated with (cholesterol, pure chitosan, cholesterol, chitosan brown, cholesterol and chitosan white truffles) compared to cholesterol group.

The results of the current study indicated that there was a significant increase (P<0.05) in the concentration of the LDL level in the blood male rabbits infected with the hypercholesterolemia compared with the control group. It also indicated that there was a significant decrease (P<0.05) in the groups of animals treated with (pure chitosan, brown truffle chitosan, white truffle chitosan) compared with the control group. There was also a significant decrease in the groups of animals treated with (cholesterol, pure chitosan, cholesterol, brown truffle chitosan, cholesterol and white truffle chitosan) compared to cholesterol group.

The significant increase in the concentration levels of total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL), and very low-density lipoprotein (VLDL), and the significant decrease in the level of high-density lipoprotein (HDL) concentration in the blood of male rabbits with induced hypercholesterolemia, this result is a normal of feeding animals with cholesterol, which may be due to the increase in cholesterol esters resulting from hypertriglyceridemia, and this confirms the ability of cholesterol to raise the concentration of triglycerides, also may be due to changes in the absorption process

and the elimination of steroids, or a decrease in the level of bile salts, and may be due to the increase in cholesterol due to the presence of a disease affecting the liver, which leads to its inability to benefit from cholesterol to convert it to HDL and LDL [25].

HDL plays an inhibitory or cholesterol-lowering role as it picks up excess cholesterol and cholesterol esters from the blood and peripheral tissues to the liver, where it is broken down into bile acids [28]. The increase in HDL with the treatment is due to the activation and increase of the effectiveness of the enzyme Lecithin-Cholesteryl acyl transferase (LCAT), which is the enzyme responsible for combining cholesterol with HDL, and inhibition of the enzyme Hepatic triglycerides lipase (HTL), which leads to rapid lipid catabolism through the hepatic tissues [29], as well reverse stimulation of cholesterol transport and competitive inhibition of epithelial cell mediated LDL uptake and prevention of oxidative LDL formation [30], [31]. The significant increase in the HDL level of the groups of animals treated with truffle chitosan may be due to the nature of the extracted chitosan [32], as chitosan acts as antioxidants that have a role in increasing the biosynthesis of apoA-1 with the change in the activity of proteins transporting cholesterol esters, thus raising the level of HDL in plasma and liver [33].

LDL is responsible for transporting TC to the cells of the body, as it carries about 60-70% of total cholesterol, so the increase in TC levels is followed by an increase in LDL that cannot be eliminated in the process of fat metabolism [34].

The decrease significant in the levels of cholesterol, triglycerides, LDL, and VLDL, and an increase in HDL levels in the groups treated with chitosan may be due to the effect of chitosan that reduces the absorption of fats and cholesterol, and that chitosan decomposes into multiple sugars in the intestine, which causes an increase in viscosity and a reduction in the absorption of fat and cholesterol [35], in addition, the hydrolysis of chitosan to glucose amine reduces the formation of triglycerides in the liver [36]. Studies indicate that the positive charge carried by the chitosan molecule (amino groups) causes it to bind to negatively charged substances such as fat, cholesterol and lipoproteins, mixed Chitosan with fat in food in the stomach an efficient emulsification process and then interferes with the fat droplets to form a Chitosan-lipid complex, the convert complex into a gel in the small intestine and thus prevents lipolysis and thus the secretion of undigested fats, including cholesterol [37], [38]. The results of the current study are agreement with (Bahijri and others) through their study when rats were given a food containing chitosan for a week, which led to a decrease in the average values of cholesterol, triglycerides and LDL [39], [40], It also agreed with (Park and others) as they found that feeding 2% chitosan led to a decrease cholesterol in rats after 8 weeks of the experiment, and as the triglyceride and LDL values decreased, there was also a significant increase in HDL values [41],[42].

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التقييم العلاجي للكايتوسان المستخلص من الكمأ المحلي ضد فرط دهون الدم المستحث تجريبياً في ذكور الارانب

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الملخص

هدفت الدراسة الحالية الى التعرف على التقييم العلاجي للكايتوسان المستخلص من فطر الكما والكايتوسان النقي على سكر الكلوكوز والدهون في مصل الدم لذكور الارانب المحلية المصابة بفرط الدهون المستحث فيها تجريبياً بواسطة الكوليسترول وتضمنت الفحوصات هي تقدير مستويات الكلوكوز والكوليسترول الكلي (TC) والدهون الثلاثية (TG) والبروتينات الدهنية عالية الكثافة (HDL) والبروتينات الدهنية واطئة الكثافة (LDL) والبروتينات الدهنية واطئة الكثافة جداً (VLDL). تم استحداث فرط الدهون في ذكور الارانب المستخدمة في الدراسة التي تم تجريعها فمويا بالكوليسترول بتركيز 150mg/kg من وزن جسم الحيوان. حيث قسمت ذكور الارانب الى ثمانية مجاميع: السيطرة، الكوليسترول، الكايتوسان النقي، كايتوسان الكمأ البني، كايتوسان الكمأ الابيض، الكوليسترول والكايتوسان النقي، الكوليسترول وكايتوسان الكمأ البني والكوليسترول وكايتوسان الكما الابيض. 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 تراكيز HDL، مقارنة مع مجموعة السيطرة. أن معاملة ذكور الارانب بالكايتوسان النقي أدت الى عدم وجود فروق معنوية في تراكيز الكلوكوز، HDL، بينما حصل انخفاضا معنويا في تراكيز TC، TG، LDL، VLDLمقارنة مع مجموعة السيطرة. اما معاملة الحيوانات بالكوليسترول والكايتوسان النقي أدت الى ارتفاعا معنويا في تراكيز HDL، بينما حصل انخفاضا معنويا في تراكيز الكلوكوز، TC، TG، LDL، VLDL، مقارنة مع مجموعة الكوليسترول. عند معاملة ذكور الارانب بكايتوسان الكمأ البني لم تحدث فروقا معنوية في الكلوكوز، HDL، بينما حصل انخفاضا معنويا في TC، TG، LDL، VLDL مقارنة مع مجموعة السيطرة. اما المعاملة بالكوليسترول وكايتوسان الكمأ البني أدت الى ارتفاعا معنويا في تراكيز HDL، فيما لم يحصل اي فرق معنوي في الكلوكوز، بينما حدث انخفاضا معنويا في TC، TG، LDL، VLDL مقارنة مع مجموعة الكوليسترول. ان معاملة ذكور الارانب بـ كايتوسان الكمأ الابيض لم تحصل فروقا معنوية في الكلوكوز، HDL، بينما حصل انخفاضا معنويا في TC، TG، LDL، VLDL مقارنة مع مجموعة السيطرة. اما المعاملة بـ الكوليسترول وكايتوسان الكمأ الابيض اظهرت ارتفاعا معنويا في تراكيز HDL، فيما لم يحدث اي فرق معنوي في الكلوكوز، بينما حدث انخفاضا معنويا في TC، TG، LDL، VLDL، مقارنة مع مجموعة الكوليسترول. ويتبين من الدراسة الحالية أن الكايتوسان المستخلص له دوراً مهم للحدّ من ارتفاع مستويات الدهون ومضاعفاتها، كما له دورا مباشرا على معظم الفعاليات الكيموحيوية الحاصلة في الجسم وبدون تأثيرات جانبية يجعل بالإمكان الاستفادة منه في الوقاية وعلاج العديد من الحالات المرضية وخصوصاً الأمراض القلبية والوعائية.