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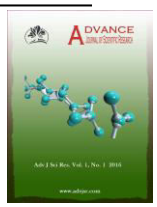

Isolation and modification of chitin and chitosan from shrimp shells and study as inhibitors of hypoglycemia compounds

Zainab Abdulelah,[1] Dawood Ali, [2] Salah Shakir [2]

Department of Chemistry, Science College University of Basrah, Basrah, Iraq

ABSTRACT

Chitosan is a nontoxic, biodecomposable and bioconsistent polysaccharide of β (1-4) -linked D-glucosamine and N-acetyl-D-glucosamine. It can be derived of raw Chitin (shrimp shelles) which offers outstanding features that have introduced the Chitosan in the pharmaceutical and bio-medical fields. This study is concern with extracted and synthesis of some polymers from natural sources from local shrimp shells such as, Chitin, Chitosan and their derivatives: Ethyl amine of Chitin, Ethyl amine of Chitosan, Carboxymethyl of Chitin and Carboxymethyl of Chitosan. Those polymers have been noted as (I-IV) respectively for simplicity. The degree of deaceylation of chitosan was calculated from IR spectrum and it was found 91.6 %. Chitosan's molecular weight was calculated by viscosity approach and it was found that the MW (1.995 k D) a study of the effective of the polymers (I-IV) has been performed on diabetes in blood of the rabbits and it was found that ethyl amine of chitosan was more active to decrease blood glucose level in the rabbits from 248.72 mg/100ml to 104.51 mg/100ml in 2 hours period and after 24 hours it declined to 101.11 mg/100ml. Additionally, the carboxymetyl of chitosan decreased the blood glucose level in the rabbits from 133.49 mg/100ml to 121.95 mg/100ml in 2 hours period and after 24 hours it decreased to 115.55 mg/100ml. In summary, all the polymers (I-IV) were effective in decreasing of blood glucose level in the rabbits, while the ethyl amine of chitosan was more active to decrease blood glucose level than the other polymers (I-IV).

Keywords: Chitosan, polysaccharide, chitin, shrimp shells

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1. Introduction

Chitosan is a polysaccharide which is simply obtainable in nature from the shells and the other sea shellfish product from deacetylation of chitin. Chitosan is a cationic polysaccharide with linear chain consisting of β-(1, 4)-linked 2-acetamino-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β- D-glucopyranose

1.2 Treatment of diabetes using modern drug delivery systems and special properties of nanoparticles

Diabetes mellitus is a group of metabolic diseases characterized by rise blood glucose levels (hyperglycemia) resulting from deficiency in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment

One of the essential applications of nanotechnology in the medical field is the use of it in drug delivery system such as, used chitosan for drug delivery system as a target tissue for therapy of disease i.e. diabetes, cancer and wound healthy.

Chitosan makes a continued issue because hyperlipidemia may play an important role in the development of diabetic complications. However, the results are inconclusive because chitosan has poor solubility and low absorbability *in vivo*. On the other hand, chitosan oligosaccharide (CS) has soluble and absorbable properties and various functional activities, including free radical scavenging activity [1,2,3]

A few reports on CS are also available on the anti-diabetic effects, such as increased glucose tolerance and insulin secretion, improved antioxidant capacity, reduced loss of pancreatic β-cells and accelerated proliferation of pancreatic islet cells [4,5]

The previous results with CS are not conclusive to date, because it shows its biological activity under the toughest condition, especially depending on the molecular weight, degree of deacetylation and the solubility and viscosity of chitosan used [6,7,8] Therefore the present study was designed to investigate the longterm report about material foundation of CS *in vivo*. For this purpose, we prepared the highly deacetylated CS with a dose of 500 mg/kg

2. Results and Discussion

2.1 Characterization of polymers (I-VI)

The extraction and preparation polymers (I-VI) were characterised by using FTIR and the spectrums were recorded and discussed as following:

2.1.1 FTIR spectrum of Chitin I

The IR spectrum of chitin showed characteristic bands at absorptions 1660.71, 1624.06, 1554.63 cm^{-1} have been assigned as amide I bands, while the latter the amide II bands of chitin, the absorption bands of the hydroxyl and amine groups at 3419.79 cm^{-1}, the C-H stretching band at 2891.30 cm-1 , the bridging oxygen – stretching band at 1157.29 cm^{-1} , the C-O stretching bands at 1072.42, 1026.13, 894.97 cm^{-1} 15,16

2.1.2 FTIR spectrum of chitosan II

The IR spectrum of chitosan 17 showed characteristic bands absorptions at 3128.54 cm^{-1} and 3442.94 cm-1 have been assigned as the stretching vibration of OH and NH groups, the band at 896.90 cm-1 have been assigned as (C-H) bands of chitosan, the bands at 1035.77, 1155.36 and 1251.80 cm^{-1} corresponds to the asymmetric stretching vibration (C-O-C) and symmetric stretching vibration (C-O-C). The band at 2879.72 cm^{-1} indicates symmetric stretching vibration (C-H)

2.1.3 FTIR spectrum of Ethyl amine of Chitin III

The IR spectrum of ethyl amine of chitin showed that the characteristic band at 3479.58 cm^{-1} is attributed to stretching vibration of –NH group the absorption band at 2877.79 cm-1 which attributed to aliphatic C-H stretching.The spectrum was characterized by three significant bands at 1653.00, 1624.06, 1558.48 cm^{-1} , which correspond to the amide I and amide II. It is important to note that the amide I band of chitin splits at 1653.00 and 1624.06 cm^{-1} , which is attributed to the two types of H-bonds formed by amide groups in the antiparallel alignment present in crystalline region of ethyl amine of chitin. the sharp band at 1379.10 cm^{-1} corresponds to a symmetrical deformation of the CH3 group and at 1074.35 cm^{-1} showed C-O-C vibration inside chitin ring and produced many peaks caused by the presence of hydroxide from chitin which contains a single bond C=O .

2.1.4 FTIR spectrum of Ethyl amine of Chitosan IV

The IR spectrum of ethyl amine of chitosan showed characteristic bands at absorptions broad band at 3402.43 cm^{-1} due to the stretching vibration of NH and OH groups, the band at 1558.48 cm^{-1} correspond to NH2, the band at 894.97 cm^{-1} correspond to the (C-H) .The band at 1058.92 , 1151.50 and 1259.52 cm^{-1} corresponds to the asymmetric stretching vibration (C-O-C). The band at 1421.54 cm^{-1} corresponds to C-H. Meanwhile the band at 2881.65 cm^{-1} indicates symmetric stretching vibration (C-H).

2.1.5 FTIR spectrum of Carboxymethyl of chitin V

The FTIR spectrum of carboxymethyl of chitin 18. showed characteristic bands at absorptions of –COOH group at 1760 cm^{-1}, while the bands at 1720.71 cm^{-1} and 1413.82 cm-1 corresponding to the carboxy group (which overlaps with N-H bend) and –CH2COOH group , respectively . The bands at 1078.21 -1028.06 cm^{-1} (C-O stretch)

2.1.6 FTIR spectrum of Carboxymethyl chitosan VI

The FTIR spectrum of carboxymethyl of chitosan showed abroad absorption band between 3448.72 cm^{-1}, due to O-H stretching vibration, N-H. The band at 1730 cm^{-1} attributed to the –COOH group. The signals at 2895.15 and 2980.87 cm^{-1} could be assigned to the vibration of methyl and methylene.A band at 1575.84 cm^{-1} was observed, which is attributed to the angular deformation of the N-H bonds of the amino group. A band at 1396.46 cm^{-1} due to the symmetric angular deformation of CH3 and the amide III band at 1317.38 cm^{-1} were observed. The band corresponding to the polysaccharide skeleton, including vibrations of glycoside bonds, C-O and C-O-C stretching in range 1010.70, 1153.43 cm^{-1}, were observed [19]

2.2 Determination of Degree of deactylation DD

Several IR absorption band ratios are possible to determine the % of deacetylation. The amide band I at 1660 cm^{-1} is preferred over the amide II band for analysing sample of low N-acetylation content. This is because at low N-acetylation ,the NH_2 band contered at 1590 cm^{-1} predominates and obscures the amide II ,making its estimation difficult . The 3450 cm^{-1} band is prominent and relatively isolated. The 2878 cm^{-1} band is dependent on the level of N-acetylation as originates from the C-H band vibrations and the A_{1655}/A_{3450} ratio is the preferred ratio as gives %N-acetylation

Degree of Deacetylat (DD) at following:

%N-acetylation = A_{1655}/A_{3450} *115 [20,21]

Deacetylation =100 – N-actylation

=91.6 %

2.3 Measurement of $\overline{M_v}$

The weight average molecular viscosity (M_v) of chitosan measure by viscometric measurements and the result as shown in table (1)

$[\eta]= KM^{\alpha}$ [22] Mark-Houwink-Sakurada-equation

Relative viscosity, $\eta rel = t/t_s$ [23]

Specific viscosity $\eta_{sp} = (t/t_s)-1$ [24]

Reduced viscosity $\eta red = \eta_{sp}/c$ [25]

Table (1) Result from viscosity measurement of chitosan solution at 25 ° C

Solution	Concentration (C) (g/ml)	Time (sec)	η_{rel}	η_{sp}	η_{red}
C1	0.5	155.21	4.57	3.57	7.14
C2	0.4	130.70	3.84	**2.84**	7.1
C3	0.3	120.55	3.55	2.55	8.5
C4	0.2	70.82	2.08	**1.08**	5.4
C5	0.1	50.55	1.48	**0.48**	4.8

2.4 Effect of polymers (I-VI) in blood glucose of rabbits

The present study investigated and compared the hypoglycemic activity of differently regioselective chitosan in alloxan-induced diabetic Rabbit. Compared with the normal control rabbit, it was significantly higher blood glucose levels were observed in the alloxan-induced diabetic Rabbit [25].

Table (2): It illustrates the results of the effect of polymers prepared on the level of glucose in the blood of rabbits infected with hyperglycemic, as can be seen from this table drop in the level of blood glucose dramatically

Considerably in this study found that chitin/chitosan has definite hypoglycemic effects. The assumed mechanism indicated that chitosan of a certain molecular weight stimulated the beta-cells proliferation [26] the excretion and release of insulin, and limited the glucagon secretion of islet α cells. Furthermore chitosan was active in the liver: it inhibited hepatic gluconeogenesis and the *in vivo* absorption of sugar; reduced sugar output; enhanced the utilization of sugar by the surrounding tissue, thus decreasing the level of blood sugar. Another hypothesis is that Chitosan could raise the amounts of insulin and glucose receptors, improve insulin sensitivity, and strengthen the biological activity of the receptor. Thereafter, the intracellular oxidase system was inhibited followed by tissue hypoxia. As a result, glucose metabolism was increased, and the blood sugar decreased.

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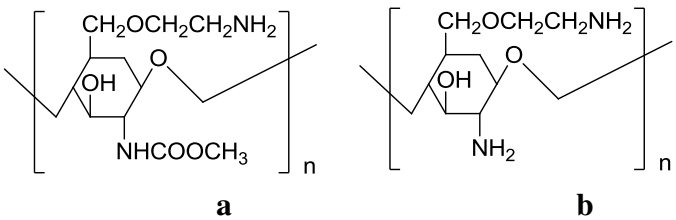
3. Experimental details

3.1 Preparation of Ethyl amine of Chitin (III) and of Chitosan (IV)

A round contain 20 ml of mono ethanolamine added two drops of phosphoric acid, then added drop wise from for half an hour 1 gm of (chitin or chitosan) dissolved in 15 ml of mono ethanolamine then the mixture heated to 50 ° C, and then the heat was increased gradually up to 80 ° C this reaction at reflexes for 5 or 6 hours.

In the same previous method preparation ethyl amine of chitosan. The chemical structure of ethyl amine of chitin and chitosan is shown in Figure 1.



a **b**

Figure 1: (**a**) The chemical structure of ethyl amine of chitin (**b**) the chemical structure of ethyl amine of chitosan.

Table(2) The glucose conc. In (mg/l) in blood of the rabbit at different time

Comp.	%glucose 0 time	% glucose at 2hr	% glucose after24 hr
I	135.95	127.63	125.55
II	134.76	120.79	112.44
III	157.35	120.146	115.99
IV	248.72	104.51	101.11
V	154.31	127.89	118.88
VI	133.49	121.95	115.55

Glucose concentration(mmol/l) = $A_{sample}/A_{standard}$ *standerd conc.(mmol/l), % glucose at Rabbit control = 127.15, A_{sample} = absorbency of sample, $A_{standard}$ = absorbency of standard

The preparation procedure of Carboxymethyl of Chitin (V) and chitosan (VI) has been taken from referance 11 and 12 respectively.

3.2 Determination of viscosity – Average Molecular Weight (M_V) of Chitosan [13]

Five solution of chitosan powder was prepare at concentration (0.5, 0.4, 0.3, 0.2, 0.1) gm/ml (0.3 ml of HAC dissolved in 25ml of distilled water and 0.3 gm of ammonium acetate dissolved in 25 ml of distilled water)(Figure 2)

Viscosity – average molecular weight was determined by using of viscometric measurements an Ubbelohde Capillary viscometer 531 over 10 I. this value was calculated from $[\eta] = KM^{\alpha}$ Mark-Houwink-Sakurada-equation

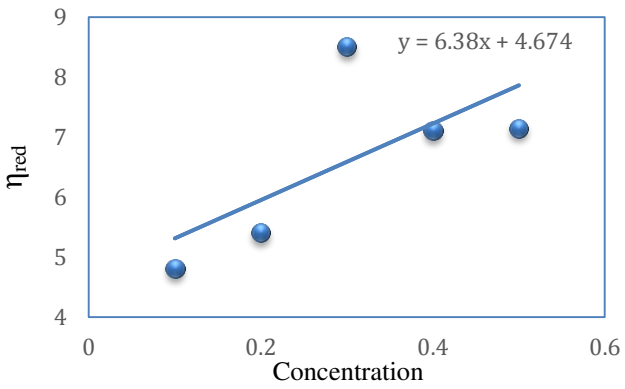


Figure 2: Relation between η_{red} and concentration of chitosan solution

Equation, where K=9.66 * 10-5 (dm3/g) and a=0.742 in 0.15 M Ammonium Acetate and 0.2 M Acetic Acid solution at 25° C

Where K and the exponent a both are constants for a given well-defined polysaccharide solvent system

The values of the parameters K and a depend on both the polymer-solvent system and the temperature

3.2 Experimental animals

The experimental animals used in this study included:

-Adult male domestic rabbits

In this study 12 adult domestic male rabbits weighed between 1-1.5 kg and aged 6 months were bought from a local market of Basra City. They were managed and housed in the animal cage of the College of Science / University of Basra and were house at temperature 25 ± °C and 12/12light/dark cycle under controlled environment.

3.3 Induction Diabetes [14]

The male rabbits were rendered diabetes by a single intravenously injection of alloxan 150 mg/ kg body weight in the marginal vein of the ear after an overnight fast, 1 ml insulin syringe and alloxan mono-hydrate in freshly prepared normal saline 0.5ml is used.

Alloxan injected animals exhibited a massive hyperglycemia (by glucose enzymatic method) within a few days. (3 - 4 days) after injection with alloxan, the rabbits were fasted for 12 h. and then blood samples were collected for measuring blood glucose concentration through 24 h. period. Postprandial blood glucose levels greater than 150 mg/dl were considered diabetic and then the animals used for experiment, the rabbits given the following treatment.

250 mg from the compounds induction the rabbit and after 2 h was measure blood glucose and after 24 h also measure blood glucose

Use 50% of glucose solution drinking for rabbits injected and then began the process of withdrawing animal blood samples from the ear veins of rabbits using a special syringe for glaucoma insulin blood samples collected in centrifuge test tube and underwent a process of separation by centrifuge and quickly (3000 cycle/min) for 10 min. Then separate the serum and saves at 4° C and then was appointed the ratio of glucose

3.4 Estimate the level of glucose in the blood serum

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase, Glucose (GLUC-PAP)

The hydrogen peroxide formed reacts, under catalysis of peroxide, with phenol and 4-aminophenazone to form a red – violet quinoneimine dye as indicator

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

The mixing of the contents are left for ten minutes at room temperature (Table 3)

Table 3: Prepared solutions for glucose conce. Measurment

SGS	Sample	Stander	Blank
Serum	10µl	10µl	10 µl H_2O
WS	1000µl	1000µl	1000µl

SGS: Stander glucose solution, WS: Working solution

The calculation of mix absorbency (A) (500 nm) are used to calculate the glucose concentration of blood as shown in equation 1

Reading of sample /Reading of standard *100 = (X) mg/100 mL of Blood …….. (1)
100 = conc. of standard solution (mg/100mL)

4. Conclusion

According to the results of this study the following conclusions can be summarized:

1. The polymers (I-VI) are non-toxic, prevents the incidence of side effects and Cheap, chitosan is biodegradable and biocompatible
2. The new polymers (III, IV) were found more active from the others because the medications containing amine and carboxyl groups be more effective in the drug
3. The compound (IV) has effectively worked in treatment of HIV. It has been found that ethyl amine of chitosan has inhibited HIV-1 replication in cell culture at concentration of 100 µM.
4-The polymers have a clear impact in reducing the level of blood glucose in rabbits where the compound (IV) has the ability to lower the level blood glucose dramatically

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