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Study the effect of *β-Sitosterol* (from *Passiflora incarnata* L. Seeds) and chitosan (from shrimp shell) on plasma lipid profile in hypercholesterolemic and cholecystectomy in male rabbits

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Abstract
β-Sitosterol (of *Passiflora incarnata* L. seeds) and chitosan (of shrimp shell) have been isolated and characterized by several techniques such as FT-IR,^{1}H-NMR,^{13}C-NMR, CHN, GC-MS and HPTLC for *β-Sitosterol* and FT-IR and X-Ray for chitosan. This study was conducted to determine the effect of *β-Sitosterol* and chitosan on plasma lipid profile in hypercholesterolemic and cholecystectomy in male rabbits. Results show that *β-Sitosterol* significantly decrease total cholesterol (TC), low density lipoprotein cholesterol (LDL) and triglyceride (TG) levels with increase in high density lipoprotein cholesterol (HDL) compared with chitosan. The same effect in the second experiment (cholecystectomy in male rabbits).

Keywords: Hypercholesterolemic, cholecystectomy, lipid profiles, *β-Sitosterol*, chitosan, *Passiflora incarnata* L.

1. Introduction
Passiflora incarnata L., commonly known as passionflower, maypop, or maracuja, belongs to the family Passifloraceae. The genus *Passiflora* L. consists of around 520 species, most of which are vines and commonly found in Central or South America, North America, Southeast Asia, and Australia [1]. *Passiflora incarnata* is recorded and described as the first passionflower in the mid-16th century. Among all the reported species of the genus *Passiflora*, *Passiflora incarnata* L .and *Passiflora edulis* Sims have been the most widely investigated with regards to their chemical composition and biological activities [2]. Of these two, *Passiflora incarnate* L. is the most extensively used herbal drug in the current Western phytotherapy, for its sedative and anxiolytic activity; it is usually used alone, or in combination with other known sedative constituents such as valerian, hawthorn, and kava kava [3-5] Because of its extensive clinical applications, *Passiflora incarnata* has been included as an official plant in the pharmacopoeias of many countries, including Great Britain, United States, India, France, Egypt, Germany, and Switzerland [6]. Hypercholesterolemia is defined as elevated levels of cholesterol. Cholesterol is a lipid which, together with cholesterol esters, phospholipids, and triglycerides, is transported in the blood as part of larger molecules called lipoproteins. They can be assigned to different categories and the five major families of lipoproteins are low-density lipoproteins (LDL), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and chylomicrons [7]. Chitosan, a natural cationic polysaccharide, has received considerable attentions as a functional, renewable, nontoxic and biodegradable biopolymer for diverse applications, especially in pharmaceutics [8] food [9] and cosmetics [10]. In the medical field, chitosan has been developed not only as artificial skin, absorbable surgical suture, and a wound healing accelerator, but also as new physiological materials due to their antitumor, immune enhancing, antimicrobial and hypocholesterolemic properties [11]. These functions have been revealed to be dependent on both their chemical structure and the molecular size. This study was designed to (1) isolate and characterize of *β-Sitosterol* from seeds of *Passiflora incarnata* L. seeds and chitosan from shrimp shell (2) Assess the potentials effect of *β-Sitosterol* and chitosan on plasma lipid profile in hypercholesterolemic and cholecystectomy in male rabbits. The findings from this work may add to the overall value of the medicinal potential of the plant.

2. Materials and Methods

2.1 Chemicals

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO), and solvents were from E. Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water to eliminate the contamination of metal ions.

2.2 Materials of the study

Seeds of *Passiflora incarnata* L. and fresh shrimp were bought from a local market in Basrah city, January 2015. The plant was botanically authenticated and voucher specimens were deposited in the Herbarium of Basra (Iraq, Basra, College of Science, University of Basra). Seeds were ground by hand mill and kept polyethylene bags until time of use. Head and skin of the shrimp were separated. The collected shrimp wastes were then washed with tap water and crushed with mortar pastille. Crushed shrimp waste was kept in polyethylene bags at ambient temperature for (24h) to facilitate chemical extraction of chitosan to improve the quality of chitosan.

2.3 Animals

Healthy male rabbits (1-1.5 kg) body weight and (6-7 months) of age were brought from local market/Basrah and were used for the present study. The animals, with no prior drug treatment, were housed in polypropylene cages (five in cage) under a 12 h light/12h dark cycle in a controlled temperature room (25 ± 2°C). All the animals were acclimatized to the laboratory conditions for a week before use. They had free access to food and water. The rabbits were fasted for 12hr. before collection of blood samples.

3. Methods

3.1 Isolation of *β-Sitosterol* from *Passiflora incarnate* L. Seeds

Powdered (100gm) seeds parts of *Passiflora incarnata* L. seeds were continuously extracted by soxhelt using 500ml of n-hexan (24h) then the solvent was removed under rotary evaporator to afford (16.15 gm) of oil [12]. Then, 100ml of alcoholic potassium hydroxide (5%w/v) was added to the oil extract, refluxed and heated on water bath for 3hr. The solution was extracted, while just warm, three times with ethyl acetate (100ml), poured each ethyl acetate extract into another separating funnel containing (40ml) of distilled water, The acetate extract were combined, and then dried Na_2SO_4 poured into weighted flask and evaporated. The pale yellow oily material was (1.34 gm) [13]. The isolated compound were identification by IR, ^{1}H-NMR, ^{13}C-NMR, GC-MS and HPTLC. The compound was concluded as *β-Sitosterol*.

3.2 Isolation of Chitosan from shrimp shell

Chitin and chitosan were prepared from shrimp shell according to [14] Dried shell waste was washed with tap water and deproteinized by boiling in 3% aqueous sodium hydroxide for 15 min. After draining the alkali, the process was repeated for the removal of residual protein from the shell and washed with tap water. The deproteinized shell was demineralised by HCl (1.25 N) at room temperature for 1 h. The acid was drained off and washed thoroughly with tap water followed with distilled water. The chitin was dried at ambient temperature (30±2 °C). The dried chitin was pulverised into powder using a dry grinder. The chitosan was prepared by deacetylation of chitin by treating with aqueous sodium hydroxide (1:1; w/ w) at 90 to 95 °C for 2 h. After deacetylation the alkali was drained off and washed with tap water followed by distilled water. Finally, the chitosan was dried at ambient temperature (30 ± 2 °C).

3.3 Experimental Design

This study included two main experiments as following:

4. Experiment one

4.1 Effect of oral administration of cholesterol on plasma lipid profile

Twelve male rabbits were used in this experiment which allocated randomly into two groups (6 rabbit/group).

Group I: Received orally (0.5gm/kg B.W.) cholesterol dissolved in 5ml soybean oil (cholesterol free) daily for two weeks for induction of hypercholesterolemia [7]

Group II: received only 5ml normal saline (0.9% NaCl).

4.2 Effect of *β-Sitosterol* and chitosan on plasma lipid profile in hypercholesterolemic rabbits

Forty two male hypercholesterolemic rabbits were randomly and equally divided into seven groups (6rabbit/group) received the following treatments:

Group 1: Received orally (0.075g/kg B.W.) of *β-Sitosterol*.

Group 2: Received orally (0.075g/kg B.W.) of chitosan.

Group 3: Received orally (0.4g/kg B.W.) of rosuvastatin. This Drug is used clinically in the treatment of hypercholesterolemic.

Group 4: Received orally (2ml) normal saline (0.9% NaCl) to hypercholesterolemic animals and served as hypercholesterolemic control.

5. Experiment two

5.1. Effect of cholecystectomy on plasma lipid profile

Preparations of animals to operation include clapping and shaving of abdominal region then the site of operation were cleaned and then disinfected. This is followed by intramuscular injection of (10 mg/kg B.W.), acepromazin mleate to sedate (tranquilize) the animal. Then animals were anaesthetized by intramuscular injection of Ketamine hydrochloride (10mg/kg B.W.) and Zylazine (5mg/kg B.W.) The animal interred into surgical anesthesia for 30-45 minutes [15]. The anaesthetized animals were lied and casted on their back. An incision was made in linea albua, after the gall bladder has been exposed, the peritoneum was splited and the gall bladder is freed by blunt dissection. Two forceps were applied to the duct of the gall bladder the pedicle is served between them. A ligature was placed around the duct beneath the first forceps. A second ligature was done in front the second forceps. Hemorrhage from exposed liver surface can be controlled by pressure with a guze sponge [16]. Twelve rabbits were divided randomly into two groups:

Group I: Rabbits (6) in this group were subjected to cholecystectomy operation.

Group II: In this group, the normal animals (6) were placed as control group.

5.2 Effect of *β-Sitosterol* and chitosan on plasma lipid profile in cholecystectomy rabbits

The rabbits (42) were subjected to cholecystectomy operation used in this experiment. These animals were allocated randomly into seven groups. Group 1 to 5 were received the same doses as described in the experiment one with served Group 4 as cholecystectomy control group.

5.3 Statistical Analysis

The data were expressed as mean values ± SEM and tested with analysis of variance followed by Dunnett's t-test. P-values <0.05, 0.01 were considered to be statistically significant.

6. Results and Discussion

6.1 Characterization of Isolated compounds

The IR spectra of the isolated compound from *P. incarnata* L. show absorption bands at 3549.99 and 1063.34 cm^{-1} indicating presence of hydroxyl group. Other prominent peaks were at 2935.73, 2867.38, 1465.50 and 1377.14 cm^{-1}. The absorption band at 1637.63 and 801.31 is due to presence of C=C group as shown in Figure 3.1and Table 1.

Table 1: Infra-Red (IR) absorption spectra of the isolated compound of *P. incarintia* L.

Isolated compound (ύ cm^{-1})	Group
3549.99	(Br, OH)
2935.73	(C-H str. in CH_2)
2867.38	(C-H str. in CH3)
1637.63	(C=C str.)
1465.50	(C-H deformation in CH3)
1377.14	(C-H deformation in gem dimethyl)
1063.34	(C-O str. of secondary alcohol)
801.31	(C=C str.)

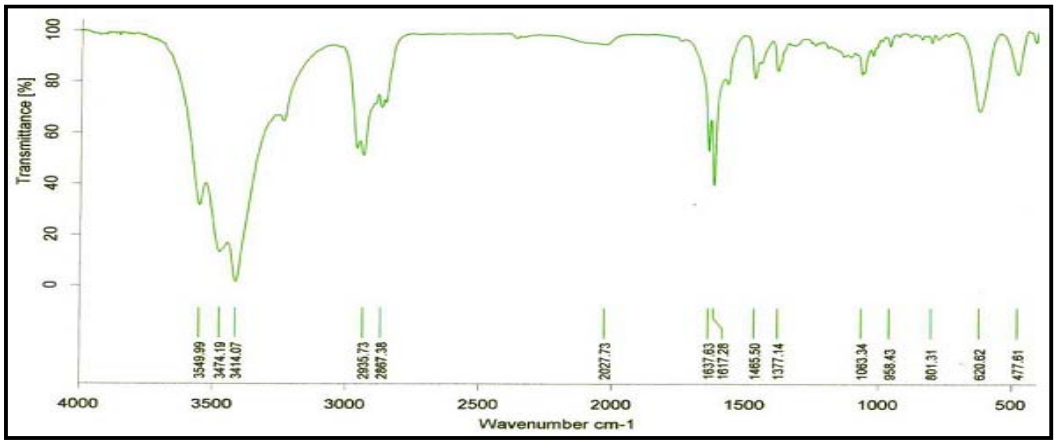

Fig 3.2: IR spectra of the isolated compound from seeds of p. incarintia L.

Table 2: Infra-Red (IR) absorption spectra of the isolated chitosan from shrimp shells.

Isolated compound (ύ cm^{-1})	Group
3444	(Br, OH str. & NH str.)
2852	(CH_2 str.)
3363 & 3245	(NH_2 str.)
1024	(C-O str. of secondary alcohol)
2921	(C – H ste.)
1377	Indicate the decreased in acetyl group
1415	(O – H bend)
1558	NH_2 bend

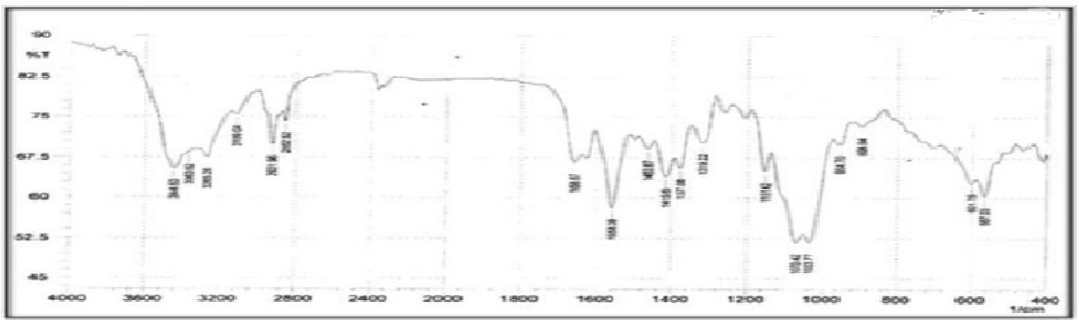

Fig 2: IR spectra of the chitosan isolated from shrimp shells.

The isolated compound was subjected to ^{1}H-NMR and ^{13}C-NMR spectrum and the spectrum was shown in Table 3 and Figures 3.2 to 3.5, respectively. ^{1}H-NMR spectra showed peaks at δ_H 5.35 corresponding to H-6 of an olefinic proton, δ_H 3.79 to H-3 in addition to six methyl group singles at δ_H 1.00, 0.67 (s each, Me-19, -18), 0.90, 0.85, 0.80 (d each Me-21, 26, 27) and 0.87 (t- Me-29). ^{13}C-NMR showed 29 signals including six methyls (CH_3), eleven methylenes (CH_2), nine methane (CH) and three quaternary carbons (C). The signals at δ 19.0 and 15.6 corresponds toangular carbonatom (C_{19} & C_{18}). The forementioned data with the ^{13}C-NMR indicated the presence of *β-Sitosterol*, the common triterpene compound. This was supported by the comparison with those reported in literature [17]. The compound has previously been isolated from some plants such as *Raulinoa echinata* Cowan [17, 19, 20]. and other *Acacia* species such as *A. farnesiana* [18]. Moreover, the compound has also been previously isolated from some the aerial parts of *Satureja khuzestanica*, *Mentha cordifolia Opiz*, *Vitex negundo* and *Croton membranaceus* [21, 22, 17].

Table 3: Chemical shifts of ^{1}H-NMR and ^{13}C-NMR of the isolated compound from *p. incarintia* L seeds.

Position	Group	δ ppm	
		^{1}H-NMR	**^{13}C-NMR**
1	CH_2	1.49	37.81
2	CH_2	1.25	32.21
3	CH	3.50	72.35
4	CH_2	2.23	40.33
5	C	-	141.31
6	CH	5.35	122.25
7	CH_2	2. 02	32.21
8	CH	1.44	29.72
9	CH	1.43	50.70
10	C	-	37.81
11	CH_2	1.49	21.63
12	CH_2	1.56	40.33
13	C	-	42.86
14	CH	1.43	56.62
15	CH_2	1.29	21.85
16	CH_2	1.63	21.85
17	CH	1.48	56.62
18	CH_3	0.63	12.40
19	CH_3	1.02	19.33
20	CH	1.63	36.69
21	CH_3	0.93	19.58
22	CH_2	1.25	32.46
23	CH_3	1.25	28.79
24	CH	1.49	42.45
25	CH	1.82	29.72
26	CH_3	0.86	21.63
27	CH_3	0.86	21.63
28	CH_2	1.51	23.62
29	CH_3	0.69	12.52
30	OH(at C_3 position)	1.99	-

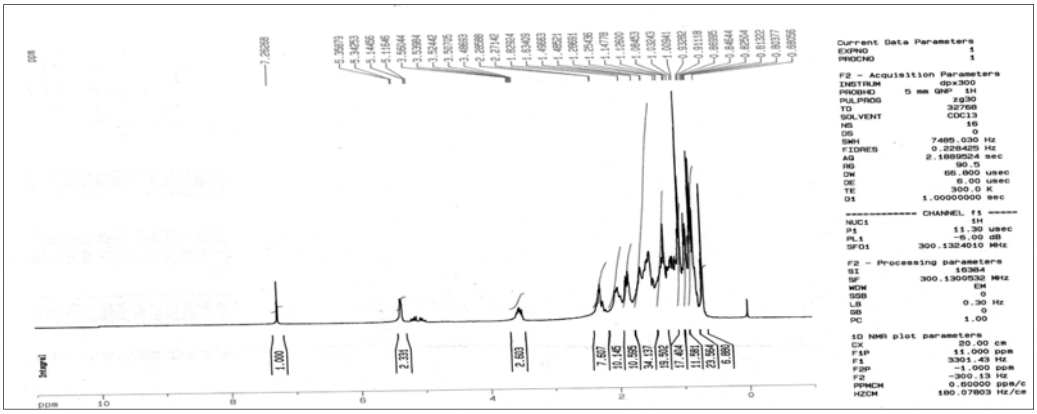

Fig 3: ^{1}H-NMR spectra of the isolated compound from seeds of *p. incarintia* L.

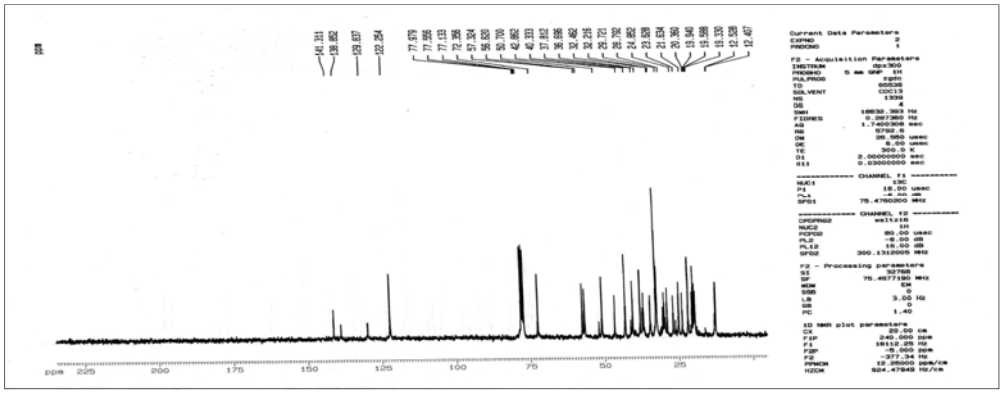

Fig 4: ^{13}C-NMR spectra of the isolated compound from seeds of *p. incarintia* L.

6.2 Mass Spectra of isolated compound from *P incarintia* L. seeds:-

Mass spectrum shows molecular ion peak at m/z 414 (M^+, $C_{29}H_{50}O$, 54%), other significant peaks were at 397 ($C_{28}H_{45}O$, 18%), 329($C_{23}H_{37}O$, 12%), 303($C_{21}H_{35}O$, 10%), 288($C_{20}H_{32}O$4%), 273 ($C_{19}H_{29}O$10%), 255 (M^+ - $C_{19}H_{27}$: Side chain +H_2O, 6%), 231 ($C_{17}H_{27}$, 10%), 199($C_{15}H_{19}$, 20%), 161 ($C_{12}H_{17}$, 30%), 147($C_{11}H_{15}$, 34%), 133 ($C_{10}H_{13}$, 24%), 105 (C_8H_9, 50%), 91 (C_7H_7, 76%). The ion peaks m/z 288, 273 are Due to the formation of carbocation by β-bond cleavage of side chain leading to the loss of C_9H_{18} and $C_{10}H_{21}$ that corresponds to the M-126 and M-141. The molecular weight and fragmentation pattern indicate that the compound presenting is β-sitosterol. The dehydration of fragment at m/z 273 would yield m/z 255, which on successive dealkylation would yield the other ions (12-14, 17), as shown in Figure 5.

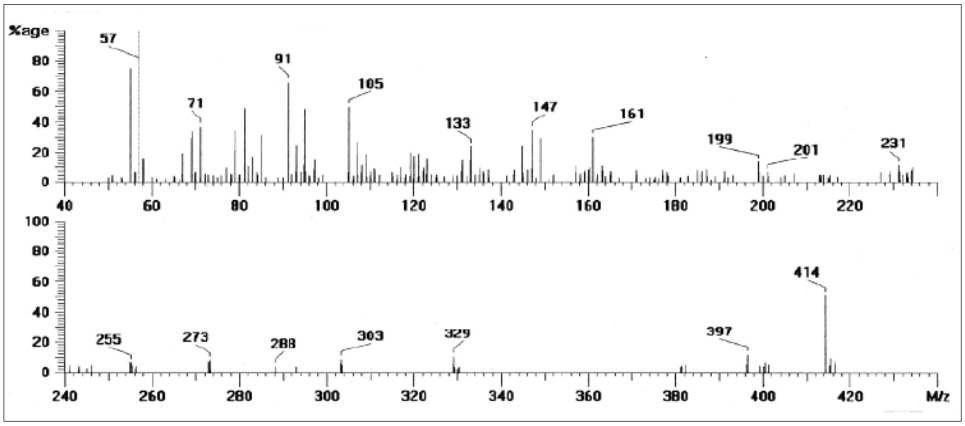

Fig 5: Mass spectra of the isolated compound from seeds of *p. incarintia* L.

6.3 CHN Spectra of isolated compound from *P incarintia* L. seeds:-

Racial analysis results showed good conformity flour practical and theoretical values for each of the carbons and hydrogens, As shown in the table below.

Table 4: result of CHN

Compound	Theortical value to compound			practical value to compound		
β- Sitosterol	C%	H%	N%	C%	H%	N%
	38.99	12.15	-	39.01	12.22	-

HPTLC offers many advantages over other chromatographic techniques (like TLC, HPLC...etc) such as unsurpassed flexibility (esp. stationary and mobile phase), choice of detection, user friendly, rapid and cost effective. Thus, HPTLC is most widely used at industrial level for routine analysis of herbal medicines [23, 24] Thus, the objective of the present study was to develop and validate a HPTLC method for estimation of *β-Sitosterol* in *p. incarintia* L. seeds. The results of this study are presented in Figures 3.8 and 3.9, respectively. The results revealed that the acquired chemical and spectral data of isolated compound and comparison with references standard, it was confirmed that the isolated compound is *β-Sitosterol*.

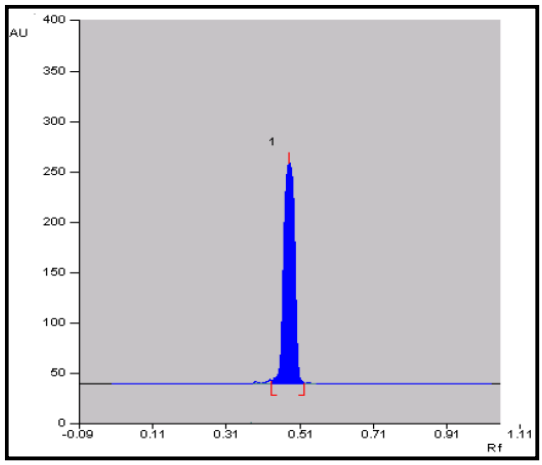

Fig 6: HPTLC chromatogram of standard *β-Sitosterol*

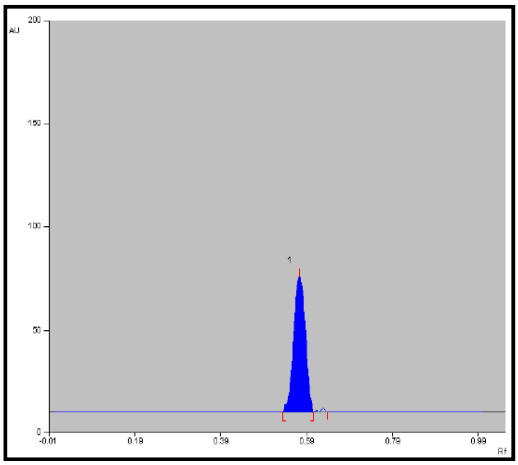

Fig 7: HPTLC chromatogram of isolated compound

6.4 X-Ray Diffractometry

X-ray spectroscopy is unarguably the most versatile and widely used means of characterizing materials of all forms. The XRD data of compound chitosan is shown in Figure (8). From the spectrum, it was obvious that chitosan is a crystal polymer to some degree. The spectra of chitosan show the characteristic peaks at 10 and 20, which suggest the formation of inter- and intra-molecular hydrogen bonds in the presence of free amino groups in chitosan

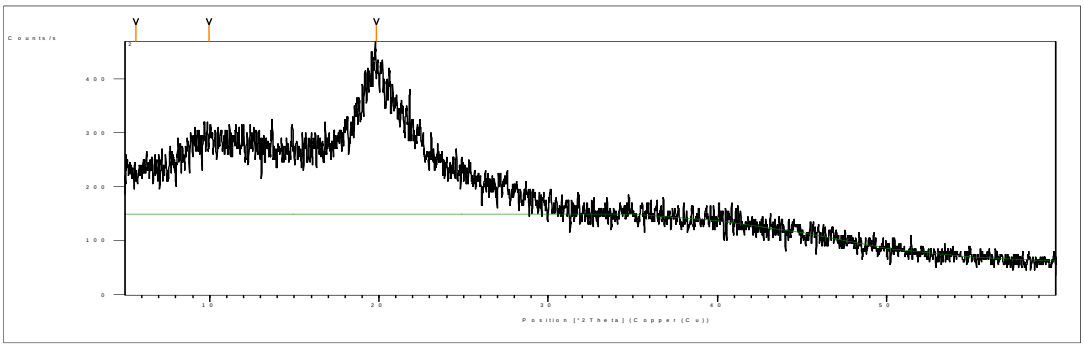

Fig 8: X-Ray Diffractometry of the chitosan isolated from shrimp shells.

6.5 Effect of *β-Sitosterol* and chitosan on plasma lipid profile in hypercholesterolemic and cholecystectomy rabbits

Plasma TC, TG, HDL, LDL and VLDL concentrations was changed as shown in tables (5,6,7,8,9,10,11,12), at 4weeks, There was significant decrease in TC, TG, LDL and VLDL concentration and significant increase in HDL concentration as compared with normal control group (p<0.001) in two experiments.

Table 5: Effect of control on plasma lipid profile parameters (mg\dl) on weeks 0, 1, 2, 3, 4 in hypercholesteromic rabbits

Lipid profile parameters	W0	W1	W2	W3	W4
TC	1140.43±142.303^{a}	1103±118.58^{b}	1051.11 ±94^{c}	967.20±79^{d}	889.50±102^{e}
TG	219.88+20.55^{a}	219.30+47.43^{b}	218.76+18.97^{c}	218.70+20.79^{d}	218.29+20.42^{e}
HDL	18.66±1.74^{a}	19.10±1.73^{b}	18.90 ± 1.82^{c}	19.61 ± 1.83^{d}	19.49 ±1.66^{c}
LDL	1077.664±134.48^{a}	1040.22±94.87^{b}	988.458±88.54^{c}	903.85±86.96^{d}	826.352±126.49^{e}
VLDL	43.976±7.15^{a}	43.86±5.41^{b}	43.752 ± 4.39^{c}	43.74 ± 3.90^{d}	43.658 ±3.40^{e}

Values are exbressed as mean ± SD, n=6/ group, high significant differences are presented by lowercase letters in each column (p<0.001) vs.normal

Table 6: Effect of *β-Sitosterol* on plasma lipid profile parameters (mg\dl) on weeks 0, 1, 2, 3, 4 in hypercholesteromic rabbits

Lipid profile parameters	W0	W1	W2	W3	W4
TC	1032.56±110.68^{a}	720.50±110.68^{b}	470.12±79.06^{c}	339.53±82.22^{d}	129.60±12.65^{e}
TG	217.77±39.52^{a}	199.27±31.62^{b}	183.88 ± 23.71^{c}	161.70 ± 18.9^{d}	137.94±15.81^{e}
HDL	16.47±1.58^{a}	17.58±1.26^{b}	18.00 ± 0.79^{b}	18.70 ± 1.34^{c}	20.08 ±1.74^{d}
LDL	963.536±86.96^{a}	663.066±79.06^{b}	415.344±63.24^{c}	288.49±59.06^{d}	81.932±6.32^{e}
VLDL	43.55±4.35^{a}	39.854±3.47^{b}	36.776±4.80^{c}	32.34± 3.66^{d}	27.588±2.75^{e}

Valus are exbressed as mean ± SD, n=6/ group, high significant differences are presented by lowercase letters in each column (p<0.001) vs. control

Table 7: Effect of rosuvastatin on plasma lipid profile parameters (mg\dl) on weeks 0, 1, 2, 3, 4.

Lipid profile parameters	W0	W1	W2	W3	W4
TC	1118.16±142.3[a]	797.57±104.3[b]	494.69 ±71.21[c]	299.66 ±63.25[d]	90.76 ±4.74[e]
TG	218.30±31.6[a]	180.72±23.7[b]	157.65 ± 20.5[c]	135.71 ± 18.24[d]	117.41±15.8[e]
HDL	16.48±0.47[a]	19.84±1.26[b]	21.30 ± 1.74[c]	23.28 ± 1.11[d]	24.04 ±0.79[d]
LDL	1058.02±147[a].	741.59±118.5[b]	394.57±67.99[c]	249.24±63.24[d]	43.238±4.74[e]
VLDL	43.66±7.63[a]	36.14±5.58[b]	28.28 ± 3.89[c]	27.14 ± 4.71[d]	23.482±4.85[e]

Valus are exbressed as mean ± SD, high significant differences are presented by lowercase letters in each column (p<0.001) vs. control

Table 8: Effect of rosuvastatin on plasma lipid profile concentration in cholecystectomy rabbits

Lipid profile parameters	W0	W1	W2	W3	W4
TC	124.24±14.45[a]	120.40 ±14.40[b]	98.80±9.50[c]	72.00±8.09[d]	68.00±7.00[e]
TG	188.20±14.33[a]	170.20 ±16.74[b]	136.94±13.00[c]	115.88±10.40[d]	95.80±8.70[e]
HDL	16.60±.0.40[a]	19.80±1.20[b]	20.40±1.70[b]	22.20±1.80[d]	24.50±2.00[e]
LDL	70.00±18.09[a]	61.56±20.00[b]	51.012±13.4[c]	26.624±5.5[d]	24.34±3.44[e]
VLDL	37.64±.4.55[a]	34.04±9.54[b]	27.388±5.5	23.176±1.6[d]	19.16±1.5[e]

Valus are exbressed as mean ± SD, n=6/ group, high significant differences are presented by lowercase letters in each column (p<0.001) vs. control

Table 9: Effect of control concentration in cholecystectomy rabbits

Lipid profile parameters	W0	W1	W2	W3	W4
TC	122.80±16.04[a]	114.40 ±14.60[b]	109.68±10.00[c]	104.93±12.09[d]	100.00±11.00[e]
TG	171.60±6.30[a]	169.14 ±7.60[b]	169.90±7.70[c]	166.50±9.00[d]	166.00±8.09[d]
HDL	17.38±.1.40[a]	17.90±1.60[b]	18.70±1.57[c]	18.90±1.77[c]	19.80±1.5[e]
LDL	71.1±19.97[a]	62.672±15.5[b]	56.8,±13.00[c]	52.73±15.90[d]	47.00±12.90[e]
VLDL	34.32±.3.55[a]	33.828±3.00[b]	33.98±4.5[c]	33.3±2.3[c]	33.2±2.5[c]

Valus are exbressed as mean ± SD, n=6/ group, high significant differences are presented by lowercase letters in each column (p<0.001) vs. normal

Table 10: Effect of chitosan on plasma lipid profile parameters (mg\dl) in hypercholesteromic rabbits

Lipid profile parameters	W0	W1	W2	W3	W4
TC	993.18±79.06[a]	910.40±118.58[b]	780.04 ±63.24[c]	500.40 ± 47.4[d]	317.60 ±6.32[e]
TG	218.04±28.66[a]	207.66±23.71[b]	195.00 ± 22.93[c]	186.50 ± 20.5[d]	159.46 ±18.97[e]
HDL	14.50±0.95[a]	14.80±1.26[a]	15.13±1.42[c]	16.20± 1.52[d]	16.94±1.58[d]
LDL	875.072±94.87[a]	854.068± 158.1[b]	725.91±71.15[c]	446.9±79.06[d]	268.768±12.65[e]
VLDL	43.608±8.02[a]	41.532±7.13[b]	39 ± 6.70[c]	37.3 ± 6.8[d]	31.892 ±5.51[e]

Valus are exbressed as mean ± SD, n=6/ group, high significant differences are presented by lowercase letters in each column (p<0.001) vs.control

Table 11: Effect of *β-Sitosterol* on plasma liped profile concentration in cholecystectomy

Lipid profile parameters	W0	W1	W2	W3	W4
TC	189.41±19.40[a]	173.70±17.50[b]	162.30±13.90[c]	140.00±11.30[d]	114.20±10.00[e]
TG	222.00±19.04[a]	206.80 ±16.5[b]	200.0±15.00[c]	150.00 ±7.09[d]	95.00±6.00[e]
HDL	16.40±.1.50[a]	17.90±1.20[b]	19.80±0.80[c]	21.90±2.00[d]	22.40±3.09[d]
LDL	167.718±14.00[a]	154.16±15.17[b]	147.74±8.00[c]	100.1±8.99[d]	50.6±7.00[e]
VLDL	37. 82±.2.48[a]	34.74±10.00[b]	32.46±4.5[c]	28.00±3.3[d]	22.00±2.23[e]

Valus are exbressed as mean ± SD, n=6/ group, high significant differences are presented by lowercase letters in each column (p<0.001) vs. control

Table 12: Effect of chitosan on plasma lipid profile concentration in cholecystectomy

Lipid profile parameters	W0	W1	W2	W3	W4
TC	330.33±14.04[a]	302.20 ±14.50[b]	299.80±15.00[c]	260.00±8.09[d]	220.00±7.99[e]
TG	200.00±.18.00[a]	190.07±12.50[b]	143.77±12.40[c]	150.00±18.09[d]	137.00±13.44[e]
HDL	15.30±.1.10[a]	16.50±1.20[b]	17.40±1.40[c]	17.63±1.70[c]	18.99±1.5[e]
LDL	275.03±15.88[a]	247.686±19.17[b]	253.65±11.00[c]	212.37±13.22[d]	173.61±9.97[e]
VLDL	40.00±2.55[a]	38.014±4.10[b]	28.754±5.6[c]	30.00±4.3[d]	27.4±3.00[e]

Valus are exbressed as mean ± SD, n=6/ group, high significant differences are presented by lowercase letters in each column (p<0.001) vs.control

Within the time, *β-Sitosterol* extract treated group has shown a significant reduction in plasma concentration of TC, TG, LDL and VLDL, with a significant increase in plasma concentration of HDL as compared with normal control group and chitosan in two experiments. It was shown that although drugs had hypolipidemic activity. The maximum percentage reduction LDL level observed with rosuvastatine treatment. Phytosterol are well known for their ability to inhibit absorption of cholesterol and lowering of serum cholesterol by two main processes [25].

7. Conclusion

From the results obtained in the present study, it may be concluded that rosuvastatin had greater effect on plasma lipid profile than *β-Sitosterol* and chitosan in two experiments. On the other hand, *β-Sitosterol* had greater effect on plasma lipid profile than chitosan in two experiments. Moreover, *β-Sitosterol* possesses good pharmacological activities, which might be helpful in preventing or slowing the progress of hypercholesterolemia.

8. References

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