

# Comparative Evaluation between Blend from Beta-Sitosterol and Chitosan with Ration (2:1) and Rosuvastatin on Plasma Lipid Profile Changes in Hypercholesterolemic Rabbits

Nadhun A. Awad<sup>1</sup>, Adnan J. M. Al-Fartosy<sup>2</sup>, Nisreen A. Abdul A. Aali<sup>3</sup>

<sup>1,2</sup>Department of Chemistry, College of Science, University of Basrah, Iraq

<sup>3</sup>Department of Chemistry, College of Science, University of Missan, Iraq

**Abstract:** The purpose of the present survey was to determine the effects of the Blend from  $\beta$ -Sitosterol and Chitosan with ration (2:1) and rosuvastatin on plasma lipid profile changes and the influence of time on treatment process in rabbits. For the management of cholesterol induced hyperlipidemia. Eighteen healthy rabbits were randomly divided into three equal groups Group A (control) included six rabbits that were fed with cholesterol powder (0.5 g/kg B.W for 2 weeks). Group B was similar group A, but in addition rosuvastatin (0.4mg/kg) was administrated for 4 weeks after induced hyperlipidemia. Group C was similar to B, but blend (0.07g/kg B.W) was administered instead of rosuvastatin. Blood samples were collected from ear margin vein by using insulin syringe (2ml) and transferred into EDTA tubes immediately. Blood was then centrifuged at (402 Xg for 10 minutes) to remove red blood cells and recover plasma; this plasma sample was used in biochemical measurements total cholesterol TC, triglycerides TG, high density lipoprotein HDL levels were measured using standard commercial Kits. Rosuvastatin ( $p < 0.01$ ) and blend ( $p < 0.001$ ) showed more hypolipidemic activity in lowering low density lipoprotein compared with group A. In a comparison between two drugs and their effects on TG, rosuvastatin showed a significant difference with blend ( $p < 0.001$ ) Rosuvastatin ( $p < 0.01$ ) and blend ( $p < 0.001$ ) showed more activity in lowering cholesterol than the control group. The treated groups B and C had good results in lowering LDL, compared with group A, on 4 weeks ( $p < 0.001$ ). A significant difference was seen only between group A and C and on 4 weeks in increase of HDL ( $p < 0.01$ ). In conclusion, it was shown that although both drugs had hypolipidemic activity in rabbits blend from  $\beta$ -Sitosterol and Chitosan with ration (2:1) was more effective than rosuvastatin.

**Keywords:** Rosuvastatin; Rabbits; Hypercholesteremic; Lipid profiles;  $\beta$ -Sitosterol; chitosan; *Passiflora incarnata* L.

## 1. Introduction

The term hypercholesterolemia is defined as an 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<sup>(1)</sup> LDL cholesterol normally makes up 60-70% of total serum cholesterol and contains a single apolipoprotein, apo B-100 (apo B) that surrounds the fatty acids, keeping them soluble in the aqueous environment. In general, LDL transports cholesterol and triglycerides from the liver to peripheral tissues and regulates cholesterol synthesis at these sites. LDL cholesterol is well known to increase the risk of coronary heart disease (CHD) because it can be retained in arteries by arterial proteoglycans starting the formation of arterial plaques<sup>(2)</sup>. Increased levels are associated with atherosclerosis, and thereby heart attack, stroke, and peripheral vascular disease. In contrast, HDL cholesterol is inversely correlated to the risk of CVD. HDL normally carries around 20-30% of total serum cholesterol. Apo A-I and apo A-II are the major apolipoproteins of HDL<sup>(3)</sup>. Another lipoprotein subclass is VLDL, which is rich in triglycerides. They account for 10-15% of total serum cholesterol and are assembled in the liver from cholesterol

and apolipoprotein. In the bloodstream it is converted to LDL, therefore it is a precursor of LDL. The intermediate lipoproteins IDL, reside between VLDL and LDL, but are included in LDL measurements in clinical practice<sup>(1, 4)</sup>. Hypercholesterolemia does not lead to specific symptoms and is usually discovered during routine medical tests or examinations for atherosclerotic cardiovascular disease. Sometimes xanthomas, deposits of cholesterol, can be found in individuals with hereditary forms of the disorder or in people with high levels of cholesterol. Deposits are especially found around the eyes or along the achilles tendon<sup>(5)</sup>.  $\beta$ -sitosterol itself has a poor absorption from gastrointestinal track and it is essential to improve its pharmacokinetic behavior by enhancing the bioavailability in combination with phosphatidyl choline. This approach is employed to make a formulation as phyto-vesicles in treatment of alopecia<sup>(6)</sup>. However, several formulations of this compound or other phytosterols exist, which contain either plant extracts or pure sitosterol. Chitosan, a natural cationic polysaccharide, has received considerable attention as a functional, renewable, nontoxic and biodegradable biopolymer for diverse applications, especially in pharmaceuticals<sup>(7)</sup> food<sup>(8)</sup> and cosmetics<sup>(9)</sup>. 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, immunoenhancing, antimicrobial and hypocholesterolemic properties<sup>(10)</sup>. The present study was designed to assess the potential effect of  $\beta$ -Sitosterol, chitosan and blend with ratio

(2:1) of  $\beta$ -Sitosterol and chitosan on plasma lipid profile in hypercholesteremic 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

### 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.

### 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 Basrah). 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.

### 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. Healthy albino mice of either sex (20-30 g) were used for study the Median Lethal Dose (LD<sub>50</sub>) of active blend. 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.

### Methods

#### Isolation of $\beta$ -Sitosterol from *Passiflora incarnata* 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<sup>(11)</sup>. 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<sub>2</sub>SO<sub>4</sub> poured into weighted flask and evaporated. The pale yellow oily material was (1.34 gm)<sup>(12)</sup>. The isolated compound were identification by IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, GC-MS and HPTLC. The compound was concluded as  $\beta$ -sitosterol.

#### Isolation of Chitosan from shrimp shell

Chitin and chitosan were prepared from shrimp shell according to <sup>(13)</sup>. Dried shell waste was washed with tap

water and deproteinised 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 deproteinised 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. Experimental Design

This study included two main experiments as following:

### 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 <sup>(14)</sup>.

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

### Effect of $\beta$ -Sitosterol, chitosan and it's blend on plasma lipid profile in hypercholesteroleiomic rabbits

Forty two male hypercholesteremic 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 blend from  $\beta$ -Sitosterol and Chitosan with ration (2:1).

**Group 2:** Received orally (0.4g/kg B.W.) of rosuvastatin. This Drug is used clinically in the treatment of hypercholestermia.

### Median Lethal Dose (LD<sub>50</sub>) of Active Blend of $\beta$ -sitosterol and chitosan

Forty two male albino mice (Bulb C) were divided into six groups each group contains (6) mice were placed in standard cage (30×15×13 cm). The first group (control group) received orally (1ml) normal saline by using a plastic disposable syringe with a blunt needle cut to a length of 5mm. and fitted with a plastic tube, while other groups were given doses of active blend (2:1) of  $\beta$ -Sitosterol and chitosan (0.25, 0.5, 0.75, 1, 2, 3 gm/kg B.W.). These groups were left in their cages under observation for 48 hours for the presence any signs of toxicity and mortality rate.

### Statistical Analysis

Results were analyzed using SPSS software (Version 19) and the values were expressed as mean ± standard deviation (SD). All comparison were 2-tailed, and considered statistically significant when (p < 0.05), and highly significant when (P < 0.001).

## 4. Results and Discussion

The results of acute toxicity studies were given in Table 1 The active blend (2:1  $\beta$ -sitosterol:chitosan) showed no death

and no symptoms of toxicity or behavioral changes in all treated groups of mice at the maximum dose (3000 mg/kg) during 48 hours. Therefore, 150 mg/kg b.w (100,  $\beta$ -sitosterol + 50, chitosan) was chosen as a high dose for further studies.

**Table 1:** Behavioural data of a cute toxicity studies of the active blend (2:1  $\beta$ -sitosterol:chitosan)

No.	Dose g/kg b.w	Toxicity		Time of death	Observations										
		on set	stop		ANO	PTO	RESP	CNS	TRE	con	SALI	DIA	Sleep	LET	Coma
1)	0.25	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2)	0.5	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3)	1	X	X	X	X	X	X	X	X	X	X	X	X	X	X
4)	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X
5)	2.5	X	X	X	X	X	X	X	X	X	X	X	X	X	X
6)	3	X	X	X	X	X	X	X	X	X	X	X	X	X	X

(ANO-Anorexia, PTO-Ptosis, TRE-Tremor, CON-Convulsions, SALI-Salivation, DIA-Diarrhea, LET-Lethargy), X=Negative,  $\surd$ =positive.

Rosuvastatin (P< 0.01) and blend (P< 0.001) showed more hypolipidemic activity in lowering total cholesterol and triglyceride and low density lipoprotein compared with group A. In comparison between two drugs and their effects on plasma lipid profile, blend showed a significant

difference than rosuvastatin (P< 0.001), after 4weeks of treatment with blend and rosuvastatin drugs. In comparison between two drugs and their effects in increase of HDL, a significant difference was seen between groups A and B on 4weeks (P< 0.001). Tables (2,3, 4) and figures (1,2,3,4,5) shows the effects of control blend and rosuvastatin on plasma lipid profile(TC, TG, HDL, LDL and VLDL) concentration in hypercholesteremic domestic rabbits on 0,1,2,3,4 weeks after induced hypercholesteremic.

**Table 2:** effect of control on plasma lipid profile parameters (mg \dl ) on weeks 0,1,2,3,4.

Lipid profile parameters	W0	W1	W2	W3	W4
TC	1140.43±142.30 <sup>a</sup>	1103±118.58 <sup>b</sup>	1051.11±94.8 <sup>c</sup>	967.20±79.00 <sup>d</sup>	889.50±102.77 <sup>e</sup>
TG	219.88±20.55 <sup>a</sup>	219.30±47.43 <sup>b</sup>	218.76±18.97 <sup>c</sup>	218.70±20.70 <sup>d</sup>	218.29±20.42 <sup>e</sup>
HDL	18.66±1.74 <sup>a</sup>	19.10±1.73 <sup>b</sup>	18.90 ± 1.82 <sup>c</sup>	19.61 ± 1.83 <sup>d</sup>	19.49 ± 1.66 <sup>e</sup>
LDL	1077.664±134.00 <sup>a</sup>	1040.22±94.00 <sup>b</sup>	988.458±88.00 <sup>c</sup>	903.85±86.00 <sup>d</sup>	826.352±126.40 <sup>e</sup>
VLDL	43.976±7.15 <sup>a</sup>	43.86±5.41 <sup>a</sup>	43.752 ± 4.39 <sup>a</sup>	43.74 ± 3.90 <sup>a</sup>	43.658 ± 3.40 <sup>a</sup>

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

**Table 3:** effect of blend 1 on plasma lipid profile parameters (mg \dl ) on weeks 0,1,2,3,4.

Lipid profile parameters	W0	W1	W2	W3	W4
TC	1120±142.30 <sup>a</sup>	780.50 ± 110.6 <sup>b</sup>	493.60±71.15 <sup>c</sup>	290.60±63.24 <sup>d</sup>	88.70±6.32 <sup>e</sup>
TG	217.70±30.52 <sup>a</sup>	172.20±28.46 <sup>b</sup>	151.78 ± 18.81 <sup>c</sup>	124.30±20.24 <sup>d</sup>	116.43±30.07 <sup>e</sup>
HDL	16.40±0.63 <sup>a</sup>	19.59±1.34 <sup>b</sup>	21.38±1.73 <sup>c</sup>	23.45±1.107 <sup>d</sup>	25.70±0.63 <sup>e</sup>
LDL	1065.97±79.06 <sup>a</sup>	726.47±110.68 <sup>b</sup>	441.864±55.34 <sup>c</sup>	242.29±39.53 <sup>d</sup>	34.4±7.90 <sup>e</sup>
VLDL	43.54±6.62 <sup>a</sup>	34.44±4.84 <sup>b</sup>	30.356 ± 4.76 <sup>c</sup>	24.86 ± 3.80 <sup>c</sup>	23.386 ± 3.43 <sup>e</sup>

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

**Table 4:** 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 <sup>a</sup>	797.57±104.3 <sup>b</sup>	494.69±71.2 <sup>c</sup>	299.66±63.2 <sup>d</sup>	90.76±4.74 <sup>e</sup>
TG	218.30±31.62 <sup>a</sup>	180.72±23.71 <sup>b</sup>	157.65±20.5 <sup>c</sup>	135.71±18.2 <sup>d</sup>	117.41±15.81 <sup>e</sup>
HDL	16.48±0.47 <sup>a</sup>	19.84±1.26 <sup>b</sup>	21.30 ± 1.74 <sup>c</sup>	23.28 ± 1.11 <sup>d</sup>	24.04 ± 0.79 <sup>e</sup>
LDL	1058.02±147.05 <sup>a</sup>	741.59±118.58 <sup>b</sup>	394.57±67.99 <sup>c</sup>	249.24±63.24 <sup>d</sup>	43.238±4.74 <sup>e</sup>
VLDL	43.66±7.63 <sup>a</sup>	36.14±5.58 <sup>b</sup>	28.28 ± 3.89 <sup>c</sup>	27.14 ± 4.71 <sup>d</sup>	23.482±4.85 <sup>e</sup>

Valus are expressed as mean ± SD, n=6/ group, significant differences are presented by lowercase letters in each column( p<0.01) vs.control

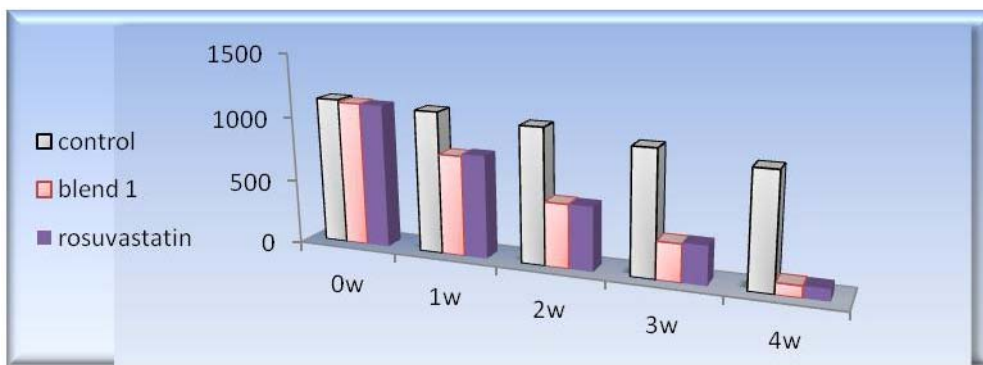


Figure 1: effect of control, blend 1 and rosuvastatin plasma total cholesterol consntration in hypercholesteremic rabbits

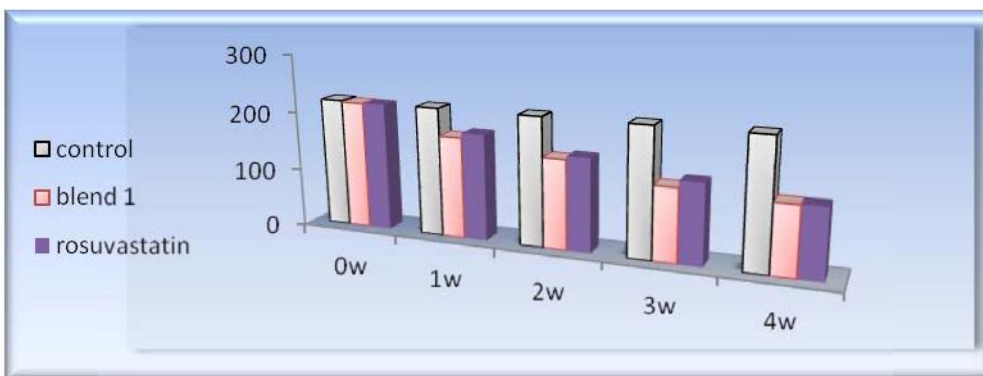


Figure 2: effect of control, blend 1 and rosuvastatin plasma triglyceride consntration in hypercholesteremic rabbits.

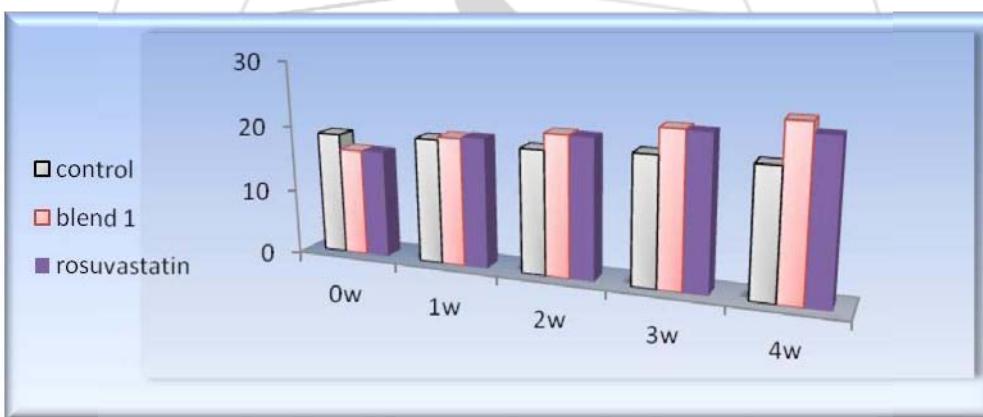


Figure 3: effect of control, blend 1 and rosuvastatin plasma HDL consntration in hypercholesteremic rabbits

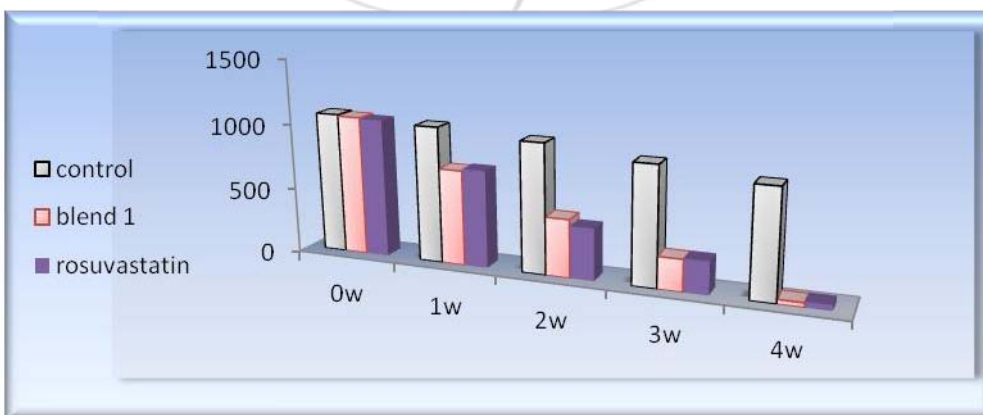


Figure 4: effect of control, blend 1 and rosuvastatin plasma LDL consntration in hypercholesteremic rabbits

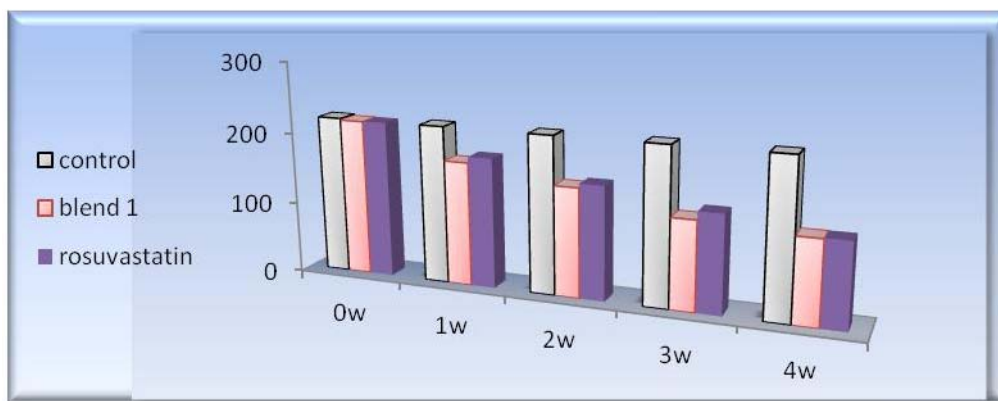


Figure 5: effect of control, blend 1 and rosuvastatin plasma VLDL consntration in hypercholesteremic rabbits

In the present survey, the effects of blend and rosuvastatin were evaluated on plasma lipid profile changes in hypercholesteremic rabbits. The results showed that rosuvastatin and blend from  $\beta$ -Sitosterol and Chitosan both hypolipidemic activity in rabbits, but blend with ration (2:1) was more effective than rosuvastatin. In comparison between two drugs and their effects on LDL level, blend showed a significant difference than rosuvastatin. Also, blend and rosuvastatin showed more activity in lowering TC and TG than the control group. Plasma HDL concentration was increased after 4 weeks of treatment blend from  $\beta$ -Sitosterol and Chitosan with ration (2:1). These results are agreement with finding of <sup>(15)</sup>, who suggested that material extracts causes elevation of HDL in male rats after 4 weeks of treatment. The decrease in plasma LDL concentration was observed in present study and decrease to normal level after 4 weeks of treatment with  $\beta$ -sitosterol and chitosan and mixed from them. In this study rosuvastatin reduce LDL level in hypercholesteremic rabbits <sup>(16)</sup> concluded that statin was increased in the synthesis of the cell surface LDL-receptor, accelerate removal of LDL and TG rich lipoprotein.

## 5. Conclusion

From the results obtained in the present study, it may be concluded that the blend from  $\beta$ -Sitosterol and Chitosan with ration (2:1) possess greater effect on plasma lipid profile than rosuvastatin, which might be helpful in preventing or slowing the progress of various diseases that results from hypercholesterolemia and improving elimination of cholesterol <sup>(17)</sup>.

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