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

Nadhum A. Awad¹, Adnan J. M. Al-Fartosy², Nisreen A. Abdul A. Aali³

¹,²Department of Chemistry, College of Science, University of Basrah, Iraq
³Department 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 β-Sitosterol and Chitosan with ration (2:1) and rosuvastatia 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 rosuvastatia (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 rosuvastatia. 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 lipoprotien HDL levels were measured using standard commercial kits. Rosuvastatia (p<0.01) and blend (p<0.001) showed more hypolipidemic activity in lowering low density lipoprotein compared with group A. In comparison between two drugs and their effects on TG, rosuvastatia showed a significant difference with blend (p<0.001) Rosuvastatia (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 β-Sitosterol and Chitosan with ration (2:1) was more effective than rosuvastatia.

Keywords: Rosuvastatia; Rabbits; Hypercholesteremic; Lipid profiles; β-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 (1). 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 (2). 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 (3). 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 they are 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 (1, 4). 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 (5). β-sitosterol itself has a poor absorption from gastrointestinal tract and it is essential to improve its pharmacokinetic behavior by enhancing the bioavailability in combination with phospatidyl choline. This approach is employed to make a formulation as phyto-vesicles intreatment of alopecia (6). 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 attentions as a functional, renewable, nontoxic and biodegradable biopolymer for diverse applications, especially in pharmaceutics (7) food (8) and cosmetics (9). 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 (10). The present study was designed to assess the potentials effect of β-Sitosterol, chitosan and blend with ratio.
were used for the present study. Healthy albino mice of (3 months) of age were brought from local market/Basrah and ethyl acetate (100ml), poured each ethyl acetate extract into solution was extracted, while just warm, three times with extract, refluxed and heated on water bath for 3hr. The Na2SO4 poured into weighted flask and evaporated. The pale water, The acetate extract were combined, and then dried yellow oily material was (1.34 g m)(12). The isolated compound were identication by IR, 1H-NMR, 13C-NMR,GC-MS and HPTLC. The compound was concluded as β-sitosterol.

Isolation of Chitosan from shrimp shell
Chitin and chitosan were prepared from shrimp shell according to (13). Dried shell waste was 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 Median Lethal Dose (LD50) 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.

Isolation of β-Sitosterol from Passiflora incarnata L. Seeds
Powdered (100gm) seeds parts of Passiflora incarnata L. 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(11). 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 Na2SO4 poured into weighted flask and evaporated. The pale yellow oily material was (1.34 gm)(12). The isolated compound were identification by IR, 1H-NMR, 13C-NMR,GC-MS and HPTLC. The compound was concluded as β-sitosterol.

Isolation of Chitosan from shrimp shell
Chitin and chitosan were prepared from shrimp shell according to (13). 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 hr. 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 hr. 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 1: Received orally (0.5gm/kg B.W.) cholesterol dissolved in 5ml soybean oil (cholesterol free) daily for two weeks for induction of hypercholesterolemia (16).
Group II: received only 5ml normal saline (0.9% NaCl).

Effect of β-Sitosterol, chitosan and it's blend on plasma lipid profile in hypercholesterolemic 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 β-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 hypercholesteremia.

Median Lethal Dose (LD50) of Active Blend of β-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 β-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 β-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, β-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 β-sitosterol:chitosan)

<table>
<thead>
<tr>
<th>No.</th>
<th>Dose g/kg b.w</th>
<th>Toxicity on set</th>
<th>Time of death</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 0.25</td>
<td>X X</td>
<td>X X</td>
<td>ANO PTO</td>
<td>CNS TRE</td>
</tr>
<tr>
<td>2) 0.5</td>
<td>X X</td>
<td>X X</td>
<td>RESP con</td>
<td>SALI DIA</td>
</tr>
<tr>
<td>3) 1</td>
<td>X X</td>
<td>X X</td>
<td>X X</td>
<td>LET</td>
</tr>
<tr>
<td>4) 2</td>
<td>X X</td>
<td>X X</td>
<td>X X</td>
<td>Coma</td>
</tr>
<tr>
<td>5) 2.5</td>
<td>X X</td>
<td>X X</td>
<td>X X</td>
<td>Coma</td>
</tr>
<tr>
<td>6) 3</td>
<td>X X</td>
<td>X X</td>
<td>X X</td>
<td>Coma</td>
</tr>
</tbody>
</table>

(ANO-Anorexia, PTO-Ptosis, TRE-Tremor, CON-Convulsions, SALI-Salivation, DIA-Diarrhea, LET-Lethargy), X=Negative, √=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 4 weeks 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 4 weeks (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 hypercholestermic domestic rabbits on 0,1,2,3,4 weeks after induced hypercholestermic.

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

<table>
<thead>
<tr>
<th>Lipid profile parameters</th>
<th>W0</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>1140.43±142.30g</td>
<td>1103±118.58g</td>
<td>1051.1±94.89g</td>
<td>967.20±79.09g</td>
<td>898.50±102.77g</td>
</tr>
<tr>
<td>TG</td>
<td>219.88±20.55g</td>
<td>219.30±47.43g</td>
<td>218.76±18.97g</td>
<td>218.70±20.70g</td>
<td>218.29±20.42g</td>
</tr>
<tr>
<td>HDL</td>
<td>18.66±1.74g</td>
<td>19.10±1.73g</td>
<td>18.90±1.82g</td>
<td>19.61±1.83g</td>
<td>19.49±1.66g</td>
</tr>
<tr>
<td>LDL</td>
<td>1077.66±134.00g</td>
<td>1040.22±94.00g</td>
<td>988.45±88.00g</td>
<td>908.35±86.00g</td>
<td>826.35±126.40g</td>
</tr>
<tr>
<td>VLDL</td>
<td>43.976±7.15g</td>
<td>43.86±5.41g</td>
<td>43.752±4.39g</td>
<td>43.74±3.90g</td>
<td>43.65±3.40g</td>
</tr>
</tbody>
</table>

Values 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.

<table>
<thead>
<tr>
<th>Lipid profile parameters</th>
<th>W0</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>412.0±142.30g</td>
<td>780.50±110.6g</td>
<td>493.60±71.15g</td>
<td>290.60±63.24g</td>
<td>88.70±6.32g</td>
</tr>
<tr>
<td>TG</td>
<td>217.0±30.52g</td>
<td>172.20±28.46g</td>
<td>151.78±18.81g</td>
<td>124.30±20.24g</td>
<td>116.43±30.07g</td>
</tr>
<tr>
<td>HDL</td>
<td>16.40±0.63g</td>
<td>19.59±1.34g</td>
<td>21.38±1.73g</td>
<td>23.45±1.107g</td>
<td>25.07±0.63g</td>
</tr>
<tr>
<td>LDL</td>
<td>1059.7±99.06g</td>
<td>126.47±110.68g</td>
<td>441.86±55.34g</td>
<td>242.29±39.53g</td>
<td>34.4±7.90g</td>
</tr>
<tr>
<td>VLDL</td>
<td>43.54±6.62g</td>
<td>34.44±4.84g</td>
<td>30.356±4.76g</td>
<td>24.86±3.30g</td>
<td>23.386±3.43g</td>
</tr>
</tbody>
</table>

Values 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.

<table>
<thead>
<tr>
<th>Lipid profile parameters</th>
<th>W0</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>1118.16±142.3g</td>
<td>797.57±104.3g</td>
<td>494.69±71.2g</td>
<td>299.66±63.2g</td>
<td>90.75±4.74g</td>
</tr>
<tr>
<td>TG</td>
<td>218.30±31.62g</td>
<td>180.72±23.71g</td>
<td>157.65±20.5g</td>
<td>135.71±18.2g</td>
<td>117.41±15.81g</td>
</tr>
<tr>
<td>HDL</td>
<td>16.48±0.47g</td>
<td>19.84±1.26g</td>
<td>21.30±1.74g</td>
<td>23.28±1.11g</td>
<td>24.04±0.79g</td>
</tr>
<tr>
<td>LDL</td>
<td>1058.02±147.05g</td>
<td>741.59±118.58g</td>
<td>394.57±67.99g</td>
<td>249.24±63.24g</td>
<td>43.238±4.74g</td>
</tr>
<tr>
<td>VLDL</td>
<td>43.66±7.63g</td>
<td>36.14±5.58g</td>
<td>28.28±3.89g</td>
<td>27.14±4.71g</td>
<td>23.48±2.85g</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n=6/group, significant differences are presented by lowercase letters in each column (p<0.001) vs.control
Figure 1: effect of control, blend 1 and rosuvastatin plasma total cholesterol concentration in hypercholestermic rabbits.

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

Figure 3: effect of control, blend 1 and rosuvastatin plasma HDL concentration in hypercholestermic rabbits.

Figure 4: effect of control, blend 1 and rosuvastatin plasma LDL concentration in hypercholestermic rabbits.

Volume 5 Issue 12, December 2016

www.ijsr.net
Licensed Under Creative Commons Attribution CC BY
In the present survey, the effects of blend and rosuvastatin were evaluated on plasma lipid profile changes in hypercholesterolemic rabbits. The results showed that rosuvastatin and blend from β-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 with blend and rosuvastatin. These results are agreement with finding of (15), who concluded that the blend from β-Sitosterol and Chitosan both possess greater effect on plasma lipid profile changes in hypercholesterolemic rabbits. The results showed that rosuvastatin reduce LDL level in hypercholesterolemic rabbits (16) concluded that stanin 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 β-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 (17).

References