Effect of using Licorice Powder (Glycyrrhiza Glabra) Supplemented with Drinking Water at Two Different Doses in Serum Lipid Profile and Blood Proteins of Local Female Rabbit

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Abstract
This study was conducted to elucidate the effects of administration of Glycyrrhiza Glabra (licorice powder) supplemented with drinking water in serum lipid profile, proteins of local female rabbits. This experiment was carried at the animal farm, college of Veterinary Medicine, University of Baghdad from 1st May to 30th June 2013. Twenty one local female rabbits included in this study at age of approximately 3-4 months, divided randomly and equally (7 each) (body weight was considered) into three groups, first group (untreated) kept as control (C), second group administrated licorice powder (GG) with drinking water and in a range of 1.75 gm/day, third group also administrated (GG) with drinking water in a range of 3.5 gm/day administration regime was continued up to end of experiment, all groups were offered alfalfa with concentrate diet and water freely. Blood samples were collected by heart puncture method biweekly and serum samples were obtained to carry out the biochemical test, serum total cholesterol concentration (TC), serum triglycerides (TG), high density lipoprotein – cholesterol (HDL-C), low density lipoprotein – cholesterol (LDL-C), total serum protein, albumin, and globulin. The result of administration Glycyrrhiza Glabra (licorice powder) with drinking water revealed statistically significant differences (P<0.05) and remarkable effect between treated and untreated groups for all biochemical parameter on serum lipid profile, serum proteins. Therefore, it could be concluded from this study that using of Glycyrrhiza Glabra (licorice powder) to treated groups it could contribute in decline the blood serum lipids, increase serum total protein and it's fractions, improving animal health when compared with untreated groups.

Keywords: Licorice, proteins, powder, health, lipid.

Introduction
The use of plants for medicinal purposes is an old as the evolution of man himself [1]. Medical plants have valuable contribution to the life, because they are considered as an important nutrition and drug sources, their leaves, stems, roots and seeds contain various chemicals and many active ingredients which can be used for the treatment of different kinds of diseases [2]. Licorice the root of the Glycyrrhiza species, is one of the most frequently employed botanicals in traditional medicine, The history of licorice, as a medicinal plant, is very old and has been used in many societies throughout the millennia [3][4] Glycyrrhiza Glabra (Licorice) that grows in various parts of the world and it has ethnobotanical history [5]. Phytochemical analysis of G. glabra root extract showed that it contains saponin, triterpenes (glycyrrhizin s, glycyrrhetinic acid and liquiritic acid.), flavonoids (liquiritin, isoflavonoids and formononnetin) and other constituents such as coumarins, simple sugar and polysaccharide like starch, pectin, amino acids, tannins, choline, phytoestrogens, mineral salts and various other substance, glycyrrhizic acid, glycyrrhizin normally being considered to be the main biologically active components [6,7], which are believed to be partly responsible for anti – ulcer, anti – inflammatory, anti – diuretic, anti – epileptic, antihypertoxic, anti – viral activities, anti – allergic and anti – oxidant, furthermore the root extracts are reported to exhibit ant- anglogenic activities and radio – protective effects, also the other important compound is glabridin, it is the major flavonoid present specially in licorice, it has various physiological activities such as antimicrobial, anti – tumor promoting, estrogenic and anti – proliferative activity against cancer cells, also affects melanogenesis inflammation, low density lipoprotein oxidation [8, 9, 10, 11]. Glabridin is reported to be a potent antioxidant toward LDL oxidation, also licorice roots contains flavonoids and polyphenolic flavonoids which have lipophilic characteristic and anti-oxidative properties, polyphenolic flavonoids can reducing plasma cholesterol level and their ability to inhibit LDL oxidation, Antihyper – lipidaemic and anti – hypertriglyceridaemic properties [12, 13, 14]. The aim of present study was undertaken to evaluate the effect of using licorice powder (Glycyrrhiza Glabra) supplemented with drinking water at two different doses in serum lipid profile, proteins and it's fractions of local female rabbit.

Materials and Methods
1. Licorice powder:
Pure licorice powder obtained from licorice extraction factory in Al-Azazia / Kut governorate (www. Licorice-iq. Com)
2. Time of the Experiment and location
This experiment was carried out at the Veterinary Medicine college (Animal farm)- Baghdad University from 1st May to 3rd June 2013 to find out the effect of using licorice powder (Glycyrrhiza Glabra) supplemented with drinking water at two different doses in serum lipid profile, Total proteins and its fractions of local female rabbit.

3. Experimental Design, Animals and sample collection:
Twenty one healthy local female rabbits were bought at age of about 3-4 Months, experimental groups were organized as three groups that include seven animals each, body weight was considered as follow:-
The first group (C) was the control group which did not receive any treatment, G1 and G2 included the animals which were dially administrated G. Glabra (licorice powder) with water at 1.75 gm (0.25%), 3.5 gm (0.5%) respectively. All groups given free access to Alfalfa, concentrate diet, water ad libitum.

21 local female Rabbits

Control group (C)        G1          G2
7 local female rabbits (untreated group) 7 local female rabbits 7 local female rabbits

Blood samples were taken biweekly by heart puncture method for biochemical analysis of serum: Total cholesterol, Triglyceride, High density lipoprotein – Cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), Total protein concentration and its fractions.

4. Biochemical assay:
Serum cholesterol [15, 16, 23], triglyceride [17], HDL-C [18,24], LDL-C [19], Total protein and its fractions [21, 22] levels were measured by commercially available kits (SYRBIO DIGNOSTIC) in spectrophotometer.

5. Statistical analysis:
Serological data were analyzed as a complete Randomized Design (3 treatments) (one way ANOVA). Least Significant Differences (LSD) was applied to detect the significant differences among different groups means at (p < 0.05) level according to [25, 26].

Results
The results of this study revealed that oral administration of licorice powder (Glycyrrhiza Glabra) supplemented with drinking water for 2 month at two different doses (1.75gm, 0.25%) , (3.5gm, 0.5%) show significant decrease in total serum cholesterol (TC) and Triglyceride (TG) when compared with the untreated group (control group), also low – density lipoprotein cholesterol (LDL-C) was markedly reduced in the treated groups in comparison with the untreated group (control) beginning from 3rd week up to the end of experimental periods as shown in table (1,2,3).

<table>
<thead>
<tr>
<th>Time</th>
<th>1st week</th>
<th>3rd week</th>
<th>5th week</th>
<th>7th week</th>
<th>9th week</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C control</td>
<td>A 135.5±12.2</td>
<td>A 58.1±12.8</td>
<td>A 68.9±13.1</td>
<td>A 101.7±5.5</td>
<td>A 89.8±4.6</td>
<td>18.2</td>
</tr>
<tr>
<td>G1</td>
<td>A 38.5±3.9</td>
<td>C 38.1±5.1</td>
<td>B 46.7±3.9</td>
<td>B 38.7±3.6</td>
<td>B 35.9±1.9</td>
<td></td>
</tr>
<tr>
<td>1.75 gm (0.25%)</td>
<td>38.1±12.4</td>
<td>A 38.9±13.1</td>
<td>A 101.7±5.5</td>
<td>A 89.8±4.6</td>
<td></td>
<td>18.2</td>
</tr>
<tr>
<td>G2</td>
<td>1.75 gm (0.25%)</td>
<td>38.1±12.4</td>
<td>A 38.9±13.1</td>
<td>A 101.7±5.5</td>
<td>A 89.8±4.6</td>
<td></td>
</tr>
<tr>
<td>3.5 gm (0.5%)</td>
<td>38.1±12.4</td>
<td>A 38.9±13.1</td>
<td>A 101.7±5.5</td>
<td>A 89.8±4.6</td>
<td></td>
<td>18.2</td>
</tr>
</tbody>
</table>

Capital letters denote Significant differences among treatment groups at (P<0.05) level.
Table (2) Effect of licorice powder (Glycyrrhiza Glabra) with drinking water in Triglyceride concentration (mg/dl) of local female rabbit (mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>1st week</th>
<th>3rd week</th>
<th>5th week</th>
<th>7th week</th>
<th>9th week</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>6.4±1.6</td>
<td>A</td>
<td>85.2±8.8</td>
<td>A</td>
<td>62.6±6.2</td>
<td>A</td>
</tr>
<tr>
<td>G1 1.75 gm (0.25%)</td>
<td></td>
<td>53.5±4.9</td>
<td>B</td>
<td>54.8±4.8</td>
<td>B</td>
<td>57.2±7.6</td>
<td>B</td>
</tr>
<tr>
<td>G2 3.5 gm (0.5%)</td>
<td></td>
<td>86.7±5.1</td>
<td>A</td>
<td>52.2±6.3</td>
<td>B</td>
<td>47.2±2.3</td>
<td>B</td>
</tr>
</tbody>
</table>

Capital letters denote Significant differences among treatment groups at (P<0.05) level.

Table (3) Effect of licorice powder (Glycyrrhiza Glabra) with drinking water in Low-density lipoprotein cholesterol (LDL-C) concentration (mg/dl) of local female rabbit (mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>1st week</th>
<th>3rd week</th>
<th>5th week</th>
<th>7th week</th>
<th>9th week</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>13.7±4.4</td>
<td>A</td>
<td>100.2±9.2</td>
<td>A</td>
<td>118.02±20.9</td>
<td>A</td>
</tr>
<tr>
<td>G1 1.75 gm (0.25%)</td>
<td></td>
<td>26.1±0.9</td>
<td>A</td>
<td>68.4±9.4</td>
<td>B</td>
<td>90.3±7.7</td>
<td>B</td>
</tr>
<tr>
<td>G2 3.5 gm (0.5%)</td>
<td></td>
<td>33.4±8.6</td>
<td>A</td>
<td>101.3±14.2</td>
<td>B</td>
<td>109.5±14.9</td>
<td>B</td>
</tr>
</tbody>
</table>

Capital letters denote Significant differences among treatment groups at (P<0.05) level.

While the table (4) revealed a significant (p < 0.05) higher values in High – density lipoprotein cholesterol (HDL-C) in treated groups at 5th week of experiment when compared with untreated group (control) up to end of experiment.

Table (4) Effect of licorice powder (Glycyrrhiza Glabra) with drinking water in High - density lipoprotein cholesterol (HDL-C) concentration (mg/dl) of local female rabbit (mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>1st week</th>
<th>3rd week</th>
<th>5th week</th>
<th>7th week</th>
<th>9th week</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>22.7±1.9</td>
<td>83.7±23.1</td>
<td>A</td>
<td>43.2±6.7</td>
<td>57.1±3.4</td>
<td>69.1±1.8</td>
</tr>
<tr>
<td>G1 1.75 gm (0.25%)</td>
<td></td>
<td>23.8±0.8</td>
<td>86.5±15.2</td>
<td>B</td>
<td>40.6±4.6</td>
<td>46.9±3.7</td>
<td>69.2±1.9</td>
</tr>
<tr>
<td>G2 1.5 gm (0.5%)</td>
<td></td>
<td>18.8±1.2</td>
<td>80.5±8.6</td>
<td>A</td>
<td>77.3±7.1</td>
<td>93.6±3.9</td>
<td>96.7±2.8</td>
</tr>
</tbody>
</table>

Capital letters denote Significant differences among treatment groups at (P<0.05) level.

Table (5, 6, 7) demonstrates the effect of oral administration of licorice powder supplemented with drinking water on serum total protein, albumen, globulin, the treated groups recorded significantly (p<0.05) higher values in all these fraction than those of control group from beginning up to the end of experimental period.

Table (5) Effect of licorice powder (Glycyrrhiza Glabra) with drinking water in total serum protein concentration (g/dl) of local female rabbit (mean ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>1st week</th>
<th>3rd week</th>
<th>5th week</th>
<th>7th week</th>
<th>9th week</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>5.6±0.1</td>
<td>5.3±0.01</td>
<td>6.4±0.06</td>
<td>6.3±0.09</td>
<td>6.5±0.01</td>
<td>B</td>
</tr>
<tr>
<td>G1 1.75gm(0.25%)</td>
<td></td>
<td>5.8±0.2</td>
<td>6.2±0.05</td>
<td>6.8±0.07</td>
<td>7.1±0.05</td>
<td>7.3±0.03</td>
<td>A</td>
</tr>
<tr>
<td>G2 3.5gm(0.5%)</td>
<td></td>
<td>6.1±0.1</td>
<td>6.5±0.03</td>
<td>6.9±0.05</td>
<td>7.4±0.03</td>
<td>7.7±0.04</td>
<td>A</td>
</tr>
</tbody>
</table>

Capital letters denote Significant differences among treatment groups at (P<0.05) level.
Table (6) Effect of licorice powder (Glycyrrhiza Glabra) with drinking water in serum albumin concentration (g/dl) of local female rabbit (mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>1\textsuperscript{st} week</th>
<th>3\textsuperscript{rd} week</th>
<th>5\textsuperscript{th} week</th>
<th>7\textsuperscript{th} week</th>
<th>9\textsuperscript{th} week</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>B</td>
<td>2.6±0.1</td>
<td>B</td>
<td>2.9±0.03</td>
<td>3.0±0.1</td>
<td>3.2±0.03</td>
</tr>
<tr>
<td>G1</td>
<td>AB</td>
<td>2.9±0.2</td>
<td>B</td>
<td>2.9±0.2</td>
<td>3.1±0.2</td>
<td>3.3±0.07</td>
</tr>
<tr>
<td>G2</td>
<td>A</td>
<td>3.0±0.06</td>
<td>A</td>
<td>3.3±0.06</td>
<td>3.5±0.06</td>
<td>3.7±0.01</td>
</tr>
</tbody>
</table>

Capital letters denote Significant differences among treatment groups at (P < 0.05) level.

Table (7) Effect of licorice powder (Glycyrrhiza Glabra) with drinking water in serum globulin concentration (g/dl) of local female rabbit (mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>1\textsuperscript{st} week</th>
<th>3\textsuperscript{rd} week</th>
<th>5\textsuperscript{th} week</th>
<th>7\textsuperscript{th} week</th>
<th>9\textsuperscript{th} week</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>B</td>
<td>1.5±0.09</td>
<td>B</td>
<td>1.9±0.05</td>
<td>1.7±0.10</td>
<td>2.0±0.08</td>
</tr>
<tr>
<td>G1</td>
<td>A</td>
<td>2.1±0.05</td>
<td>B</td>
<td>2.4±0.03</td>
<td>2.7±0.01</td>
<td>2.9±0.17</td>
</tr>
<tr>
<td>G2</td>
<td>A</td>
<td>2.4±0.03</td>
<td>A</td>
<td>2.9±0.07</td>
<td>3.2±0.07</td>
<td>3.6±0.08</td>
</tr>
</tbody>
</table>

Capital letters denote Significant differences among treatment groups at (P < 0.05) level.

Discussion

The significant decrease in (TC, TG and LDL – cholesterol) and the significant increase (p < 0.05) in (HDL – Cholesterol) in rabbits receiving licorice powder (Glycyrrhiza Glabra) (treated groups) as compared to the untreated group (Control). High level of total serum cholesterol is one of the major risk factors for many vascular and heart diseases like (coronary heart diseases) and it is well known for hyperlipidemia, incidence of atherosclerosis, increase in diabetes and hypertension [27,28]. Because the liver play a pivotal role in lipid homeostasis with some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acid, synthesis of cholesterol and phospholipids and secretion of some specific classes of plasma lipoprotein [29, 30]. Lowering the level of serum lipid through dietary or drug therapy this lead to decrease the risk of vascular disease and many complications, though there was a large class of hypolipidemic drugs can be used, none of the existing ones available worldwide is fully effective, absolutely safe and free from side effect [31]. Many efforts are being made to find out effective and safe agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases. Among natural materials, medical plants have been shown to have properties like anti-hyperlipidemic [14, 32, 33].

The result of this study reveals significant reduction in total serum Cholesterol, Triglyceride, LDL-C as shown in table 1, 2, 3 this is agreement with many study [4,13,14,33], that attributed the hypocholesterimic effect of using licorice powder Glycyrrhiza glabra) to the presence of many phytochemical constituents isoflavones and flaronids which act as anti – oxidant via inhibition of LDL – cholesterol oxidation, also the glycosides of licorice prevent accumulation of cholesterol in cells [4,5,32,34]. The significant increase (p < 0.05) in HDL – Cholesterol level in treated group compared with control group (table 4) is considered beneficial because the high HDL – C levels could potentially contribute to anti – atherogenesis including inhibition of LDL oxidation to protect the endothelial cells from the cytotoxic effects of oxidized LDL [4,27,35].

Also the significant decline in serum LDL – Cholesterol in treated groups could be correlated with the saponin content of licorice powder that enhances the hepatic LDL- receptor levels and increase hepatic uptake of (LDL-C) and aids it catabolism to bile acid [36], also the saponin is known to lower triglyceride by in habiting pancreatic lipase activity, and on the other hand the effect of phytosterol content of licorice on triglyceride metabolism through a decreased absorption of dietary cholesterol [12, 37]. The phytosterols and saponine in Glycyrrhiza Glabra could be important in cholesterol elimination, and the phytosterols are reported to displace intestinal cholesterol and reduce cholesterol absorption from intestine [38,39], saponins are capable of precipitating cholesterol from micelles and interfere with enterohepatic circulation of bile acid, making in unavailable for intestinal absorption [13,14,33].

On the other hand, the flavonoids as natural materials and phytochemical constituents were suggested
to lower blood cholesterol level by two mechanisms initially it enhance the phosphorylation of (HMG - COA) reductase (β – hydroxyl- β – methylglutataryl – COA reductase) indirectly thus diminishes endogenous cholesterol production, Secondly flavonoides probably exert their influence on steroid metabolism by binding to cytoplasmic steroid receptor due to hypophobicity of their aglycones protein . Alternatively , the flavonoids may intercalate it between the base of DNA segments , leading to transcription of gene involved in lowering blood cholesterol level [40] . However , the inhibitory mechanism of flavonoides on cholesterol synthesis are yet unknown [34,40]. Either, the hypothyroidism is a well established cause of hyperlipidemia and elevated (LDL-C) in animals and human [41,42] . Thyroid hormone (T3) influence hepatic cholesterol metabolism and play an important role in the regulation of cholesterol 7- hydroxylase , the rate limiting enzyme in bile acid synthesis [43].

As for serum total protein and it's fraction albumin , globulin were slightly and gradually increased with time progress of treatment up to the end of experimental period table [5,6,7] showed that the treated groups significantly (p < 0.05) higher values than untreated group (control), this could be attributable to the phytochemical component of Glycyrrhiza Glabra like saponin , isoflavonoides , flavonoids , amino acids , phytosterols , and various other substances that normally being considered to be the main biologically active components which support the body health either systems or organs like liver as antihepatotoxic and anti – inflammatory , in addition to this the liver play a pivotal and main organ in protein synthesis [5,6,9,12] , also the flavonoides as anatural materials act as antioxidant that prevent and protect the liver from any injury or stress and due to this, the synthesis of proteins and it's component could be due to an increase in the metabolic process of liver and all cells of body specially the center of protein synthesis in cells like ribosomes [32 , 37 , 44].

Also the increase in the serum globulin as was noticed in the study could be due to that phytochemical component of Glycyrrhiza Glabra that supplemented to treated groups may plays positive effect and stimulate the humeral immunity to produce globulines and this agreed with those found by [4,5,7,8] when using herbal therapy as alternative medicine .

Conclusion:
It could be concluded from this study reveals that using of the licorice powder (Glycyrrhiza Glabra ) supplemented with drinking water at two different doses had various effect on female rabbit to reduction of serum lipide profile , increase serum total protein and it's fraction and this could be contribute to improving animals health.

References
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