Effect of Feeding Gum Arabic on Serum Total and Lipoproteins Cholesterol in Hypercholesterolemic Rats

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In

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DEDICATION

To my parents ..............

My brothers ..............

My sister ............

I dedicate this work with my love.....

Nagm
List of contents

List of contents........................................................................................................ i
List of tables................................................................................................................ iv
List of figures............................................................................................................... v
Acknowledgements.................................................................................................... vi
English abstract........................................................................................................ vii
Arabic abstract.......................................................................................................... viii
Introduction............................................................................................................... 1

Chapter one

Literature review

1.1 Lipids.................................................................................................................... 3
  1.1.1 Triacylglycerol............................................................................................... 3
  1.1.2 Cholesterol.................................................................................................... 4
  1.1.3 Lipoproteins.................................................................................................. 4
     1.1.3.1 Very low density lipoproteins (VLDL)................................................. 5
     1.1.3.1 Low density lipoproteins (LDL)............................................................ 5
  1.1.3.2 High density lipoproteins (HDL)............................................................. 6
1.2 Diseases associated with elevated level of plasma cholesterol......................... 6
  1.2.1 Atherosclerosis............................................................................................ 6
  1.2.2 Coronary heart disease (CHD)..................................................................... 7
1.3 Hypolipidemic agents.......................................................................................... 7
  1.3.1 Drugs............................................................................................................. 7
  1.3.2 Plants............................................................................................................ 8
     1.3.2.1 Cinnamon............................................................................................... 8
     1.3.2.2 Flaxseed (Linum usitatissimum)........................................................... 8
     1.3.2.3 Garlic (Allium sativum L.)................................................................. 8
     1.3.2.4 Green tea............................................................................................... 9
1.3.2.5 Guar gum…………………………………………………. 9
1.3.2.6 Mangiferin……………………………………………….. 9
1.3.2.7 *Nigella sativa*…………………………………………….. 10
1.3.2.8 Soybean genistein……………………………………... 10
1.3.2.9 *Trigonella foenum graecum* (fenugreek)……………… 10
1.3.2.10 *Zingiber officinale Roscoe* (Ginger)………………….. 10

1.4 Gum Arabic (acacia gum)………………………………… 11
1.4.1 General taxonomy of *Acacia Senegal*………………….. 11
1.4.2 Chemical composition of Gum Arabic………………….. 12
1.4.3 Folkloric uses of Gum Arabic………………………….. 12
1.4.4 Adverse effects and toxicity of Gum Arabic…………… 13
1.4.5 Effect of Gum Arabic on cholesterol………………….. 13

**Chapter two**

*Materials and Methods*

2.1 Experimental animals……………………………………… 15
2.2 Experimental design……………………………………… 15
2.3 The composition of rats basal diet………………………... 15
2.4 Gum Arabic………………………………………………... 16
2.5 Cholesterol supplementation in the diet………………….. 16
2.6 Blood sampling…………………………………………… 16
2.7 Analytical methods………………………………………… 16
2.7.1 Total cholesterol estimation……………………………. 16
2.7.2 Low density lipoprotein-cholesterol (LDL-c) estimation… 17
2.7.3 High density lipoprotein-cholesterol (HDL-c) estimation... 17
2.7.4 Triacylglycerol estimation…………………………….. 18
2.8 Procedure of the analyzer machine……………………….. 18
2.9 Calculation of very low density lipoprotein-cholesterol… 19
2.10 Statistical analysis……………………………………….. 19
## Chapter three

### Results

3.1 The induction of hypercholesterolemia

3.2 The effect of feeding Gum Arabic on serum total cholesterol level

3.3 The effect of feeding Gum Arabic on serum LDL-c level

3.4 The effect of feeding Gum Arabic on serum HDL-c level

3.5 The effect of feeding Gum Arabic on serum Triacylglycerol and VLDL-c level

## Chapter four

### Discussion

4.1 The induction of hypercholesterolemia

4.2 The effect of feeding Gum Arabic on serum total cholesterol level

4.3 The effect of feeding Gum Arabic on serum LDL-c level

4.4 The effect of feeding Gum Arabic on serum HDL-c level

4.5 The effect of feeding Gum Arabic on serum Triacylglycerol and VLDL-c level

### Conclusion

### References
## List of tables

<table>
<thead>
<tr>
<th>NO.</th>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The effect of feeding Gum Arabic mixed diet on serum total cholesterol, LDL-c, HDL-c and Triacylglycerol level in induced hypercholesterolemic Wistar albino rats</td>
<td>21</td>
</tr>
</tbody>
</table>
## List of figures

<table>
<thead>
<tr>
<th>NO.</th>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The level of total cholesterol, HDL-c, and LDL-c in group B compared to group A after supplementation of 1% cholesterol for 30 days</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>The effect of feeding Gum Arabic mixed diet on serum level of total cholesterol in induced hypercholesterolemic Wistar albino rats</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>The effect of feeding Gum Arabic mixed diet on serum level of LDL-c in induced hypercholesterolemic Wistar albino rats</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>The effect of feeding Gum Arabic mixed diet on serum level of HDL-c in induced hypercholesterolemic Wistar albino rats</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>The effect of feeding Gum Arabic mixed diet on serum level of triacylglycerol in induced hypercholesterolemia Wistar albino rats</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>The effect of feeding Gum Arabic mixed diet on serum level of VLDL-c in induced hypercholesterolemia Wistar albino rats</td>
<td>28</td>
</tr>
</tbody>
</table>
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English Abstract

The objective of this study was to evaluate the effect of Gum Arabic mixed diet on serum concentration of total cholesterol (TC), very low density lipoprotein cholesterol (VLDL-c), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), and triacylglycerol (TAG) in cholesterol fed rats.

Twenty five Wistar albino rats were divided into five groups named A, B, C, D, and E each of five rats. Group A was provided with basal diet only and served as control, group B received 1% cholesterol powder mixed with the basal diet. Group C received 1% cholesterol powder and 10% (GA) mixed with the basal diet, Group D received 1% cholesterol powder and 20% GA mixed with the basal diet, and group E received 1% cholesterol powder and 30% GA mixed with the basal diet.

After 30 days, blood samples were collected from all groups for analysis. There was non-significant reduction on TC in group C, D and E compared to group B. The level of LDL-c was found to be significantly decreased in group C, D and E compared to group B. In group C, D and E HDL-c level was significantly increased compared to group B. Serum TAG and VLDL-c were reduced in dose response manner of Gum Arabic in group C, D and E compared to group B, although the decrease was significant (P< 0.05) only in group D and E.

The present study concluded that the addition of Gum Arabic to rat’s diet has positive effect in lowering serum cholesterol and triacylglycerol levels.
المستخلص

هدفت هذه الدراسة لقيام تأثير الصمغ العربي المخلوط مع الطعام على مستوى الكوليسترول الكلى وไกลسترول الليموزرتونين (الدهن البروتيزي) ذو الكثافة المخفضة جداً والليبروتيتين منخفض الكثافة والليبروتيتين عالت الكثافة والجليسريدات الثلاثية في دم الفئران المغذية اضافياً بالكوليسترول.

خمسة وعشرون فاراً تم تقسيمهم إلى خمسة مجموعات أ، ب، ج، د و ه، كل مجموعة تحتوي على خمسة فئران. كانت المجموعة (أ) هي مجموعة التحكم وأعطت الوجبة الأساسية فقط، المجموعة (ب) أطعنت الوجبة الأساسية إضافة 1% كوليسترول وتبدير الصمغ العربي بنسبة 10% المجموعة (د) أعطت الوجبة الأساسية إضافة 1% كوليسترول وتبدير الصمغ العربي بنسبة 20%، أما المجموعة (ه) فأعطت الوجبة الأساسية إضافة 1% كوليسترول وتبدير الصمغ العربي بنسبة 30%.

بعد 30 يوماً من بداية الدراسة أخذت عينات من الدم من جميع المجموعات وذلك لإجراء التحليل.

كان هناك انخفاض في مستوى الكوليسترول الكلي في المجموعات (ج)، (د)، و (ه) بمقارنة بالمجموعة (ب).

لكن، لم يكن معنوناً مستوى الليموزرتونين منخفض الكثافة الانخفاض معنوباً في المجموعات (ج)، (د)، و (ه) بمقارنة بالمجموعة (ب). مستوى الليموزرتونين عالت الكثافة زاد معنوباً في المجموعات (ج)، (د)، و (ه) بمقارنة بالمجموعة (ب)، انخفاض مستوى الجليسريدات الثلاثية وكمليسترول الليموزرتونين ذو الكثافة المنخفضة جداً اعتماداً على تركز الصمغ العربي في المجموعات (ج)، (د)، و (ه) بمقارنة بالمجموعة (ب)، بارغم من ان الانخفاض كان معنوناً في المجموعات (د) و (ه) فقط.

خلصت هذه الدراسة إلى أن إضافة الصمغ العربي إلى غذاء الفئران له أثر إيجابي على تخفيض مستوى الكوليسترول وجليسريدات الثلاثية في الدم.
Introduction

Coronary heart disease (CHD) is a global health problem. High intake of fats and dyslipidemia are risk factors for its development. Certain food items which are rich in dietary fiber in association with exercise may be protective (Buriro and Tayyab, 2007).

The association of raised serum cholesterol with Coronary heart disease is well known, some studies suggest that the increase of serum Triacylglycerol may also be a hazard (Sharma et al., 1996).

There are many chemical drugs that lower cholesterol level in the body such as: statins, fibrates, ezetimibe and nicotinic acid, but most of them are expensive and have undesirable effect (Thomas, 2003). So there are increasing interest in alternative/herbal medicine for the prevention and treatment of hypercholesterolemia.

Gum Arabic (GA) is a dried exudates obtained from the branches and stems of *Acacia senegal* and closely related species (FAO, 1999). It is a complex polysaccharide of high molecular weight which contains neutral sugars as rhamnose, arabinose, and galactose; acids such as glucuronic acid; minerals such as calcium, magnesium, potassium, sodium, and phosphorous (Leung, 1980). Gum Arabic has wide industrial uses as a stabilizer, thickening agent and emulsifier, mainly in the food industry also in the textile, pottery, lithography, cosmetics and pharmaceutical industries (Verbeken et al., 2003).

In folk medicine, GA has been reported to be used internally for the treatment of inflammation of the intestinal mucosa, and externally to cover inflamed surfaces (Gamal el-din et al., 2003). Some recent reports have claimed that GA possesses anti-oxidant, nephro-protectant and other effects (Rehman et al., 2001, Gamal el-din et al., 2003, Ali et al., 2008).

Clinically, GA has been tried in patients with chronic renal failure, and it was claimed that it helps to reduce urea and creatinine plasma
concentrations and reduces the need for dialysis from 3 to 2 times per week (Suliman et al., 2000).

Ross et al., (1983) and Sharma (1985) reported reductions of total serum cholesterol by 6% and 10.4%, respectively when human subjects received 25 g/day and 30 g/day of GA for periods of 21 and 30 days. The decrease was confined to LDL and VLDL cholesterol only, with no effect on HDL and Triacylglycerol. In contrast, consumption of GA at a dose of 15 g/day for 4 weeks by healthy human subjects or hypercholesterolemic subjects had no significant effect on plasma lipids (Haskell et al., 1992., Jensen et al., 1993).

In rats, the results were as contradictory. Topping et al. (1985) has shown that plasma cholesterol concentrations were unaffected by feeding GA, but plasma triacylglycerols were significantly lower than in controls.

In another study, GA was fed to rats replacing cellulose in purified diets supplemented with cholesterol and cholic acid, no significant effects of increasing concentrations of GA were found on the concentrations of plasma cholesterol when compared to levels found in rats that consumed control diet containing cellulose alone. Plasma triacylglycerol concentrations were, however, higher in rats fed GA (Annison et al., 1995). These findings are not universally accepted and their confirmation, validity, reliability and mode of action await further studies.

The aim of this study is to evaluate the effect of feeding Gum Arabic mixed diet on the level of serum total and lipoproteins cholesterol in induced hypercholesterolemic Wistar albino rats with respect to:-

1. Serum total cholesterol (TC).
2. Very low density lipoprotein cholesterol (VLDL-c).
3. Low density lipoprotein cholesterol (LDL-c).
4. High density lipoprotein cholesterol (HDL-c).
Chapter one
Literature review

1.1 Lipids

Lipids are a heterogeneous group of substances of biological origin that are easily dissolved in organic solvents such as chloroform, and benzene. By contrast, they are either insoluble or only poorly soluble in water (Koolman and Roehm, 2005).

Four main classes of lipids can be recognized from a metabolic standpoint. These are free fatty acids, triacylglycerol, phospholipids, and cholesterol and its esters (Forrester et al., 1987).

The principle functions of lipids are to act as energy stores and to serve as important structural component of cells. To fulfill these functions, lipids have to be transported in plasma from one tissue to another, from the intestine or the liver to other tissues such as muscular or adipose tissue, or from the other tissues to the liver (Bishop et al., 2000).

Combination of fat and protein (lipoproteins) are important cellular constituents occurring in the cell membrane and in the mitochondria, and serving as the means of transporting lipids in the blood (Murray et al., 2003).

There are complex mechanisms that control the release of lipids from tissues into plasma, and the uptake of lipids by the tissues from the plasma. Abnormalities of these mechanisms may be associated with the development of disease, particularly ischemic heart disease (Champe et al., 2005).

1.1.1 Triacylglycerols

Triacylglycerols are esters of glycerol with fatty acids; they represent the most important class of dietary fats which constitute more than 90% of the dietary lipids (Sheriff, 2004). They are transported as lipoproteins from the intestine and the liver to various tissues, such as adipose tissue. Plasma triacylglycerol concentrations rise after a fatty meal and remain increased for several hours (Stein and Gary, 1994). Liver and intestine are the major sites
of triacylglycerol synthesis. When there is an excess in body's calories, the liver synthesizes the triacylglycerol, then transported and stored in adipose tissue. A recent study showed that having high level of triacylglycerol, increase the risk of having heart attack (Mirkin, 2000).

1.1.2 Cholesterol

Cholesterol is a lipid found in the cell membrane of all animal tissues, and it is transported in the blood plasma of all animals. It's the best known steroid; because of its association with atherosclerosis. Cholesterol is the parent molecule from which all steroids in the body are synthesized, which includes bile acids, adrenocortico-hormones, sex hormones, and vitamin D (Murray et al., 2003).

Cholesterol from the liver and intestine is transported in plasma. About 75% esterified with fatty acids and the rest is unesterified. It is taken up from plasma by different tissues. Its main route of metabolism is to bile acids, which are secreted into bile as conjugated with glycine or taurine. Unesterified cholesterol is also secreted into bile, and both undergo an entero-hepatic-circulation with some loss of cholesterol and bile acids occur daily in the faeces. Unlike that of triacylglycerol, plasma concentration of cholesterol does not rise after a fatty meal (Johnson et al., 1991).

Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins. Complex spherical particles which have an exterior composed mainly of water-soluble proteins; fats and cholesterol are carried internally (Brunzell, 2008).

1.1.3 Lipoproteins

Fat absorbed from the intestine or those synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. Lipoproteins are the main transported form of lipids. They are classified based on their density to five fractions including: high density lipoproteins (HDL), low density lipoproteins (LDL), intermediate
density lipoproteins (IDL), very low density lipoproteins (VLDL) and chylomicrones (Davidson and Sittman, 1994).

Chylomicrones are derived from the intestinal absorption of triacylglycerol. The VLDL is synthesized in the liver for exportation of triacylglycerol to the extra-hepatic tissues; LDL is the final stage in the catabolism of VLDL; IDL is a transient lipoprotein formed during the conversion of VLDL to LDL, it contains both triacylglycerol and cholesterol, IDL is usually undetectable in normal plasma (Zilva et al., 1994). HDL is involved in VLDL and chylomicrones metabolism and also in the transport of cholesterol to the liver (Murray et al., 2003).

1.1.3.1 Very-low-density lipoprotein (VLDL)

Very low-density lipoproteins (VLDL) are produced in the liver. They are composed predominantly of triacylglycerol, and their function is to carry this type of lipids from the liver to peripheral tissues (Murray et al., 2003). The imbalance between hepatic triacylglycerol synthesis and secretion of VLDL can cause hepatic steatosis (fatty liver) as in uncontrolled Diabetes mellitus and Obesity. VLDL is converted to LDL in the circulation after triacylglycerol degraded by the enzyme lipoprotein lipase and transferred to HDL in exchange reaction that concomitantly transfers cholesterol esters from HDL to VLDL. This exchange is accomplished by cholesterol ester transfer protein (Champe et al., 2005).

1.1.3.2 Low-density lipoprotein (LDL)

Low-density lipoprotein (LDL) is a lipoprotein that transports cholesterol and triacylglycerol from the liver to peripheral tissues. LDL also regulates cholesterol synthesis at these sites. It commonly appears in the medical setting as part of a cholesterol blood test, and since high levels of LDL-cholesterol can signal medical problems like cardiovascular disease. Each native LDL particle contains a single apolipoprotein B-100 molecule (Apo B-100, a protein with 4536 amino acid residues) that circulates the
fatty acids, keeping them soluble in the aqueous environment. In addition, LDL has a highly-hydrophobic core consisting of polyunsaturated fatty acid linoleate, and about 1500 esterified cholesterol molecules (Segrest, 2001). Conditions with elevated concentrations of oxidized LDL particles, especially "small dense LDL" (sdLDL) particles, are associated with atheroma formation in the walls of arteries, a condition known as atherosclerosis, which is the principal cause of coronary heart disease and other forms of cardiovascular disease (Lewington et al., 2007).

1.1.3.3 High-density lipoprotein (HDL)

HDL is the smallest of the lipoproteins. It is involved in the transport of cholesterol from the peripheral tissues to the liver. They are the densest because they contain the highest proportion of protein. They contain the A class of apo-lipoproteins. The liver synthesizes these lipoproteins as complexes of apo-lipoproteins and phospholipids, which resemble cholesterol-free flattened spherical lipoprotein particles. They are capable of picking up cholesterol, carried internally, from cells they interact with. A plasma enzyme called lecithin-cholesterol acyltransferase (LCAT) converts the free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol) which is then sequestered into the core of the lipoprotein particle eventually making the newly synthesized HDL spherical. They increase in size as they circulate through the blood stream and incorporate more cholesterol molecules into their structure (Kwiterovich, 2000).

1.2 Disease associated with elevated level of plasma cholesterol

1.2.1 Atherosclerosis

Atherosclerosis is a progressive inflammatory disorder of arterial wall that is characterized by the deposition of cholesterol and cholesterol ester of lipoprotein containing ApoB-100 in connective tissues of the arterial wall which lead to the formation of fatty fibrous plaque that narrow the lumen of
arteries and may lead to thrombosis. Atherosclerosis correlates with a high plasma LDL-c: HDL-c ratio (Davidson, 2002).

Complication of atherosclerosis could be diminished when plasma cholesterol level lowered by hypocholesterolemic agent (Frich et al., 1987)

1.2.2 Coronary heart disease (CHD)

Coronary heart disease is the most common form of heart disease and is almost always due to atheroma and its complication resulting from inadequate blood flow. CHD raises with high plasma LDL-c concentration which carries the most of the plasma cholesterol (Davidson, 2002).

LDL-c particles are often termed "bad cholesterol" because they have been linked to atheroma formation. On the other hand, high concentrations of functional HDL-c "good cholesterol", which can remove cholesterol from cells and atheroma, offer protection. The balance between LDL-c and HDL-c can be changed by medications, food choices and other factors (Durrington, 2003).

1.3 Hypolipidemic agents

1.3.1 Drugs

Cholesterol reducing drugs are medication that lowers the level of fat in the blood, including total cholesterol, LDL-c, and triacylglycerol. High level of these fats in the blood stream increases the risk of atherosclerosis, heart attack, and other heart related conditions. Therefore, cholesterol reducers and other hypolipidemic medication are often prescribed for people with high cholesterol level or with elevated level of other type of lipids (Ozkan et al., 2004).

Hypolipidemic drugs such as Statins, fibrates (including bezafibrate, gemfibrozil and fenofibrate), ezetimibe, colestide, and nicotinic acid have severe side effect, like interfering with the absorption of other substances including other medications, or flushing as seen in nicotinic acid at high doses (Thomas, 2003).
1.3.2 Plants

A plant-based diet that is rich in fruit, vegetables, legumes and low in saturated fat, along with regular aerobic exercise program, is a typical prescription for anyone with elevated risk of cardiovascular disease. In addition, there are many herbs available that provide some help for persons with hyperlipidemia (Winston, 1999).

1.3.2.1 Cinnamon

Cinnamon significantly reduced the triacylglycerol levels in diabetic individuals. This effect of cinnamon is particularly important for hyperlipidemic individuals. The lipid lowering effect of cinnamon might be due to insulin potentiating action of cinnamon. Usually, when glucose metabolism is improved, lipid metabolism is also improved. Some constituents of cinnamon are blocking the synthesis of cholesterol or facilitating the clearance of cholesterol from the body. The insulin potentiating property of cinnamon may help to reduce cholesterol level. High fibers amount present in cinnamon decrease fat absorption by the gut resulting in a decrease level of triacylglycerol in the blood (Alam et al., 2003).

1.3.2.2 Flaxseed (*Linum usitatissimum*)

Flour derived from flaxseed (*Linum usitatissimum*) is popular for use in bread and bakery products. It provides a nutty flavor and also increases the nutritional and health benefits of the final product. Flaxseed consumption may lower both total- and LDL-c concentrations because of its low–saturated fat content, high polyunsaturated fat, phytosterol content and mucilage content (Cunnane et al., 1993).

1.3.2.3 Garlic (*Allium sativum* L.)

The administration of 4% and 8% of fresh crushed garlic bulbs to Wistar albino rats resulted in a significant reduction of total cholesterol, LDL-c and triacylglycerol levels, whereas, the level of HDL-c were reported
to be significantly elevated (Elmahdi et al., 2008). Regular use of garlic can be effective in reducing the risk of heart attack and stroke because it lowers total and LDL-c and triacylglycerol concentrations without affecting HDL-c concentrations (Warshafsky et al., 1993). Garlic is reported to decrease serum cholesterol, LDL-c and triacylglycerol, possibly through inhibition of HMG-CoA reductase activity and increases the excretion of cholesterol as bile acids (Kleijnen et al., 1989).

1.3.2.4 Green tea

Several epidemiologic studies have suggested that drinking either green or black tea may lower blood cholesterol concentrations and blood pressure, thereby providing some protection against cardiovascular disease. When rats were fed green tea polyphenones, blood cholesterol concentrations declined in hypercholesterolemic animals and blood pressure decreased in spontaneously hypertensive animals (Dreosti, 1996). Potential mechanisms include reduced micelles solubility and intestinal absorption of cholesterol increased fecal excretion of fat and cholesterol, reduced hepatic cholesterol concentration and up-regulation of the LDL receptor in liver cells (Bursill et al., 2001).

1.3.2.5 Guar gum

Guar gum at dose rate of 30% reduced total cholesterol and triacylglycerol in plasma and liver of diabetic and non-diabetic rats when fed for 2 weeks. The mechanism is due to high amount of fibers that bind to bile acids and excreted them out of the body (Yamamoto, 2001).

1.3.2.6 Mangiferin

Feeding mangiferin roots at dose rate of 20% mixed with mice diet for 14 days, increases bile acid excretion and reduced blood cholesterol levels in hypercholesterolemic mice, this is due to the fiber content of the plant (Miura et al., 2001).
1.3.2.7 *Nigella sativa*

*Nigella sativa*-treated rats had lower fasting plasma levels of cholesterol and triacylglycerol, and higher HDL-c as compared to pair-fed controls. The hypocholesterolemic function of *Nigella sativa* is either by reducing the synthesis of cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine (Ali *et al*., 2004).

1.3.2.8 *Soybean genistein*

Administration of genistein to rats at dose of 5 to 8mg/day/100mg (B. wt) for 12 days inhibited the incorporation of acetate into cholesterol and fatty acids in the liver, also the activity of the enzyme hydroxyl-methyle-glutaryl-CoA reductase (HMG-CoA reductase) and cholesterol hydroxylase was decreased, and the microsomal enzyme acyl-CoA cholesterol acyl transferase (ACAT) showed a dramatic decrease. The genistein enhances bile formation and biliary lipid secretion (Kojima *et al*., 2002).

1.3.2.9 *Trigonella foenum graecum* (fenugreek)

The seeds of *Trigonella foenum graecum* (fenugreek) have been reported to have antidiabetic and hypocholesterolaemic properties in both animal models and humans (Hannan *et al*., 2003). Activity has been attributed largely to fenugreek’s saponin and high fiber and unsaturated/saturated fatty acid ratio contents of the seeds, and is probably not related to its major alkaloid trigonelline. Fenugreek administration may increase plasma insulin levels in vivo. The hypocholesterol-emic effect has been attributed to the increased conversion of hepatic cholesterol to bile salts which are lost in the faeces together with fenugreek fiber and saponins. Fenugreek treatment selectively reduces the LDL-c and increases HDL-c (Molham and Amala, 1998).

1.3.2.10 *Zingiber officinale Roscoe* (Ginger)

Daily oral administration of ginger at a dose of 35 and 70mg/kg B. wt to rats for 10 weeks decreased significantly the level of cholesterol,
phospholipids and free fatty acid in serum and tissues. Level of serum triacylglycerol was also significantly reduced. Supplementation of ginger increased the concentration of HDL-c and decreased the concentration of LDL-c and VLDL in the serum, and therefore reduces the risk of atherosclerosis (Murugaiah et al., 1999).

In experimental mice, ginger significantly impaired cholesterol biosynthesis and lowered serum cholesterol concentrations (Tanabe et al., 1993).

1.4 Gum Arabic (acacia gum)

Gum Arabic is the most important polysaccharide gum of commerce. It appears in commerce under a variety of names depending on its quality and the location of its origin. The highest quality gum that constitute the great bulk of the trade is derived from Acacia senegal which grows in Sudan and Senegal regions, and to much lesser extent in Nigeria, Uganda, Tanzania, and recently Niger (Anderson et al., 1991).

1.4.1 General taxonomy of Acacia Senegal:

Kingdom: Planate
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Genus: Acacia
Species: A. Senegal (L) Wild.

Gum Arabic is odourless and tasteless. The best shape is the tears drop or globular shape of various grades of gum Arabic. The best grades of Gum Arabic are almost colourless with slight trace of yellow, some possess pink lines. Gum Arabic has an extremely high solubility in water, in contrast to its high molecular weight; Gum Arabic has low viscosity (Glicksman, 1969).
1.4.2 Chemical composition of Gum Arabic

In recent study, Gum Arabic was separated into a high molecular weight glycoprotein containing 90% carbohydrate, and a lower molecular weight heterogeneous polysaccharide fraction. The sugar components of Gum Arabic are galactose, arabinose, rhamnose, and glucuronic acid (Lamport and Fong, 1991).

It was established by Akiyama et al. (1984) that the protein part of Gum Arabic is probably covalently bound to carbohydrate. They indicated that the amino acid composition of Gum Arabic is rich in hydroxyproline and serine while the alanine content is low.

Anderson and Weiping (1990) reported that Gum Arabic contains aspartic acid, glutamic acid, glycine, histidine, leucine, proline, threonine, and phenylproline.

Buffo et al. (2001) reported that Gum Arabic contains aluminum, iron, magnesium, phosphorus, copper, and zinc.

1.4.3 Folkloric uses of Gum Arabic

Major uses of Gum Arabic are in food and pharmaceutical industries, where their stabilizing, thickening and gel forming properties are the main physical requirements.

Bulk quantities are employed in the mining industry and in the manufacture of paper and textiles. Gums are used also in bakery, meat products, beverages, confectionery, sauces, adhesives, cosmetics, printing, foundry ceramics, fertilizers and binder to explosives. Gum Arabic is also an effective emulsifier allowing a homogenous distribution of fats in different products. It's widely used for diet pastry and low calorie sweets. In deep frozen milk products Gum Arabic prevents the crystallization of sugar and water. It's also used to thicken soft drinks, high-quality glues, for colours in dyes and as palletizing agents (Glicksman, 1982; Walker, 1984; and Szczeniak, 1986). In pharmaceutical industries Gum Arabic serves as an
emulsifying agent and gives viscosity to powdered drug material; and used a binding agent in making bills and tablets and particularly cough drops and lozenges (Morton, 1977).

Gum Arabic has several medical uses, such as demulcent to smooth irritation, especially of mucous membrane. It was noticed that supplementation with Gum Arabic fibers increase fecal nitrogen excretion and lower serum urea nitrogen concentration in chronic renal failure patients consuming a low protein diet (Bliss et al., 1996). It is used to treat diarrhea, dysentery, coughs, throat irritation and fevers (Morton, 1977).

1.4.4 Adverse effects and toxicity of gum Arabic

Number of reports said that GA causing contact dermatitis and asthma (e.g. Ilchysn and Smith, 1985). In all these reports, the offending allergen has not been identified. However, in 1998, a carbohydrate specific IgE was identified in a chocolate confectioner with cough and dyspnea (Fötisch et al., 1998). More recently, Sander et al. (2006) found that sensitization to GA carbohydrate structures occurs casually in atopic patients with pollen sensitization without obvious exposure to GA, and that allergy to GA is mediated preferentially by IgE antibodies directed to polypeptide chains of GA.

Doi et al., (2006) reported that administration of acacia gum to both sexes of rats at dietary levels of different concentrations had no effects on clinical signs, survival, body weights, and food and water consumption, or on findings of urinalysis, ophthalmology, hematology, or blood biochemistry. Gross pathology and histopathology exhibited no differences of toxicological significance between control and treated rats.

1.4.5 Effect of Gum Arabic on cholesterol

Gum Arabic is a water-soluble, and as such belongs to a group of compounds known to have the potential to lower plasma cholesterol in humans and to be fermented by the large bowel microflora. Annison et al.
(1995) reported that liver triacylglycerols were lower in rats fed gums compared to control. It is thought that, at the population level, the lowering of plasma cholesterol and triacylglycerol concentrations is associated with a decreased risk of cardiovascular disease. How Gum Arabic lower plasma lipids is still unclear, but the mechanism is related possibly to increased fecal bile acid and neutral sterol excretion or a modification of lipid digestion and absorption (Annison et al., 1995).

Ross et al. (1983), reported that Gum Arabic administered to men for 3 weeks has little effect on glucose tolerance, but decreases the serum cholesterol.

In animal experiments, Gum Arabic was noticed to reduce serum cholesterol in rats, suggestion gum interference with dietary cholesterol absorption (Kelley and Tsai, 1978). El-khier et al. (2009) reported that increasing the ratio of the Gum Arabic (from 5- 15%) in the basal laying hen diet significantly reduced serum cholesterol in a gradual manner and consequently egg with lowered yolk cholesterol were obtained.
Chapter two
Materials and Methods

2.1. Experimental animals

Twenty five Wistar albino rats obtained from the National Center for Research, Khartoum were used in this study. The rats were housed identically in stainless steel cages in an air room under suitable conditions. All of the rats were initially fed a standard laboratory diet for 7 days as adaptation period. Tap water was freely available.

2.2 Experimental design

The animals were divided into five groups of five animals each. These groups were named as A, B, C, D, and E. Group A was given the basal diet and served as control, group B received 1% cholesterol added to the basal diet. Group C received 1% cholesterol and 10% Gum Arabic (GA) added to the basal diet, Group D received 1% cholesterol and 20% GA added to the basal diet, and group E received 1% cholesterol and 30% GA added to the basal diet. Blood samples were collected at the end of the study period for the determination of lipid fractions concentration.

2.3 The rat basal diet

The rats were given a basal diet which fulfilled their requirement. The composition was as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>657g</td>
</tr>
<tr>
<td>Dry meat</td>
<td>220g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3g</td>
</tr>
<tr>
<td>Oil</td>
<td>120g</td>
</tr>
</tbody>
</table>
2.4 Gum Arabic

Gum Arabic powder was purchased from (the Gum Arabic Company limited, Khartoum), which are pre-identified, and then added to the rats diet according to the different groups.

2.5 Cholesterol supplementation in the diet

Cholesterol powder (Lab Tech Chemicals) was supplemented to the basal diet of the rats so as to induce hypercholesterolemia in all groups except the control group according to (Son et al., 2003).

2.6 Blood sampling

Blood was collected from the rat's orbital plexus (1.5 ml) after an overnight fasting by capillary tubes and was put in test tubes; the blood was centrifuged at 5000 rpm for 10 minutes. Then the serum was placed into + containers and used immediately.

2.7 Analytical methods

All lipid fractions were estimated in the Research and Laboratory Unit, Khartoum Teaching Hospital, using Roche diagnostic Hitachi 902 analyzer, Germany, which is fully automated and computerized machine to report test results on various body fluid samples for wide range of analysis. It uses spectrophotometric principle.

2.7.1 Total cholesterol estimation

Principle

In the presence of cholesterol esterase, the cholesterol esters in the sample are hydrolyzed to cholesterol and free fatty acids. The cholesterol produced is oxidized by cholesterol oxidase to cholesterol and hydrogen peroxide. Hydrogen peroxide is detected by chromogenic oxygen acceptor, phenol – ampyrone, in the presence of peroxidase. The red quinine formed is proportional to the amount of cholesterol present in the sample.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids} \\
\text{Cholesterol} + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{cholesterol oxidase}} \text{cholestnone} + \text{H}_2\text{O}_2
\]
$2\text{H}_2\text{O}_2 + 4\text{- Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quainoneimine dye} + 4\text{H}_2\text{O}$

**Reagents:**

Reagent (1)  
- Buffer pH 6.9  
- Phenol 26 mmol/L  
Reagent (2)  
- Cholesterol esterase 300 U/L  
- Cholesterol oxidase 300 U/L  
- Phenol-ampyrone/peroxidase 1250 U/L  
- 4-Aminoantipyrine 0.4 mmol/L

**2.7.2 Low density lipoprotein-cholesterol (LDL-c) estimation**

**Principle**

LDL-c particles in the sample are precipitated with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant. The LDL-c is then spectrophotometrically measured by means of the coupled reactions as described for cholesterol.

**Reagents**

Polyvinyl sulphate 3g/L, and polyethylene glycol 3g/L.

**2.7.3 High density lipoprotein-cholesterol (HDL-c) estimation**

**Principle**

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample are precipitated with phosphotungstate and magnesium ions. The supernatant contains HDL-c. The HDL-c is then spectrophotometrically measured by means of coupled reactions described for cholesterol.

**Reagents**

50 ml phosphotungstate (0.4 m mol/L) and magnesium chloride (20 m mol/L).

HDL-c standard concentration = 40 mg/dl.
2.7.4 Triacylglycerol estimation

Principle

Triacylglycerol in the sample are hydrolyzed enzymatically to glycerol and fatty acids. The glycerol formed is converted to glycerol phosphate by glycerol kinase. Glycerol phosphate is oxidized to dihydroxyacetone phosphate by glycerol phosphate oxidase. The liberated hydrogen peroxide is detected by a chromogenic acceptor, chlorophenol and 4-amino antipyrine in the presence of peroxidase. The red quinone formed is proportional to the amount of triacylglycerol present in the sample.

\[
\text{Triacylglycerol} + \text{H}_2\text{O} \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids} \\
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-P} + \text{ADP}. \\
\text{Glycerol-3-P} + \text{O}_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + \text{H}_2\text{O}_2. \\
2\text{H}_2\text{O}_2 + 4\text{- Aminoantipyrine} + 4\text{- Chlorophenol} \xrightarrow{\text{POD}} \text{Guinoneimine dye} + 4\text{H}_2\text{O}.
\]

Reagents

<table>
<thead>
<tr>
<th>Reagent (1)</th>
<th>Buffer PH (7.5)</th>
<th>45 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophenol</td>
<td>6 mmol/L</td>
</tr>
<tr>
<td>Reagent (2)</td>
<td>Magnesium chloride</td>
<td>5 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Lipase</td>
<td>&gt;100 u/ MI</td>
</tr>
<tr>
<td></td>
<td>Glycerol kinase</td>
<td>&gt;1.5 u/mL</td>
</tr>
<tr>
<td></td>
<td>Glycerol -3- oxidase phosphate</td>
<td>&gt;4 u/ mL</td>
</tr>
<tr>
<td></td>
<td>Peroxidase</td>
<td>&gt;0.8 u/mL</td>
</tr>
<tr>
<td></td>
<td>4- aminoantipyrine</td>
<td>0.75 mmol/L</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>0.9 mmol/L</td>
</tr>
</tbody>
</table>

2.8 Procedure of the analyzer machine

For the determination of each parameter, 0.5 ml of serum was put in Hitachi sample cups, then the analyzer automatically adds an equal quantity from samples and reagents to the reaction tubes, and the analytes
concentration of each sample did appear directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.9 Calculation of Very low density lipoprotein-cholesterol (VLDL-c)

Very low density lipoprotein-cholesterol (VLDL-c) in samples was calculated according to the formula of (Friedewald, et al., 1972) which state that: VLDL-c = TG/5

2.10 Statistical analysis

Data are expressed as the means ± SE. The effect of Gum Arabic was determined by the one-way analysis of variance (ANOVA) procedure, using SPSS version 17 software. Differences were considered significant at p < 0.05.
Chapter three

Results

3.1 The induction of hypercholesterolemia

Figure (1) Shows significant increase (P<0.05) in the total cholesterol level as well as LDL-c, and a significant decrease (P<0.05) in HDL-c in group B compared to group A (control).

3.2. The effect of feeding Gum Arabic mixed diet on serum total cholesterol level in induced hypercholesterolemic Wistar albino rats

Table (1) and Figure (2) Show the results of serum total cholesterol of groups A, B, C, D and E. The level of serum total cholesterol in group B is significantly (P< 0.05) higher than the level of serum total cholesterol in group A, but there is non-significant (P> 0.05) difference between groups B, C, D and E.

3.3. The effect of feeding Gum Arabic mixed diet on serum LDL-c level in induced hypercholesterolemic Wistar albino rats

Table (1) and Figure (3) Show the results of serum LDL-c of groups A, B, C, D and E. The level of serum LDL-c in group B is significantly (P< 0.05) higher than the level of serum LDL-c in groups A, C, D and E. In group C the level of serum LDL-c is significantly (P<0.05) different compared to the level of serum LDL-c of group A. However, there is non-significant difference between groups A, D and E.
Table (1): The effect of feeding Gum Arabic mixed diet on serum total cholesterol, LDL-c, HDL-c, VLDL-c and triacylglycerol (TAG) level in induced hypercholesterolemic Wistar albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>TC (mg/ dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
<th>TAG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>87.40 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.60 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.20 ± 9.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.40 ± 10.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.00 ± 6.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>122.2 ± 10.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.20 ± 9.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.36 ± 8.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.21 ± 9.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.20 ± 10.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>120.20 ± 6.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.20 ± 7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.92 ± 8.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.08 ± 11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.40 ± 10.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>116.0 ± 9.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.20 ± 7.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.76 ± 08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.04 ± 4.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.20 ± 6.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>107.20 ± 9.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.80 ± 5.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.12 ± 6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.28 ± 8.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.40 ± 5.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± SE within the same column having different superscript small letters are significantly different at (P < 0.05) based on t-test.
Figure (1): The level of total cholesterol, HDL-c, and LDL-c in group B compared to group A after supplementation of 1% cholesterol for 30 days.

Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
Figure (2): The effect of feeding Gum Arabic mixed diet on serum level of total cholesterol in induced hypercholesterolemic Wistar albino rats.

Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
Figure (3): The effect of feeding Gum Arabic mixed diet on serum level of LDL-c in induced hypercholesterolemic Wistar albino rats.

Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
3.4. The effect of feeding Gum Arabic mixed diet on serum HDL-c level in induced hypercholesterolemic Wistar albino rats

Table (1) and Figure (4) Show the results of serum HDL-c of groups A, B, C, D and E. The level of serum HDL-c in group B is significantly (P<0.05) lower than the level of serum HDL-c in groups A, C, D and E. In group C the level of serum HDL-c is non-significantly (P>0.05) different compared to the level of serum HDL-c of group A, but the level of serum HDL-c of group D and E is significantly (P < 0.05) higher than that of group A (the control group).

3.5. The effect of feeding Gum Arabic mixed diet on serum Triacylglycerol and VLDL-c level in induced hypercholesterolemic Wistar albino rats

Table (1) and Figures (5 and 6) Show the results of serum triacylglycerol (TAG) and VLDL-c level of groups A, B, C, D and E. Since they are dependent (VLDL-c equal 20% of TAG), the level of serum TAG and VLDL-c in group B are non-significantly higher than that of groups A and C, but significantly (P< 0.05) higher than those of groups D and E. Serum TAG and VLDL-c in group D and E are significantly (P< 0.05) lower than that of group A (control group).
Figure (4): The effect of feeding Gum Arabic mixed diet on serum level of HDL-c in induced hypercholesterolemic Wistar albino rats.

Bras having different superscript small letters are significantly different at (P < 0.05) based on t-test.
Figure (5): The effect of feeding Gum Arabic mixed diet on serum level of triacylglycerol in induced hypercholesterolemia Wistar albino rats.

Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
Figure (6): The effect of feeding Gum Arabic mixed diet on serum level of VLDL-c in induced hypercholesterolemia Wistar albino rats.

Bars having different superscript small letters are significantly different at (P < 0.05) based on t-test.
Chapter four

Discussion

The objective of this study was to evaluate the effect of feeding Gum Arabic mixed diet on serum levels of total cholesterol (TC), VLDL-c, LDL-c, HDL-c and triacylglycerol (TAG) in induced hypercholesterolemic Wistar albino rats.

4.1 Induction of hypercholesterolemia:

The results showed that the serum total cholesterol and LDL-c levels were significantly (P<0.05) increased following administration of 1% cholesterol powder mixed with the basal diet in group B compared to group A (control), but serum HDL-c level was significantly (P<0.05) decreased. These results were in agreement with the study of Son et al. (2003), who reported that administration of 1% cholesterol powder resulted in an increase in TC and LDL-c, but the HDL-c was reported to be decreased. Also the results were in line with the results obtained from other studies that administration of cholesterol powder or other high fat diet resulted in an increase in TC as well as LDL-c, but decreased HDL-c level (Chithra and leelaman, 1997; Hwang et al., 2001)

4.2 The effect of feeding Gum Arabic on serum total cholesterol level

The level of serum total cholesterol was decreased, but non-significantly after the administration of 10%, 20% and 20% Gum Arabic compared to the hypercholesterolemic group (group B). This result was in line with the result obtained by Ross et al. (1983) who reported that, administration of Gum Arabic to men for 3 weeks result in modest fall in serum cholesterol, and he reported that the mechanism of the decrease in cholesterol with GA is not by adsorption of cholesterol metabolites as the fecal bile acids and neutral sterols did not increase. The findings in the present study are also consistent with those reported by Kelley and
Tsai, (1978) they noticed that Gum Arabic reduces serum cholesterol in rats, suggesting that gum interferes with dietary cholesterol absorption. In chicken, El-khier et al. (2009) reported that Gum Arabic in the basal laying hen diet significantly reduced serum cholesterol in a gradual manner.

But these results disagree with Topping et al. (1985) who showed that plasma cholesterol concentration was unaffected by feeding GA, and Annison et al. (1995) who reported that GA fed to rats replacing cellulose in purified diets supplemented with cholesterol and cholic acid, has no significant affect on plasma cholesterol when compared to levels found in rats that consumed control diet containing cellulose alone, its is also not in agreement with of Tageldin et al. (2006) who reported increase on cholesterol level in rabbits fed GA, and that GA associated with an increase total cholesterol biosynthesis.

Al-Othman et al. (1998) reported that, the soluble fraction of various dietary fiber sources, as found in oat bran and barley, seem to have the potential for lowering plasma total and LDL cholesterol levels, and Gel-forming soluble fibers, such as pectin and guar gum, have also been observed to be effective hypocholesterolemic agents, on the other hand, insoluble fibers, such as cellulose or wheat bran, have to be relatively ineffective in lowering serum cholesterol levels.

Gum Arabic (soluble dietary fiber) was observed to be effective in lowering the total plasma cholesterol level compared with insoluble fiber (cellulose) when rats were fed diets supplemented with 1% cholesterol (Al-Othman et al. 1998).

According to the above mentioned results the hypocholesterolaemic effect of GA may be due to its interference with dietary cholesterol absorption as suggested by Kelley and Tsai (1978).

4.2.2. The effect of feeding Gum Arabic on serum LDL-c
The level of Plasma LDL-c decreased significantly (P<0.05) after the administration of 10%, 20% and 20% Gum Arabic. These results were in line with the results stated by Ross et al., (1983) and Sharma (1985) who reported reduction of serum cholesterol when human subjects received GA, and that the decrease was confined to LDL and VLDL cholesterol. The results were also in agreement with that obtained by Al-Othman et al. (1998), but it disagree with Jensen et al. (1993) who reported administration of GA in usual beverages of hypercholesterolemic males daily for 4 weeks showed no change in any plasma lipid parameters.

Hwang et al. (2001), reported that feeding of 5% *Allium sativum* whole fruits to rats after high fat diet decreased LDL-c level significantly, and that was due to the fiber content of the fruit, that increases LDL-c receptor activity.

**4.2.3 The effect of feeding Gum Arabic on serum HDL-c**

In the present study addition of 10%, 20% and 30% Gum Arabic to the basal diet of induced hypercholesterolemic Wistar albino rats resulted in a significant increase of serum HDL-c. The finding in the present study is consistent with that reported by Al-Othman et al. (1998), who stated that supplementing Gum Arabic to diet with 1% cholesterol resulted in a significantly higher HDL-c content.

Hannan et al. (2003) reported that the administration of the soluble dietary fiber (SDF) fraction of *Trigonella foenum graecum* to rats orally twice a day for 28 days increased the serum HDL-c level. But the present study is not in agreement with Ross et al., (1983) and Sharma (1985) who reported no effect on HDL when human subjects received 25 g/day and 30 g/day of GA for periods of 21 and 30 days, respectively, it is also disagree with that Davidson et al. (1998) who state that soluble dietary
fiber supplement containing Gum Arabic and Pectin in apple juice did not change HDL-c in hypercholesterolemic men and women.

According to the above mentioned findings the elevated level of HDL-c is attributed to the soluble fiber nature of GA which led to decrease LDL-c and increase HDL-c production.

4.2.4 The effect of feeding Gum Arabic on serum triacylglycerol and VLDL-c

In the present study addition of 20% and 30% Gum Arabic to the basal diet of induced hypercholesterolemic Wistar albino rats resulted in a significant decrease in serum triacylglycerol level, although 10% of GA resulted in non-significant decrease. These findings were in line with the result obtained by Ross et al. (1983) and Sharma (1985). These results are also in agreement with the results obtained by Topping et al. (1985) who showed that, in rats fed GA plasma triacylglycerols were significantly lower than in controls. McNaughton (1978) observed that plasma triacylglycerol decreased as dietary fiber level increased in diet fed to laying hens.

But these results disagree with Jensen et al. (1993) who reported that consumption of GA for 4 weeks by hypercholesterolemic subjects had no significant effect on plasma lipids, and Annison et al. (1995) who reported that GA fed to rats replacing cellulose in purified diets supplemented with cholesterol and cholic acid resulted in higher triacylglycerol.
CONCLUSION

Soluble fraction of various dietary fiber sources seem to have the potential for lowering plasma total and LDL cholesterol levels, and observed to be effective hypocholesterolemic agents.

From the present results and previous findings, mixing of Gum Arabic with diet has diverse effects on atherogenic lipids among animal species including human, with most studies concluded that Gum Arabic has positive effect in lowering serum cholesterol.

Accordingly further studies are needed to specify the effective dose and the treatment period of GA that exerts protective and/or curative response to human. Further studies are also needed to target other lipid classes, and other biochemical parameters, with more focusing on how Gum Arabic exert it is mechanism in lowering or increasing certain biochemical parameters.
REFERENCES


