Effect of Prebiotic, Probiotic Bacteria and Symbiotic Diets Containing Bacillus Coagulans and Gum Arabic on Lipid Profile in Hypercholesterolemic Rats

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Abstract
Purpose: Hypercholesterolemia is a major problem for many societies as well as for health professionals due to the close association between cardiovascular disease and lipid abnormalities. The current study was conducted to determine the effect of prebiotic, probiotic bacteria and symbiotic diets containing Bacillus Coagulans and Gum Arabic on lipid profile in hypercholesterolemic rats.

Methodology: The spore suspension was prepared and Gum Arabic was purchased as the natural product. 35 male albino rats (170 ± 5 g) were fed a cholesterol-supplemented diet to induce hypercholesterolemia. The rats were divided into 5 groups (n = 7). The group 1: basal diet (negative control), and the other four groups were given hypercholesterolemia diet supplemented with 25% lard and 2% cholesterol for six weeks to induce hypercholesterolemia in rats. They were divided as follows: Group 2: (positive control), Group 3: fed a supplemented basal diet with 5% Gum Arabic (prebiotic), Group 4: fed a supplemented basal diet with coagulase spores, and group 5: fed a basal diet supplemented with 5% Gum Arabic and spores per day of B. coagulans (symbiotic).

Results: The findings showed a significant increase in the levels of total cholesterol and triacylglycerol (mg/dL) (P<0.05) in (positive control) fed the basic diet (B.D), compared to (negative control). Unique Contribution to Theory, Practice and Policy: The Anti-Inflammatory Theory and The theory of using prebiotics to lower cholesterol and reduce its absorption. This study indicates that the theory used in the study has been validated. The best results that showed significant differences with the negative control group were achieved in the hypercholesterolemia groups treated with Gum Arabic (GA5%), where the highest mean value was recorded (28.54 ± 3.71 mg/dL). As for the groups that took B. coagulans and GA 5% + B. coagulans, a decrease in LDL-c, (VLDL-c), AST and AST levels, and the best result recorded was in the group GA 5% + B.coagulans. Histological examinations also showed that the liver sections in the fifth group showed a normal structure, except for slight degeneration of the liver cells. The best result was in the group of GA 5%+ B. coagulans treatment group compared to the other groups.

Keywords: Bacillus Coagulans Bacteria - Gum Arabic- Hypercholesteremic Rats

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INTRODUCTION

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It has been reported by Rerkasem et al. (2008) as one of the most important risk factors in the development and progression of atherosclerosis that lead to cardiovascular diseases (CVDs). Hypercholesterolemia poses a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities (Matos et al. 2005; Ramachandran et al., 2003). Clinical trials have demonstrated that intensive reduction of plasma LDL-C levels could reverse atherosclerosis and amounts of saturated fats and cholesterol are believed to be directly related to hypercholesterolemia and susceptibility to atherosclerosis (Asashina et al., 2005). Furthermore, dietary trials have revealed that the concentration of serum cholesterol is affected by both the content and source of proteins (Serounge et al., 1995; Forsythe, 1995). Lipid structure, composition, configuration, in addition to excessive fat and cholesterol consumption are also believed to affect the lipid profile in the plasma (Zulet et al., 1999). Hypercholesterolemic animals are useful models for studies on cholesterol homeostasis, and drug trials to better understand the relationship between disorders in cholesterol metabolism, atherogenesis as well as possible treatments for the reduction of circulatory cholesterol levels (Pellizon, 2008; Jang and Wang, 2009).

The WHO has predicted that by 2030, cardiovascular diseases will remain the leading causes of death, affecting approximately 23.6 million people around the World (WHO; 2010). It was reported that hypercholesterolemia contributed to 45% of heart attacks in Western Europe and 35% of heart attacks in Central and Eastern Europe from 1999 to 2003 (Yusuf et al., 2004). The risk of heart attack is three times higher in those with hypercholesterolemia, compared to those who have normal blood lipid profiles. The WHO delineated that unhealthy diets such as those high in fat, salt and free sugar, and low in complex carbohydrates, fruits and vegetables, lead to increased risk of cardiovascular diseases (WHO; 2003).

People affected with hypercholesterolemia may avert the use of cholesterol-lowering drugs by practising dietary control or supplementation of probiotics and/or prebiotics. Probiotics are defined as ‘living microbial supplements that beneficially affect the host animals by improving its intestinal microbial balances’ (FAO and WHO; 2001). Prebiotics are ‘indigestible fermented food substrates that selectively stimulate the growth, composition and activity of microflora in gastrointestinal tract and thus improve hosts’ health and well-being’ (Roberfroid; 2007). When probiotics and prebiotics are used in combination, they are known as ‘synbiotics’. The use of probiotics and prebiotics has only acquired scientific recognition in recent years although their applications as functional foods have been well-established throughout generations. In the interest of their promising effects on health and wellbeing, probiotics and prebiotics have become increasingly recognized as supplements for human consumption. In addition to improving gut health, probiotics have also been documented to exert other health-promoting effects such as strengthening of the immune system (Galdeano et al., 2007), antihypertensive effects (Yeo and Liong; 2010), prevention of cancer (Hirayama and Rafter; 2000), antioxidative effects, reduction of dermatitis symptoms (Weston et al., 2005), facilitation of mineral absorption (Scholz et al., 2007), amelioration of arthritis (Baharav et al., 2004), reduction of allergic symptoms (Ouwehand; 2007) and improvement of vulvovaginal candidiasis in women (Falagaset al., 2006). Probiotics have also been studied for their cholesterol-lowering effects (Pereira and Gibson; 2002). Although many controversial studies have demonstrated the cholesterol-lowering effects of probiotics, prebiotics and synbiotics in
animals and humans, there is also limited information on cholesterol-lowering effects of *B. coagulans* spores.

Gum Arabic or GA (Acacia Senegal) is a natural supplement obtained from the exudate of tubers or tears of Acacia species (Abdelkareem et al., 2016). The U.S. Food and Drug Administration have recognized GA as one of the safest dietary fibers for humans (Babiker et al., 2012). GA is considered to be a natural prebiotic as a result of containing indigestible fibers such as polysaccharides, neutral sugars (rhamnose, arabinose, and galactose), and glucuronic acid that selectively stimulate the growth and activity of beneficial bacteria by fermentation of the caecum besides containing organic matter, amino acids, and minerals (Khalid et al., 2014). Previous research has shown that GA at levels up to 6% as a broiler feed ingredient effectively acts as a prebiotic in improving growth performance and gut health (Abdulla et al., 2015). Dietary supplementation with GA (0.1 to 2%) improved biochemical serum indicators in rabbits (El-Rat et al., 2019) and rats (Longdet et al., 2018). Sharma et al. (2014) reported that GA at 0.25 to 2% has antimicrobial activity. However, increasing beneficial bacteria and eliminating pathogens by GA leads to a healthy gut (Lockyer and Stanner, 2019). A study by Teng and Kim (2018) indicated that GA as a natural prebiotic lead to improved gut health (Jha and Kim, 2021).

This study was conducted to evaluate the in vivo effects of *B. coagulans* and arabic gum, separately and in combination on lipid profile using a rats.

Therefore, the aim of this study was conducted to determine the effect of prebiotic, probiotic bacteria and symbiotic diets containing Bacillus Coagulans and Gum Arabic on lipid profile in hypercholesterolemic rats.

**Theoretical Framework**

The study follows the application of the following theories:

**The Anti-Inflammatory Theory**

This theory states that foods with anti-inflammatory properties can reduce the inflammation and oxidative stress associated with hypercholestremic and its complications. This theory can be used to compare the effects of different prebiotic, probiotic bacteria and symbiotic diets containing Bacillus Coagulans and Gum Arabic on lipid profile in hypercholesterolemic rats on the inflammatory markers and oxidative (Mohammed et al., 2021).

The theory of using prebiotics to lower cholesterol and reduce its absorption

There are two suggested mechanisms that have been attributed to hypocholesrolemic effect of prebiotics such as Gum Arabic; decreased cholesterol absorption by enhancing cholesterol excretion via feces and the selective fermentation by intestinal bacterial microflora causing production of short-chain fatty acids (Abdulla et al., 2015).

**METHODOLOGY**

**Preparation of Spore Suspension of Probiotic Bacteria**

Freeze-dried probiotic *B. coagulans* was purchased from the National Research Center, Cairo, Egypt. It was grown aerobically on Yeast Nutrient Salt Medium (NYSM) agar [10] at 37°C for 24 min. H. A single colony from a NYSM plate was inoculated into 500 ml of NYSM broth. They were incubated at 37°C with shaking at 250 rpm for 48 h. The bacterial suspension was pelleted three times. Times by centrifugation at 3,000 × g for 20 min, and washed with sterile normal saline. Last the pellet was re-suspended in 100 ml of sterile normal saline. To determine
the spore per ml suspension, the solution was heated at 80 °C for 15 min to kill the plant cells before appropriate serial dilution and plating on NYSM agar. Finally, the spore suspension was prepared at a concentration of 1 x 10⁹ spores /ml in sterile saline solution and kept in the refrigerator until use.

**Chemical Composition Analysis**

Gum Arabic or GA (Acacia Senegal) was purchased as a natural product from the Institute of Food Technology, Giza, Egypt. It was ground to a fine powder in the laboratory, and nutritional composition analysis of the GA powder samples was performed according to the method described by (Saleh et al., 2021), (Gashua et al., 2015) and (El-Sayed et al., 2020).

**Biological study**

**Preparation of Diets**

The standard diet was formulated according to AIN-93 (Reeves et al., 1993). The salt mixture was prepared according to Viviani et al., (1964). The vitamin mixture was prepared according to A.O.A.C. (1975).

**Experimental Design**

Thirty-five male albino rats (170 ± 5 g) were purchased from the National Research Center, Cairo, Egypt. After the adaptation period (2 weeks), diets supplemented with cholesterol to evoke hypercholesterolemia. rats were distributed into four food groups (n = 7/group) and housed in groups in a temperature-controlled environment (24 ± 2 °C) with 55 ± 10% relative humidity and controlled lighting.

The rats were divided into 5 groups, the first group 1: a basic diet (negative control), the other groups was given a hypercholesterolemic diet (HPC) enriched with 25% lard and 2% cholesterol for six weeks to induce of hypercholesteremia in rats. and were divided as follows: group 2: the positive control group, group 3: fed on a basal supplemented diet with 5% gum Arabic (prebiotic), group 4: fed on a basal supplemented diet with 109 spores/day B. coagulans (feed 1 ml of spore suspension prepared using a blunt needle) (probiotic), and group 5: fed on a basal supplemented diet containing 5% with weight of gum arabic and 109 spores per day for B. coagulans (Synbiotic). Food and distilled water were provided in sufficient quantities to accommodate the experimental conditions. The control and prebiotic groups were fed 1 ml of sterile normal saline once daily.

**Biochemical Analysis of Serum**

**Blood Collection and Serum Separation**

Blood Sample will be withdrawn from each animal, on fasting state collected blood samples to determination the biochemical analysis as well as will be centrifuged to obtain the serum for biochemical analysis in National Research center, Dokki, Egypt.

**Lipid Profile Determination**

**Determination of Blood Cholesterol**

1. Triglyceride (TG) will determine in the Serum TG will calculate according Total Cholesterol (TC) Determination: Cholesterol was determined by the enzymatic colorimetric method described by Allain et al. (1974).
2. High Density Lipoprotein (HDL) will determine in the serum according to the method described by (Lopes et al., 1977)
3. Low Density Lipoprotein (LDL) will determine in the Serum LDL will calculate according to (Fiedwald et al., 1972)

4. Very Low Density Lipoprotein (VLDL) will determine in the Serum VLDL will calculate according to (Fiedwald et al., 1972)

**Liver Function**

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) will determine in the serum according to the method describe by (Reitman and Frankel, 1957).

**Histopathological Examination**

Histopathological examination of liver tissues will be performed at Pathology Dept., Faculty of Veterinary Medicine, Cairo University., according to Bancroft et al. (1996) to detect any changes.

**Statistical Analysis**

The obtained result will be presented as mean ± SD. Data will be subjected to one way analysis of variance (ANOVA) using the SPSS statistic computer program. The mean difference was significant at the (p<0.05) level according to (SPSS; 2007)

**RESULTS**

The data in Table (1) shows the chemical composition of Gum Arabic/100g. Estimation of Macronutrient contents and proximate principles of Acacia Senegal. Nutrients (per 100g) Moisture 14.5 g, Energy 347.33Kcal, Carbohydrate 81.87g. Protein 0.51 g, Lipids 0.15 g, Crude Fiber 0.17 g and ash was 2.8 g.

<table>
<thead>
<tr>
<th>Nutrients (%)</th>
<th>Amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (g)</td>
<td>14.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.51</td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>347.33</td>
</tr>
<tr>
<td>Lipids (g)</td>
<td>0.15</td>
</tr>
<tr>
<td>Total carbohydrates (g)</td>
<td>81.87</td>
</tr>
<tr>
<td>Crude Fiber (g)</td>
<td>0.17</td>
</tr>
<tr>
<td>Ashe (g)</td>
<td>2.8</td>
</tr>
</tbody>
</table>

**Effect of the Treatments on Lipid Profile of Hypercholestremic Rats**

**Serum Total Cholesterol and Triacylglycerol**

Table (2) illustrates the effect of treatments from Gum Arabic (GA5%), B. coagulans and GA 5%-+B. coagulans on the serum cholesterol and triacylglycerol levels of the hypercholestremic rats.

Data in this table showed that, total cholesterol and triacylglycerol levels (mg/dl) were increased significantly (P<0.05) in un-treated hypercholestremic rats (positive control) fed on basal diet (B.D.), compared to healthy rats (negative control) which also fed on B.D. (77.05 ± 2.64 and 156.17±1.36) vs. (59.00 ± 1.00 and 11.14 ± 0.50). The mean values of serum cholesterol and triacylglycerol in hypercholestremic rat groups which fed on basal diet with supplemented diet treatments decreased gradually with intake. Results of the effect of treatments from Gum Arabic (GA5%), B. coagulans and GA 5%-+B. coagulans on the mean
values of serum cholesterol of hypercholesteremic rats showed significant improvement in the total cholesterol level at compared to positive control group. While, there was a gradual significant decrease (P<0.05) recorded in the values of triacylglycerol in all treated hypercholesteremic groups, as compared to un-treated hypercholesteremic group (positive control). The best results that showed non-significant difference with negative control group were achieved in the hypercholesteremc groups treated with Gum Arabic (GA5%), B. coagulans and GA 5%+B. coagulans groups.

**Serum Lipoprotein-Cholesterol**

Results in table (2) exhibits the effects of Gum Arabic (GA5%), B. coagulans and GA 5%+B. coagulans on cholesterol functions i.e., high density lipoprotein (HDL-c), low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c).

**High Density Lipoprotein-Cholesterol (HDL-c)**

Result in Table (2) showed that, there was significant difference in serum HDL-c of positive control (un-treated hypercholesteremic rats) that fed on basal diet, as compared to negative control (normal rats) which also fed on B.D. High-density lipoprotein cholesterol (HDL-c) among all treated groups showed significant differences, moreover there were differences between the all treated groups and the control groups. From this table, it could be noticed that, hypercholesteremc rat groups treated with GA 5%-B. coagulans recorded the highest mean value (28.54 ± 3.71mg/dl) which was near to the negative control (36.33 ±5.00 mg/dl).

**Low Density Lipoprotein-Cholesterol (LDL-c)**

Data illustrated that, there was significant difference in serum low density lipoprotein-cholesterol (LDL-c) of (+) control and (−) control group. Results also showed that, significant differences were observed in the levels of serum LDL-c among all groups fed on basal diet with Gum Arabic (GA5%), B. coagulans and GA 5%+B. coagulans , and mean values of serum LDL-c of treated hypercholesteremic rat groups were decreased gradually with intake the treatments. Moreover, there were differences between the all treated groups and the control groups.

From the results presented in table (2), and the above mentioned data it could be concluded that, treating hypercholesteremc rat group with the highest two treatments of B. coagulans and GA 5%+B. coagulans achieved the best reduction in serum LDL-c.

**Very Low Density Lipoprotein-Cholesterol (VLDL-c)**

Table (2) shows the mean values of Very low-density lipoprotein-cholesterol (VLDL-c) in serum of the hypercholesteremic rat groups which fed on B.D. with Gum Arabic (GA5%), B. coagulans and GA 5%+B. coagulans. Data in this table showed that, the mean value of VLDL-c increased significantly (P<0.05) in positive control group (un-treated hypercholesteremic rats) which fed on B.D. (29.81 ± 0.28), as compared to negative control group (healthy rats) (3.07 ± 0.10) which also fed on B.D. Results revealed that, all hypercholesteremic rats treated with Gum Arabic (GA5%), B. coagulans and GA 5%+B. coagulans led to a significant reduction in serum VLDL-c, when compared with positive control. The obtained results showed that, significant changes were observed in the levels of serum VLDL-c in Gum Arabic (GA5%), B. coagulans and GA 5%+B. coagulans groups, as compared to negative control group. It could be concluded from the above mentioned results that, using B. coagulans and GA 5%+B. coagulans tend to bring the parameter (VLDL-c) toward the normal value, especially in treating with GA 5%+B. coagulans that given the best results.
From the above mentioned data it could be concluded that, the greatest improvement in the values of total cholesterol, triacylglycerol, DL-c and VLDL-c were in hypercholestremic rats which fed on B. coagulans and GA 5%+B. coagulans.

**Table 2: Effect of Gum Arabic (GA5%), B. Coagulans and GA 5%+B. Coagulans on Serum Lipid Profile (Mg/Dl) in Hypercholestremic Rats**

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Total cholesterol Mean ± SD</th>
<th>Triacylglycerol Mean ± SD</th>
<th>HDL-c Mean ± SD</th>
<th>LDL-c Mean ± SD</th>
<th>VLDL-c Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (-)</td>
<td>59.00 ± 1.00B</td>
<td>11.14 ± 0.50AB</td>
<td>36.33 ±5.00A</td>
<td>7.22 ± 1.00C</td>
<td>3.07 ± 0.10C</td>
</tr>
<tr>
<td>Positive control (+)</td>
<td>77.05 ± 2.64A</td>
<td>156.17 ± 1.36A</td>
<td>22.50 ± 0.50D</td>
<td>34.55 ± 0.61A</td>
<td>29.81 ± 0.28A</td>
</tr>
<tr>
<td>GA 5%</td>
<td>65.57 ± 18.58AB</td>
<td>121.90 ± 1.00AB</td>
<td>25.50 ± 0.51D</td>
<td>30.56 ± 7.03B</td>
<td>24.71± 1.00AB</td>
</tr>
<tr>
<td>B. coagulans</td>
<td>64.33 ± 1.00B</td>
<td>116.20 ±79.42AB</td>
<td>27.23 ± 1.00PHC</td>
<td>19.05 ± 5.07C</td>
<td>22.90±15.73AB</td>
</tr>
<tr>
<td>GA 5%+B. coagulans</td>
<td>62.66 ± 5.81B</td>
<td>71.65 ± 4.78CB</td>
<td>28.54 ± 3.71B</td>
<td>11.63 ± 0.45S</td>
<td>13.15± 0.93CB</td>
</tr>
<tr>
<td>L.S.D</td>
<td>15.18</td>
<td>60.33</td>
<td>4.96</td>
<td>8.65</td>
<td>13.01</td>
</tr>
</tbody>
</table>

* Non significant differences between the values had the same letter.
* LSD: Least Significant Difference at level 0.05

Effect of Gum Arabic (GA5%), B.coagulans and GA 5%+B.coagulans on liver function of hypercholestremic rats. Results of aspartate amino transferase (AST) and alanine amino transferase (ALT) are presented in table (3). Results indicated that, feeding hypercholestremic rats on basal diet (PC) resulted in significant increase in serum AST and ALT, as compared to healthy rats (NC) fed on basal diet (336 ± 6.71& 135.70 ± 1.03 vs. 91.53 ± 11.31& 51.63 ± 16.22 U/L), respectively. Data in table (3) showed that, serum AST levels revealed significant gradual decrease with intake the treatment with Gum Arabic (GA5%), B.coagulans and GA 5%+B.coagulans, and the best result was recorded at GA 5%+B.coagulans, because this group showed significant change in AST enzyme activity, compared to control negative group.

Alanine amino transferase (ALT) in all treated hypercholestremic rat groups with Gum Arabic (GA5%), B.coagulans and GA 5%+B.coagulans, induced marked reduction towards the normal value, meanwhile, the lowest level of ALT enzyme was found in group of rats that take GA 5%+B.coagulans. From the above mentioned data, it could be concluded that, treating hypercholestremic rats with GA 5%+B.coagulans significantly (P<0.05) normalized liver function at the end of the experiment.
Table (3): Effect of Gum Arabic (GA5%), B. Coagulans and GA 5%+B. Coagulans on Liver Functions in Hypercholesteremic Rats

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Liver function</th>
<th>AST (U/L) Mean ± SD</th>
<th>ALT (U/L) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (-)</td>
<td></td>
<td>91.53 ± 11.31&lt;sup&gt;C&lt;/sup&gt;</td>
<td>51.63 ± 16.22&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive control (+)</td>
<td></td>
<td>336 ± 6.71&lt;sup&gt;A&lt;/sup&gt;</td>
<td>135.70 ± 1.03&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>GA 5%</td>
<td></td>
<td>172.66 ± 6.91&lt;sup&gt;B&lt;/sup&gt;</td>
<td>113.27 ± 1.33&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>B. coagulans</td>
<td></td>
<td>168.91 ± 1.56&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>71.88 ± 5.39&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>GA 5%+B. coagulans</td>
<td></td>
<td>152.65 ± 92.29&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>58.76 ± 53.31&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>L.S.D</td>
<td></td>
<td>67.922</td>
<td>28.165</td>
</tr>
</tbody>
</table>

* Non significant differences between the values had the same letter.
* LSD: Least Significant Difference at level 0.05

Histopathology Examinations

Effects of Gum Arabic (GA5%), B. coagulans and GA 5%+B. coagulans on liver histopathology. Sections of group 1 (control negative) rats, livers showed normal hepatocytes (Photo 1). Liver sections of control positive group showed severe vacuolar degeneration of the hepatocytes with inflammatory cell infiltrations around blood vessels (Photo 2). (Photo 3) Liver sections of group 3 Gum Arabic (GA5%), rats showed apparently normal structure except for slight vacuolar degeneration of hepatocytes with pericentral inflammatory cell infiltrations (Photo 4). Liver sections of group 4 (B. coagulans) rats showed vacuolar degeneration of hepatocytes with inflammatory cell infiltrations around the blood vessels (Photo 5). The liver sections of group 5 (GA 5%+B. coagulans) rats showed normal structure except for the slight degeneration of hepatocytes.

Figure 1: Liver Section of a Rat From Control Negative Group Showed Apparently Normal Hepatocytes and Portal Vein (Arrow) (H & E x 200).
Figure 2: Liver Section of a Rat from Control Positive Group Showed Severe Vacuolar Degeneration of Hepatocytes (Arrows) With Inflammatory Cells Infiltrations around Blood Vessel (H & E x 200).

Figure 3: Liver Section of a Rat from (Gum Arabic (GA5%), Group Showed Apparently Normal Structure Except Slightly Vacuolar Degeneration of Hepatocytes (Arrow) With Mild Pericenteral Inflammatory Cells Infiltrations (Star) (H & E x 200).

Figure 4: Liver Section of a Rat from (B. Coagulans) Group Showed Vacuolar Degeneration of Hepatocytes (Arrow) With Inflammatory Cells Infiltrations around Blood Vessel (Star) (H & E x 200).
Discussion

Alasdair et al. (1984) found that gum Arabic decreased the serum cholesterol level. (Kishimoto et al. 2006) showed that a Prevotellaruminicola-like the bacterium was the predominant organism that is most likely responsible for fermentation of GA to propionate. 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. In this respect, Eyibo et al., (2018) reported that, treating Albino rats with oral dose of gum Arabic at different concentrations (200 mg/kg, 400 mg/kg and 600 mg/kg) for nine (9) weeks of ageshowed a significant decrease in total cholesterol and triglyceride, as compared to the control group. 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. Ahmed et al (2015) reported that when taking normal fed rats on diet and treated with 0.5% aqueous solution of GA for seven days, then 10% aqueous solution for an additional six weeks demonstrated a reduction in total cholesterol and LDL. Fedail et al., (2016) reported that, treatment with GA significantly (P < 0.05) decreased plasma TG, LDL-c concentrations when compared to the diabetic rat group. In contrast, the treatment of GA significantly increased plasma HDL-c concentration compared to the diabetic rat group.

According to the results of this study, Gum Arabic played an important role in changing lipid profile effectively. An obvious decrease in the serum total cholesterol and LDL-cholesterol of rats fed with Gum Arabic in syngeneic and prebiotic groups was seen in all sampling days. Gum Arabic fed rats also demonstrated higher levels of HDL-cholesterol concentration; however this value in probiotic and control fed rats showed no significant change. There are two suggested mechanisms that have been attributed to hypocholesterolemic effect of prebiotics such as Gum Arabic; decreased cholesterol absorption by enhancing cholesterol excretion via feces and the selective fermentation by intestinal bacterial microflora causing production of short-chain fatty acids (Abdulla et al., 2015). Results of this study concerning hypocholesterolemic effect of Gum Arabic are in agreement with several studies conducted in vivo trials. (Kim and Shin: 1998).

Studies on the effects of *B. coagulans* on lipid profile have been limited to those who investigated the influence of administration of *B. coagulans* capsules (each containing 360 million spores) per day in hyperlipidemic patients for three months, they reported total serum cholesterol, LDL cholesterol and total cholesterol to HDL cholesterol, and LDL-cholesterol to HDL-cholesterol ratios were reduced significantly. They also found HDL-cholesterol was
marginally increased (Mohanet al.,1990). (Panda et al.,2006) also reported this probiotic is able to reduce total cholesterol, VLDL and triglycerides in broiler chickens. According to the results of this study, B. coagulans contributed to lipid profile changes. Although many studies have demonstrated cholesterol lowering effects of probiotics in both animals and humans, debatable results have also been reported concerning inability of a particular strain of probiotic bacteria to improve lipid profile. A study by Hatakka et al.,(2008) reported that administration of L. rhamnosus LC705 (1010 CFU/g per capsule; two capsules daily) did not influence blood lipid profiles in thirty-eight men after a 4-week treatment. Simons et al.,(2006) , Lewis and Burmeister : (2006) also refuted effect of L. fermentum, (2 109 CFU per capsule; four capsules daily) and L. acidophilus on human lipid profiles, respectively. Several possible mechanisms for hypocholesterolemic effects of probiotics are: incorporation of cholesterol into the cellular membranes by growing cells and deconjugation of bile via bile salt hydrolase. Once deconjugated, bile acids are less soluble and absorbed by the intestines, leading to increasing their rates of excretion in the feces. Cholesterol is used to synthesize new bile acids in a homeostatic response, resulting in lowering of serum cholesterol (Pereira and Gibson2002) . Ooi and Liong :(2010) in a review article attributed these controversial findings to various factors such as different strains of probiotics, administration dosage, analytical accuracy of lipid Accepted Paper analyses, duration of treatment period, clinical characteristic of subjects, inadequate sample sizes, and lack of suitable controls or placebo groups. Considering the mentioned reasons hypothesized by Ooi and Liong :(2010) , normolipidemic condition of used rat model in the current study may be a reason for failure of these probiotic bacteria in changing lipid profile. In addition to all of the above, the feeding period of 30 days may not be sufficient to observe a significant change in lipid profile. However, the strains of bacteria used as a cholesterol lowering agent must be bile tolerant, have the ability to deconjugate bile acids, and bind cholesterol. In addition, the ability of particular strain of probiotic bacteria to attach permanently to the gut wall and hence continuous supply might be necessary to exert the effects (Kumar et al .,2012) . The results of our previous study indicated that these bacteria are not able to colonize the intestine and are quickly eliminated in feces. As such, daily consumption of probiotic products is necessary for any long-term effect on metabolism. (Babiker et al., 2017) reported that, daily Gum Arabic dose for twelve weeks significantly affected the liver antioxidant activity of Sprague-Dawley rats.Also (Hamid et al., 2021) reported that dietary administration of GA has a beneficial result on the hepatic apoptosis, oxidative stress, and inflammatory response in experimentally-induced hepatotoxicity in rats. GA is reported to has robust anti-oxidant effects; it has been able to ameliorate the cardiac, renal and hepatic lipid peroxidation and toxicity, besides its anti-inflammatory, antimicrobial, antidiarrheal, anti-obesity and antihypertensive effects (Ali et al., 2009 and Elshama, 2018). Ahmed et al., (2015) reported that, Gum Arabic (GA) has strong antioxidant properties; therefore, it could beone of the mechanisms of hepatoprotective. Al-Kenanny et al., (2012) noted that elevated in ALT and AST mean a marked for liver injury. Mice treated with AG showed amelioration in enzymatic level (ALT +AST ) but not reach to normal level as in control group, these results indicated that AG have ability as a protective factor to decrease liver damage. ALT and AST are two enzymes that are the most accurate indicators of necrosis or hepatocellular damage. Their levels in a number of hepatic diseases are show elevated .of the two enzymes, ALT is believed to be more diagnosis to hepatic injurys because it is primarily found in cytosole in the liver and elsewhere in low concentrations (Giboney: 2005) , High concentrations for these enzymes are outcomes of destruction of hepatocytes and increased
cellular permeability (Sivakrishnan, and Kottaimuthu : 2014) Observed reduction in activity of all enzymes may indicate normal metabolism as well as improving liver function. In this line, liver enzyme activities are indicators of hepato-toxicity and liver function (Azab et al., 2013). The present study, shows reductions in albumin concentration may be attributed to impaired hepatocyte synthesis, as a result of oxidative stress and hepatitis, along with increased protein breakdown and kidney loss (Saad et al., 2018). In this study, it's reported that animals which sever from nephrotoxicity which is caused by gentamicin when given Gum Arabic for purpose of treatment for 4 weeks, Table (4) notes significant decrease in serum AST, ALT, ALP, of male rat and this agree with (Babiker et al., 2018) who reported that the gum Arabic has a protective effect on the liver through reducing the blood level of liver enzymes; aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) and through decreasing the oxidative stress.

Effect of Gum Arabic on histological assessment of hepatic necrosis and inflammation Histopathological examination of the liver revealed severe cellular necrosis, with extensive inflammatory cells infiltration and hepatocytes vacuolization or fatty degeneration in the rats treated, this result accepted with (Mohammed Hamid et al., 2021)

Present study, revealed that the Gum Arabic significantly decreased the activity of liver enzymes and may play a protective role against liver dysfunctions in rats (Khojah: 2017), In mice and rats, GA has been act as protecting agent against hepatic and renal toxicities (Nemmar et al., 2019). The protective effects of Arabic gum are likely to be related to its anti-oxidant and anti-inflammatory and cytoprotective properties (Helal et al., 2011).

Conclusion

Feeding rats on supplemented diet containing gum Arabic spore suspension of probiotic bacteria (B. coagulans) have the ability to reduce high levels of cholesterol and triglyceride, improved also the lipid profile and the liver enzymes in rats suffering from hypercholesteremic.
REFERENCES


