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Effects of Probiotic Bacteria from Yogurt on Enzyme and Serum Cholesterol Levels of Experimentally Induced Hyperlipidemic Wistar Albino Rats

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Abstract

The aim of the present study was to evaluate and confirm in vivo the cholesterol lowering effect of three commercialized yogurts sold in Buea, a town located in the South West Region of Cameroon. These yogurts are normally known to contain *Lactobacillus plantarum* and *Streptococcus thermophilus* bacteria. Following diet induced hyperlipidemia, animal model (wistar albino rats) was used to confirm the persistence of their probiotic cholesterol lowering effect after processing. 42 rats were divided into 7 groups: the positive control group (received hyperlipidemia diet (cholesterol /lard rich diet) + oral gavage of deionized water), the negative control group (fed with basal diet +oral gavage of deionized water), three test groups (fed with hyperlipidemia diet and 3 different types of commercialized yogurt administered orally by gavage respectively), ferment free group (received basal diet + oral administration of yogurt constituents free of ferments (culture)) and hyperlipidemia ferment free group (received hyperlipidemia diet + oral yogurt constituents free of ferments). The oral gavage was daily and the dose volume administered was 1.0 ml/kg body weight. The rats were acclimatized for one week followed by the experimental phase proper for 4 weeks. Daily food consumed and daily weight gains were recorded. At the end of the fourth week, rats were dissected and blood collected for biochemical analysis (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, albumin and transaminases activities (ASAT and ALAT)). Organs of the rats (heart, liver, spleen and kidney) were also removed and weighed. Rat growth response revealed no significant change in weight of rats as well as in weights of different organs among test groups meanwhile the weight of these organs and the weight gained by hyperlipidemic group were relatively high compared to test and negative control groups. A slight elevation of ASAT and ALT activities was observed only in Hyperlipidemia rats while the test group did not show any significant increase of these serum enzymes. Results also show that the yogurt administration to hyperlipidemic / hypercholesterolemic rats induced a significant decrease in the total serum cholesterol, VLDL-cholesterol, albumin, LDL-cholesterol and triglycerides, and a significant increase of HDL-cholesterol concentration in sera of test groups. In conclusion, our data support the consumption of potential probiotic yogurt to decrease serum cholesterol and also confirm the persistence of probiotic effects after these yogurts processing techniques.

Keywords

Cholesterol, Hyperlipidemia, Bacteria, *Lactobacillus plantarum*, *Streptococcus thermophiles*, Yogurt, Probiotic

1. Introduction

Elevated serum cholesterol level is widely recognized as a contributory risk factor for the development of cardiovascular diseases (CVD) such as atherosclerosis, coronary heart diseases, and hypertension. The World Health Organization (WHO) has predicted that by 2030, CVD will remain the leading cause of death and will affect approximately 23.6 million people globally [1]. It has been reported that even a 1% reduction in serum cholesterol could reduce the risk of coronary heart disease by 2 - 3% [2]. One of the major risk factors for CVD is hypercholesterolemia [3]. Hypercholesterolemia contributed to 45% of heart attacks in Western Europe and 35% of heart attacks in Central and Eastern Europe from 1999 to 2003 [4], [5]. The risk of heart attack is three times higher in those with hypercholesterolemia, compared to those who have normal blood lipid profiles. WHO delineated that unhealthy diets such as those high in fat, salt, and free sugar and low in complex carbohydrates lead to an increased risk of cardiovascular diseases [6].

The use of animal and human models to evaluate the effects of probiotics on serum cholesterol levels has been emphasized over the years. Human studies have shown promising evidence that well-established probiotics possess hypocholesterolemic effects, while new strains of probiotics or new type of prebiotics have been evaluated in animal models for their potential hypocholesterolemic effects. *Lactobacilli* are gram-positive bacteria able to function in the presence or absence of oxygen and are an important part of the Lactoacid bacteria. They are called by this name because the majority of them are able to change lactose and other simple carbohydrate to lactic acid. *Lactobacilli* normally live in the human intestine and vagina andare considered as a major species of the gut flora [7].

Nowadays numerous drugs that lower serum cholesterol have been developed to treat hypercholesterolemia patients. Although drug therapies effectively decrease cholesterol levels, they are expensive and can have severe side effects [8]. The undesirable side effects of these compounds have raised concerns about their long term therapeutic use. *It* is a gram-positive bacterium that is able to function anaerobically and aerobically. It is a catalase-negative, homofermentative, and non-motile bacteria. It is also considered a member of lactic acid bacteria family.

It can be found in milk and milk-based products and it is a one of the main strains needed for production of yoghurt. Another probiotic strain is *Streptococcus salivarus* subspecies of *thermophilus* type 1131.

Yoghurt is a food produced by bacteria fermentation of milk and contains lactose. The bacteria involved in the fermentation of milk are usually *Lactobacillus bulgaricus* or *Streptococcus thermophiles* which are probiotic. These probiotic organisms provide their benefit by adjusting the microflora in the intestines or by acting directly on body functions such as digestion and immune function. However, during processing techniques and treatments used to manufacture yogurts can affect their activities or destroy them. It is therefore useful to test the probiotic effect on the final food product to avoid administrating a life bacteria strain directly. The aim of this study is to determine the effect of three varieties of yogurts on the cholesterol level in hyperlipidemic rats and to confirm that their probiotic activities remain after processing marketing.

2. Materials and Methods

2.1. Study Area

The research was carried out in the life science laboratory of the University of Buea,. Buea is a town situated in Fako Division, South West region of Cameroon. Its geographical coordinates are 4º 9´ 34” North, 9º 14´ 12” East and its origin name is Buea.

2.2. Sample Collection

The yogurts for this study were commercial yogurts purchased from a super market in Molyko Fako division of the South West Region of Cameroon. They were designated “C” (sweetened yoghurt containing sugar, pasteurize milk and lactic cultures (*L. bulgaricus, S. thermophilus* and *Bifidobacteria;* “D” (sugar-free: fat free yoghurt containing water, skimmed milk and lactic acid bacteria cultures (*L. bulgaricus, S. thermophilus* and *Bifidobacteria* and no sweetener) and yogurt E (fat free yoghurt containing water, skimmed milk, sugar and lactic cultures (probiotic strain *Lb. Acidophilus* P106, *Lb. Acidophilus* P110. The yogurt were then taken to the life science laboratory of the University of Buea and stored at -20°C for future use.

2.3. Animal Conditions and Feeding

The 42 wistar albino rats (60 – 90 g) of about 21 days old obtained from the animal house of the Department of Zoology, University of Buea were separated into 7 groups (3 males, 3 females) of 6 rats each groups and housed under standard conditions with a 12 h light and 12 h dark cycle Temperature was maintained at 23 ± 2°C and relative humidity at approximately 50%. Acclimatization was for 7 days prior to use. The animals were housed in cages made from bowls containing saw dust covered with grills which were cleaned daily and new saw dust put in daily. The rats were given standard diet (commercial pellet formula adapted from Malathi et al., formula [9] with slight modifications) and water *ad libidum* throughout the study. Health status of treated rats were monitored daily throughout the experimental period. Rat body weight and feed consumption were recorded daily. Ethical guidelines were maintained in animal handling during the study and permission was obtained from the concerned Department.

2.4. Experimental Design

The 42 rats were divided into 7 groups (Table 1): the negative control group (fed with basal diet + oral gavage of deionized water) termed A, the positive control group (received hyperlipidemia diet (about 85% basal diet, 1% cholesterol, 10% lard (pig fat), 0.5% cholate, W/W + oral gavage of deionized water) termed B, three test groups (fed with hyperlipidemia diet and 3 different commercialized yogurt (as described in sample collection) administered orally by gavage) termed C, D and E respectively, ferment free group termed F (received basal diet and oral administration of yogurt constituents free of ferments) and hyperlipidemic ferment free group (received hyperlipidemia diet + oral gavage of yogurt constituents free of ferments termed G. The dose volume of oral gavage administered daily was 1.0ml/kg body weight and wasadjusted daily for recorded body weight changes during the treatment period. At the end of the experiment, the rats were fasted for 12 hours before blood collection. The composition of the various diets is shown in (Table 1).

2.5. Rat Blood Collection and Biochemical Assay

After dosing (30 days), the rats were allowed to fast overnight (12 hours), on the 31st day, anaesthetized using chloroform and sacrificed. Blood was collected by cardiac puncture into eppendoff tubes and the whole blood was centrifuged for 10 minutes at 4000 rpm to obtained serum. The sera were kept at -20 ˚C for the analysis of the lipid profile.

Serum lipid profile was determined with the use commercial kits (CHRONOLAB SYSTEMS, SL, Spain). Total cholesterol (TC) was analyzed enzymatically with CHOD/PAP Method [10-12] and HDLc was estimated with precipitation using commercial kits [13-14]. Triglyceride (TG) was determined enzymatically also by using a commercially available (CHRONOLAB SYSTEMS, SL, Spain). Serum LDLc was determined according to the Friedewald formula with use of HDLc and total cholesterol values [15]. VLDL-cholesterol (VLDL-c) was calculated by dividing triglyceride concentration by five. HDL/LDL ratio was calculated. Albumin, serum activities of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were measured using a commercially available kit via the methods described respectively by Tietz, Doumas et al., and Murray-Kaplan et al [16-18]. An ELISA plate reader (Labsystems, MultiskanEX, Finland) was used to read the absorbance. All the kits used were manufactured by CHRONOLAB SYSTEMS in Barcelona, Spain and were used according to the manufacturer’s instructions.

Table 1. *Diets composition and design.*

Ingredients	Rats groups and their respective diets						
	A	B	C	D	E	F	G
	Basal	**Hyperlipidemic**	**Hyperlipidemic + yoghurt C**	**Hyperlipidemic + yogurt D**	**Hyperlipidemic + yogurt E**	**Ferment free**	**Hyperlipidemic Ferment free**
Soy meal	20	20	20	20	20	20	20
Corn Starch	60	56	56	56	56	60	60
Sugar	10	3	3	3	3	10	3
soybean oil	5	5	5	5	5	5	5
Choline and Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Minerals	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamins	1	1	1	1	1	1	1
Cholesterol	0	1	1	1	1	0	1
Lard	0	10	10	10	10	0	10
Total:	100	100	100	100	100	100	100
Gavage substance	Distilled H_2O	Distilled H_2O	Yogurt C	Yogurt D	Yogurt E	Yogurts constituents	Yogurts constituents

2.6. Statistical Analysis

Data obtained were analyzed with the aid of Microsoft[R] Excel 2010 software for windows. Data are presented as the mean ± standard deviation and p value < 0.05 was considered significant. Comparisons were made by use of the student's T-test.

3. Result and Discussion

3.1. Weekly Feed Consumption

The results of the weekly food consumption are presented in Table 2. This table shows that the amount of food consumed during the first week in all groups of rats did not vary significantly (P> 0.05) and ranged between 24g and 30g. In the second week of experimentation the quantities consumed were significantly elevated (P<0.05) compared to the first week though non-significant between different groups. By the fourth week, food consumption was significantly high (P<0.05) compared. to previous weeks. This was probably due to the growth of the animals. Animal growth varies proportionately with stomach volume as the needs increase. According to Wang *et al*. [19] the need of the body increases with age and size. Animals do not have a particular preference for one type of food that is why consumption has remained insignificant in the same week between the different groups since the gavage substance was in minute quantity (1ml/kg body weight daily). This result is contrary to the one suggested by Trapp *et al.* in a similar study which investigated the effects of probiotic yogurt on lipid profile in the young and elderly [20]. They found that the group fed on yoghurt consumed more food statistically. In our experiment rats were gavaged with few milliliters of yogurt.

Table 2. *Weekly feed consumption of treated rats.*

Weeks	Types of diets weekly feed consumption (g) for the different groups of rats						
	A	B	C	D	E	F	G
	Basal diet	Hyperlipidemic diet	Hyperlipidemic +yogurt C	Hyperlipidemic +yogurt D	Hyperlipidemic + yogurt E	Ferment free group	Hyperlipidemic Ferment free
1	28.00±0.66^{a}	29.54±0.06^{a}	28.00±4.20^{a}	24.14±0.91^{a}	25.26±0.50^{a}	30.45±2.30^{a}	29.23±2.12^{a}
2	34.74±2.70^{b}	37.90±0.03^{b}	39.30±3.20^{b}	35.35±0.71 ^{b}	36.97±0.30^{b}	38.04±4.34^{b}	36.77±2.00^{b}
3	42.74±3.36^{c}	47.44±2.00^{c}	47.67±2.67^{c}	43.24±0.81^{c}	45.86±1.10^{c}	44.94±1.76^{c}	45.94±3.09^{c}
4	55.10±0.62^{d}	58.34±0.56^{d}	59.67±1.45^{d}	57.38±4.60^{d}	56.95±0.03^{d}	56.85±0.66^{d}	60.33±5.83^{d}

Values with different superscript in a column or line are significantly different (P < 0.05).

3.2. Body Weight Gain

Table 3 shows the weekly weight gain. The table revealed that the highest increases were recorded in the groups of rats fed with hyperlipidemia or hypercholesterolemia diets. However these increases were not significant (P> 0.05.) between test groups. The B and G groups that received hyperlipidemic diets recorded the highest body weights. This could be due to the consumption of yoghurt that limited weight gain probably by preventing the absorption of cholesterol or lipids.

Table 3. *Body weight gain of rats fed with three types of yogurts.*

Weeks	Types of diets weekly weight gain (g) for the different groups of rats						
	A	B	C	D	E	F	G
	Basal diet	Hyperlipidemic diet	Hyperlipidemic +yogurt C	Hyperlipidemic + yogurt D	Hyperlipidemic + yogurt E	Ferment free group	Hyperlipidemic Ferment free
1	15.30±1.14^{a}	16.74±1.01^{ab}	15.18±2.01^{a}	17.69±0.94^{a}	18.09±0.05^{a}	14.05±3.05^{a}	19.45±1.35^{b}
2	18.31±0.01^{a}	24.07±0.01^{bc}	18.67±0.01^{a}	20.22±2.54^{a}	22.50±1.30^{c}	18.74±3.45^{a}	25.87±.0.5^{b}
3	14.91±0.01^{a}	21.68±0.01^{b}	16.41±0.01^{a}	15.06±0.67^{a}	17.93±0.40^{a}	15.75±0.56^{a}	23.56±1.23^{b}
4	16.55±-1.06^{a}	22.87±1.00^{b}	17.96±1.71^{a}	18.28±0.99^{a}	19.43±0.68^{a}	16.53±1.76^{a}	23.89±2.04^{b}

Values with different superscript on a line are significantly different (P < 0.05).

3.3. Weight of Organs of Rats After Feeding Period

Organ weights of rats at the end of the one month feeding period and sacrifice (Table 4) showed that except for liver the weight of the rest organs did not change significantly between the groups. A slight increase in liver weight was observed in animals fed with high cholesterolemic diet without yoghurt and without ferments. This confirms the fact that excess lipid can induce fatty liver or steatosis (excess fat accumulation in the liver) [21-22] but yogurt would have protective effects on livers' weight gain.

Table 4. *Weight of organs of rats after feeding period.*

Organs	Organs weight (g) of rats fed on different types of diets						
	A	B	C	D	E	F	G
	Basal diet	Hyperlipidemic diet	Hyperlipidemic +yogurt C	Hyperlipidemic +yogurt D	Hyperlipidemic + yogurt E	Ferment free group	Hyperlipidemic Ferment free
Liver	6.90±1.00^{a}	8.20±0.01^{b}	6.20±1.14^{a}	5.35±1.29^{a}	5.21±0.05^{a}	6.45±0.30^{a}	7.99±.0.25^{b}
Kidney	0.70±0.07^{a}	1.11±0.01^{a}	2.00±1.08^{a}	1.2±0.28^{a}	1.13±0.90^{a}	0.87±0.00^{a}	1.29±0.26^{a}
Spleen	0.40±0.01^{a}	0.30±0.01^{a}	0.35±0.07^{a}	0.45±0.23^{a}	0.42±0.00^{a}	0.39±0.78^{a}	0.50±0.01^{a}

Values with different superscript on a same line are significantly different (P < 0.05).

3.4. Serum Lipid Profile and Transaminase Activities of Rats

Table 5 shows the serum biochemical analysis of rats and two transaminase enzyme activities (ALT and AST). This table reveals that the consumption of yoghurt significantly reduced total cholesterol, triglyceride, VLDL and LDL cholesterol levels, while increasing HDL levels. This reduction is most felt with yoghurt D versus control. These results were similar to those reported by Abdolamir *et al.* who found out that yoghurt containing probiotics influences lipid profile parameters [23]. The increase of total cholesterol and TAG were most observed with the group that received yoghurt D. Hyperlipidemic group B and G which did not receive any yogurt registered higher amounts of cholesterol and TAG. These results prove that the declines are not due to the constituents of the yoghurt but rather to the presence of the ferment (bacterial culture). Yoghurt free ferments group diet did not significantly affect cholesterol, TAG and HDL levels

The HDL / LDL ratios were even higher with rats fed with yoghurts compared to positive and negative control (group A and B). Although an increase in the serum total cholesterol level is associated with increased risk of atherosclerosis, recent reports indicated that the HDL/LDL ratio is a stronger index of atherogenicity of lipoproteins rather than the lipid profile of the individual lipoprotein fraction i.e. the higher the ratio the less atherogenic the lipoprotein profile is thought to be [24]. Yoghurt constituents alone, without bacterial strain, are insufficient to reduce the rate of total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol. The hyperlipidemic diet used here for positive control group was able to induce hypercholesterolemia in rats but the presence of yoghurt interfered with cholesterol absorption or accumulation or glyceride digestion. The constituents of the yoghurt alone are insufficient to significantly affect the blood lipid profile but the presence of different bacteria helps maintain an acceptable cholesterol levels.

The present study showed a significant increase ($P < 0.05$) in triglycerides with hyperlipidemic group which according to Bersot *et al.*, [25] are now considered an independent risk factor for coronary heart disease and acute pancreatitis. The feeding was conducted to investigate the effect of probiotic yogurt on cholesterol levels in albino rats. Administration of probiotic yorgurt significantly affects the lipid profile. Serum TC, HDL and VLDL cholesterol were significantly higher than that of the control group same as that obtained by Joy and Goyton [26-27] However, there was a significant increase in serum TG in hyperlipidemic group similar to that obtained by Mustari and Ahmed [28].

Various probiotic species have been reportd to decrease the serum cholesterol levels in experimental animals [29]. Similar results were obtained in this study. In

addition, serum TC, HDL and TG increased and similar to the results reported by Cavallini *et al.*, who observed that among the beneficial effects attributed to probiotics or probiotic-containing food products, the reduction of blood cholesterol is of particular interest [30]. Daily yogurt reduced serum TC or LDL cholesterol differently as similar to results of Peran *et al.*, [31]. Not all strains are equally beneficial, each may therefore have individual mechanisms of action. Furthermore, host characteristics such as flora composition or immune status may determine which probiotic species or strains may be optimal. Peran *et al.*, concluded that it would be interesting to compare different probiotics in the same experimental model, in order to establish the best profile in a given setting [31].

Table 5. *Effect of yoghurt supplement on the serum lipid profile and transaminase activities of experimentally induced hyperlipidemia in rats.*

Serum parameters	Rats Groups and diets						
	A	B	C	D	E	F	G
	Basal diet	Hyperlipidemic diet	Hyperlipidemic +yogurt C	Hyperlipidemic +yogurt D	Hyperlipidemic + yogurt E	Ferment free group	Hyperlipidemic Ferment free
TC mg/dL	120 ± 3.30^a	200.49 ± 10.83^b	98.48 ± 2.01^a	105.69 ± 2.17^a	100.48 ± 0.01^a	80.67 ± 8.39^a	220.11 ± 2.37^b
TG mg/dL	96.24 ± 0.24^a	$277.820.59^c$	106.36 ± 0.03^a	125.36 ± 1.03^a	115 ± 14.52^a	89.24 ± 4.2^a	171.01 ± 12.08^b
HDL-c mg/dL	60.83 ± 0.06^a	43.39 ± 0.01^b	62.36 ± 0.68^a	65.83 ± 0.004^a	71.83 ± 2.13^a	60.05 ± 0.06^a	48.86 ± 0.01^b
LDL-c mg/dL	11.053 ± 7.95^a	101.54 ± 5.00^b	14.848 ± 17.64^a	14.788 ± 17.95^a	5.65 ± 19.04^a	2.77 ± 0.50^a	137.04 ± 0.50^b
VLDL-c	19.248 ± 2.13^a	55.56 ± 0.004^c	21.272 ± 0.68^a	25.072 ± 0.06^a	23 ± 0.0014^a	17.848 ± 3.06^a	34.202 ± 2.01^b
HDLc/LDLc	5.50 ± 0.0014^a	0.42 ± 0.68^b	4.19 ± 0.01931^a	4.45 ± 0.06^a	12.71 ± 2.13^c	21.66 ± 0.68^d	0.35 ± 2.003^b
Albumin mg/dL	2.83 ± 0.01^a	5.05 ± 1.01^b	3.01 ± 0.01^a	3.31 ± 0.01^a	3.95 ± 0.01^a	2.83 ± 0.01^a	7.00 ± 1.41^b
AST (U/L)	131.47 ± 78.01^a	831.249 ± 44.01^b	145.05 ± 50.01^a	90.8 ± 20.01^a	155.45 ± 25.06^a	181.83 ± 55.01^a	757 ± 0.01^b
ALT (U/L)	15.17 ± 0.01^a	176.17 ± 10.01^b	11.97 ± 4.01^a	29.5 ± 8.01^a	19 ± 3.01^a	15.77 ± 5.01^a	176.17 ± 9.89^b

TC: Total cholesterol, TG: Triglyceride, HDL-c: High Density Lipoprotein-cholesterol, VLDL-c: Very Low Density Lipoprotein-cholesterol;
LDL-c: Low Density Lipoprotein-cholesterol, ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase.
Data are presented as mean ± SD
All the administered dose were 1.0 ml/kg day
Values with different superscript on a same line are Significantly different from control at $P < 0.05$.
C, D E are the different varieties of yogurt as described in feeding and experimental design section.
A, B C, D, E, F, G, are the different groups of rats fed with their respective diets

The high transaminase levels exhibited by groups that received yoghurts D and E that none of these diets was able to induce liver disease or damage. The elevation of AST and ALT levels are usually signs of liver disease because these enzymes originate or are specific to the liver. The yoghurt constituents without culture does not significantly affect the rate of ALT and AST. ALAT, ASAT, serum TC and TG reduced similar to the results obtained by Dougnon [32] because they have been demonstrated to be constituents of some tissues like the liver, kidney and heart. These moderate levels of ALAT and ASAT in serum in the absence of viral hepatitis and alcoholism result from the capability of the yogurt ferment to reduce the serum cholesterol hence maintaining cellular integrity. High levels have been shown to lead to higher risk of CVD with the risk higher in women [33].

Albumin is known for the transport of lipids in the blood in the form of serum albumin or chylomicron. Increased albumin levels with the group that did not receive yoghurt testify the presence of more fats or more transport to the organ for storage. These parameters were low with rats consuming hyperlipidemia feed and yoghurt at the same time; reflecting the effect of probiotic bacteria against absorption or digestion of lipids and cholesterol

The use of probiotic bacteria in reducing serum cholesterol levels has attracted much attention. Many researchers have suggested that probiotics have cholesterol reduction effects. However, the mechanism of this effect is not clearly understood. However, two hypotheses have been proposd to try to explain the mechanism. One of them is that bacteria may bind or incorporate cholesterol directly into the cell membrane. The other is that bile salt hydrolysing enzymes deconjugate the bile salts which are more likely to be exerted resulting in increased cholesterol breakdown [34]. Various studies have shown that some lactobacilli can lower total cholesterol and low-density lipoprotein (LDL) cholesterol but our results were contrary to this, probably due to the varied physical condition between the experiments. High levels of serum cholesterol have been associated with risks of coronary heart disease [35-38] The finding that bifidobacteria from fermented milk lower~~s~~ serum total cholesterol concentrations is in agreement with data from other studies involving various milk products containing selected strains of lactic acid bacteria. Our results are also in agreement with the results of another study done in Iran which concluded that consumption of probiotic yogurt in comparison with ordinary yogurt caused a significant decrease in serum total cholesterol [38]. This may suggest that the probiotic extract does not have any effect on albumin levels strain which was different from the present study. This result is in agreement with those of Dimcho *et al.*, who reported that probiotic bacteria consumption did not affect total protein (albumin) concentrations in the blood [40]. However our result is not in conformity with albumin concentrations obtained by Nishimoto *et al.*, in experimental animals feeding on base diet supplemented with probiotics increased compared to test experimental animals [41].

Kiessling and Schneider observed that consumption of yogurt increased the serum concentration of HDL-cholesterol which to the desired improvement of the LDL/HDL cholesterol ratio [42].

4. Conclusion

From the results of this study, continuous consumption of yogurt especially the one containing probiotic strain due to its importance in the body reduced some parameter in the lipid profile, as well as reduction of LDL/HDL cholesterol, therefore it can be used to reduce the risk factors of cardiovascular diseases. It is recommended that people who are susceptible to developing cardiovascular diseases and those suffering from hypercholesterolemia/ hyperlipidemia should consume more. Probiotics are claimed to have beneficial effects on health. However, only few well-performed studies have looked at clearly defined health effects such as serum cholesterol concentrations. Hypercholesterolemia is strongly associated with coronary heart disease and arteriosclerosis and decreasing serum cholesterol is an important treatment option. This study clearly demonstrates that ingestion of yogurt had no adverse effects on the rat behaviors and growth response; demonstrated that the cholesterol and lipid levels in the serum of rat fed with yoghurt fermented by probiotic bacteria decreased significantly. Results of the present study thererfore support the administration of probiotic yogurt as a safe and alternative method for prevention and treatment of risk factors of cardiovascular diseases.

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