ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.10 pp 388-398, 2015

Effect of consumption of Milk and Yoghurt on Lipid metabolism and Atherosclerosis in adult male rats

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Abstract: Conflicting findings have been reported about dairy food consumption and risk for cardiovascular diseases. Furthermore, some studies have revealed that high consumption of milk and dairy products may have protective effects against atherosclerosis and hence CVD. So, the aim of the present study is to examine whether consumption of milk and other dairy products such as yoghurt either from cow or buffalo is a risk factor for atherosclerosis or CVD. Fifty four adult male Albino rats of body weight ranging from 120 to 130 g were divided into nine groups (6 rats in each). The first group fed on the standard diet and served as negative control group, groups (2 & 3) fed on control diet + (15or30 %) powdered buffalo milk, groups (4&5) fed on control diet + (15 or 30 %) powdered cow milk, groups (6 &7) fed on control diet + (15or 30 %) powdered buffalo yoghurt and groups (8&9) fed on control diet + (15or 30 %) powdered cow yoghurt, respectively, for 8 weeks. Biological evaluation was carried out by determination of body weight gain, food intake and feed efficiency ratio. Then, blood samples were collected to determine lipid profile, activity of plasma catalase and concentration of plasma malondialdhyde (MDA), also to determine each of kidney and liver functions. The results obtained showed a non-significant change for all studied parameters in all groups compared to the negative control group. Also, histopathological examination of aorta showed no changes for all groups fed on either buffalo or cow milk or buffalo or cow yoghurt compared to the negative control group. In conclusion, our study showed that high consumption of milk and dairy products does not exert any adverse effect on cardiovascular health. In turn, it may be safely consumed.
Key words: Cardiovascular diseases, atherosclerosis, buffalo milk, cow milk, buffalo yoghurt, cow yoghurt, albino rats, lipid profile, liver & kidney functions, aorta histopathology.

Introduction

An adequate supply of good quality food is essential for human health and well-being. It is unsurprising then that milk and dairy products have been recognized as important human food sources from as early as 4000 B.C. Today there is a wide variety of milks and dairy products available to the consumer. In response to considerable scientific research on the nutritional value of milk, dietary guidelines around the world have recommended daily consumption of dairy products for the overall health of the population (1,2).

In fact, milk fat is considered "bad" by many health professionals and scientists are being asked to clarify the role of specific foods such as milk in health maintenance and prevention of chronic diseases. For over half a century, the concept of eating healthy has become synonymous with avoiding dietary fat and

cholesterol, especially saturated fat. A diet low in saturated fat keeps the heart healthy(3).The important contributions of dairy products in meeting human dietary requirement for energy, high quality protein and several key minerals and vitamins are well documented (4, 5).However, the nutritional importance of milk and dairy fats needs further investigations. With the increased demand for animal-derived food products as living standards improve, milk and yoghurt will undoubtedly continue to be an important dietary source of nutrients.

In case of milk and dairy products, there has been general perception that a food containing saturated fat is not beneficial to health. Yet, over the last decade, evidence has been accumulated that the composition and quantities of dietary fat is very important in determining the relative risk to diseases and that milk-derived fat may offer significant health benefits compared to some common sources of dietary fats (6).On average, milk from different sources contains lipids, free fatty acids, triacylglycerols, cholesterol and phospholipids. Milk fat is present as complex globules with structural properties distinct from other biological sources of fats. It is one of the most complex naturally occurring fats with more than 400 different fatty acids reported, however, only about 20 of these make up approximately 95% of the total. Not all fatty acids, or saturated fatty acids, have the same biological effects, it is important to understand that the saturated fatty acids in milk vary in their structure and many have no effect on plasma cholesterol. This was highlighted by The Nutrition Committee of the American Heart Association who emphasized the diversity in the biological effects of individual fatty acids and the need to evaluate specific fatty acids with respect to a range of variables related to the risk of coronary heart disease (2, 5).

About 60% of the fatty acids in milk fat are saturated and of these there is consensus that they have no effect on circulating cholesterol. However, is it was reported that lauric, myristic and palmitic acids may result in increasing the circulating HDL-cholesterol, a change that is associated with a reduced risk of coronary heart disease (7). Consequently, the bioactive properties of a number of components in milk have been examined with regard to a range of health-related variables, of special interest, are the components associated with the prevention of chronic diseases and results have demonstrated that milk contains specific proteins, peptides and fatty acids that are bioactive. Moreover, fermented milk products were reported to have beneficial effects on health-related variables.

Higher intakes of saturated and transfat were associated with increased risk of coronary heart disease (CHD), while higher intakes of monounsaturated and polyunsaturated fats were associated with reduced risk(8). Numerous epidemiological studies have revealed that high consumption of milk and dairy products may have protective effects against CHD, stroke, diabetes, certain cancers (such as colorectal and bladder cancers), and dementia. Individuals who consume a greater amount of milk and dairy products have a slightly better health advantage than those who do not consume milk and dairy products (9).

The majority of observational studies have failed to find an association between the intake of dairy products and increased risk of cardiovascular diseases (CVD), coronary heart disease, and stroke, regardless of milk fat levels. On the other side, powerful studies indicate that using low-fat dairy products such as fat-free or low-fat milk, yoghurt and cottage cheese as part of a balanced diet may combat hypertension and protect against strokes (10).Thus, the role of milk consumed by people in different parts of the world is still controversial, is it protective against atherosclerosis and hence CVD or is it abused as it increases the risk for CVD. Consequently, the aim of the present study was to investigate the association between milk and yoghurt intake, plasmatotal lipids, lipoproteins, total cholesterol and atherogenic risk.

Materials and Methods

Materials: Liquid buffalo milk and Liquid cow milk were purchased from Faculty of Agriculture, Cairo University. Most of the ingredients used for preparation of the diet were obtained from the local market. Ingredients used for formulation of vitamin and salt mixtures were obtained from Fluka (Germany) and BDH (England) Chemical Companies. Cholesterol was obtained from Laboratory Rasayan, Fine-Chem. Limited, Mumbai, India. Casein was obtained from Scerma in France. Cellulose was obtained from Sigma-Aldrich Chemi, Germany.

Animals used in the biological experiment were obtained from the Central Animal House, National Research Centre, Egypt. Diagnostic kits used for the determination of alanine amino transferase (ALT), aspartate amino transferase (AST), urea, creatinine, uric acid, total lipids and catalase were obtained from

Biodiagnostic Company, Egypt. Kits used for determination of total cholesterol, HDL-cholesterol and triacylglycerols were obtained from Salucea company, Netherlands. Chemicals used for the determination of lipid peroxide product; the thiobarbituric acid (TBA) and the trichloroacetic acid (TCA) were obtained from Merck (Germany) and BDH (England) Companies, respectively.

Methods

The fresh cow and buffalo milk were dried according to the method described by Subramonian, (2001) using spray drier in the Biotechnology & Genetic Engineering Unit, National Research Centre, Egypt. Powdered cow and buffalo milk were kept in -20ºC until being mixed with basal diet and used in the feeding experiment.

Manufacture of cow and buffalo yoghurt

Yoghurt was synthesized from either cow or buffalo milk according to the method of Soukoulis, et al., (2007).

Chemical analysis of cow milk, buffalo milk, cow yoghurt and buffalo yoghurt

Total fat, protein and moisture contents were determined according to the method A.O.A.C. (2007). Total carbohydrates were determined by difference (14).Total fatty acids (saturated, monounsaturated FA& polyunsaturated FA) were determined by "Gas Chromatography" according to the method by Luddy et al., (1960).Milk and yoghurt oils were extracted with n-hexane (boiling point 68-70°C) for 6 hr in a Soxhlet extractor and stored in glass vials at 1-5°C. Then, methyl esters of the fatty acids of milk and yoghurt oils were prepared (15).Fatty acids methyl esters were chromatographed as methyl esters on Fused Silica Capillary column DB% (60 m x 0.32 mmi.d). Analysis was performed on Perkin Elmer Auto System XL equipped with flame ionization detector (FID). Oven temperature was maintained initially at 150°C and programmed from 150 to 240°Cat rate 3°C/min., held at 240°C for 30 min. injector temp. 230°C, detector temp 250°C.Carrier gas: Helium, flow rate 1 ml/min. Then, the resulting peaks of fatty acids methyl esters were identified according to a standard mixture of fatty acid methyl esters obtained from Sigma Chemicals and finally, the percentage of fatty acids was calculated.

Formulation of the diet

The standard control diet was prepared according to the method Reeves et al., (1993).The other diets that contain milk or yoghurt were also formulated but with some modification according to the chemical composition of milk and yoghurt.

Design of animal experiments

Fifty four adult male Albino Sprague Dawely rats at body weight ranging from 120 to 130 g were adapted for one week prior to commencement of the experiment. They were housed individually in separate cages at 25 °C. The study protocol had been approved by scientific committee at National Research Center, Egypt. Prior permission for animal use, an approval of the protocol was obtained from the International Animal Ethics Committee. Rats were divided into 9 groups, 6 rats in each group and fed on diets as follows: Group1: Negative control group fed on the standard control diet. Group2: Control diet + 15% powdered buffalo milk.Group3: Control diet+ 30% powdered buffalo milk. Group 4: Control diet + 15% powdered cow milk. Group 5: Control diet+ 30 % powdered cow milk. Group 6: Control diet+ 15% powdered buffalo yoghurt. Group 7: Control diet+ 30 %powdered buffalo yoghurt. Group 8: Control diet+ 15% powdered cow yoghurt.Group9: Control diet+ 30 % powdered cow yoghurt.

The experiment lasted for 8 weeks during which, body weight was followed once a week. Feed intake of each rat was recorded daily. At the end of the experiment, body weight gain, feed intake and feed efficiency ratio (FER) were calculated.

At the end of the experimental period, blood was withdrawn from orbital vein (after overnight fasting) under slight anesthesia by diethyl ether and delivered into heparinized tubes, then centrifuged for 15 min. at 4000 rpm and the resultant plasma was kept in freezer at–20°C until analysis. The heart and the aorta were separated, washed with saline and immersed in 10% formalin solution for histopathological examination.

Biochemical analysis of blood

Plasma cholesterol was determined as described by NCEP Expert Panel (1988). HDL-cholesterol was determined according to Lopes (1977).LDL & VLDL-cholesterol were determined according to Warnick, et al., (1990). Atherogenic index was calculated as described by Goh, et al., (2004). Plasma triglycerides was assessed as described by Chowdhury, et al., (1971). Plasma total lipids were determined according to ZÖllner& Kirsch, (1962).Plasma urea was determined according to the method of Fawcett & Soctt, (1960). Plasma uric acid was detected according to Fossati, et al., (1980). Plasma creatinine was assessed according to Bartels et al., (1972). Plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were determined as described by Reitman & Frankel, (1957). Plasma catalease activity was determined according to Aebi, (1984).Plasma malondialdehyde (MDA) was detected as lipid peroxide product by the thiobarbituric acid (TBA) assay according to the method of Draper & Hadley (1990).

Histopathological examination

Specimens from aorta were examined after being cleared in xylol, embedded in paraffin, sectioned at 4-6 micrometer thickness and stained with Heamatoxylin and Eosin according to the method of Carleton (1976). Finally, they were examined under microscope.

Statistical analysis

Results were analyzed statistically using the computerized program SPSS. The one way analysis of variance "ANOVA" test was done. Data were represented as mean ± SE. Significance was considered at a level of 0.05.

Results and Discussion

Chemical constituents of milk & yoghurt from buffalo and cow

The results in table (1) were calculated on dry weight basis. The total fat, protein, carbohydrate and moisture contents were determined. The tabulated results show that the highest total fat contents were that of buffalo milk followed buffalo yoghurt and cow milk, while the lowest contents was that of the cow yoghurt (31.0; 27.6; 26.9 and 22.4% respectively). In addition, fat content of both types of yoghurt showed relatively lower value than milk, this is due to milk processing that employed in yoghurt manufacturing such as homogenization and fermentation which resulted in breakdown of fat. The highest protein percent were that of the buffalo yoghurt followed by buffalo milk and cow yoghurt while the lowest protein content was that of cow milk, 26.2; 24.3; 24.0& 23.7%, respectively. As shown in the table it can be noticed that the percentage of carbohydrates showed low values for both types of yoghurt compared with that of milk. It can be explained by the conversion of lactose which is the main sugar of milk carbohydrate into its simple forms of glucose and galactose by lactic acid bacteria, so the carbohydrates percent of yoghurt either of cow or buffalo showed relatively lower values than milk. Our results were confirmed that relative lower values of carbohydrate and fat of yoghurt than that of milk was attributed to fermentation and homogenization processes (30).

Table (1): Chemical constituents of buffalo milk, cow milk, buffalo yoghurt & cow yoghurt powder (g / 100g dry weight basis).

Macronutrients (%)	Buffalo milk	Cow milk	Buffalo yoghurt	Cow yoghurt
Total fat	31.00	26.90	27.6	22.40
Protein	24.30	23.70	26.2	24.10
Carbohydrates	45.03	39.70	43.26	37.37
Moisture	06.53	05.34	05.7	07.67

Table (2) illustrated the percent yield of various fatty acids including saturated, monounsaturated and polyunsaturated fatty acids of buffalo & cow milk, buffalo & cow yoghurt. Results showed variations of all tested samples. Saturated fatty acids included butyric, capruic, caprylic, capric, lauric, myristic, palmitic and stearic acids. Palmitic, stearic and myristic acids recorded the highest values of both buffalo and cow milk & yoghurt. Monounsaturated fatty acids were myristoleic, palmitoleic and oleic acids. Cow milk & yoghurt showed the higher value of monounsaturated fatty acid (oleic acid), than buffalo milk & yoghurt. The higher

content of stearic (C18:0) and oleic (C18:1) acids in the buffalo yoghurt have a favorable effect on human nutrition compared to cow milk(31). Cow and buffalo milk showed the higher values of polyunsaturated fatty acids than cow and buffalo yoghurt. A positive property of milk fat is its good ratio of omega-3 toomega-6 fatty acids (32).The predominant saturated fatty acids (SFA) in both yogurt and milk are myristic acid, palmitic acid and stearic acid and the predominant unsaturated fatty acids are; oleic, vaccenic, and linoleic acids(1),(33).However, lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids arepredominant in both yogurt and dairy beverage (33), (34).

Table (2): Percent of saturated; monounsaturated and polyunsaturated fatty acids for buffalo milk, cow milk, buffalo yoghurt & cow yoghurt.

FAs / Conc.	Saturated fatty acids SFAs%								Monounsaturated fatty acids MUFAs%			Polyunsaturated fatty acids PUFAs%	
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{14:1}$	$C_{16:1}$	$C_{18:1}$	$C_{18:2}$ (W6)	$C_{18:3}$ (W3)
Buffalo milk	**0.12**	**0.03**	**_**	**0.02**	**0.43**	**6.94**	**35.45**	**20.10**	**0.13**	**_**	**2.47**	**32.35**	**1.95**
Cow milk	**0.04**	**0.07**	**0.33**	**0.69**	**1.00**	**7.06**	**35.31**	**15.62**	**0.62**	**0.01**	**4.18**	**34.75**	**0.32**
Buffalo yoghurt	**2.31**	**1.13**	**0.08**	**0.69**	**2.05**	**12.52**	**36.73**	**16.21**	**_**	**_**	**3.88**	**24.33**	**0.08**
Cow yoghurt	**0.70**	**0.27**	**0.07**	**0.55**	**1.61**	**10.66**	**39.57**	**13.98**	**0.90**	**_**	**4.08**	**27.59**	**0.01**

C_4: Butyric acid; **C_6**: Capruic acid; **C_8**: Caprylic acid; **C_{10}**: Capric acid; **C_{12}**: Lauric acid;**C_{14}**: Myristic acid; **C_{16}**: Palmitic acid; **C_{18}**: Stearic acid; **$C_{14:1}$**: Myristoleic acid; **$C_{16:1}$**: Palmitoleic; **$C_{18:1}$**: Oleic acid; $C_{18:2}$: Linoleic acid;$C_{18:3}$: Linolenic acid.

Feed intake, body weight gain and feed efficiency ratio of adult rats

Data presented in table (3) showed the mean value of feed intake, body weight gain and feed efficiency ratio of adult rats fed on various ratios of milk and yoghurt from both buffalo and cow. Results indicated that, there was a non-significant change in the feed intake, body weight gain and fed efficiency ratio of all groups when compared to the control negative group. Kratz et al., (2014), reported similar results and added that consumption of full-fat dairy products might be inversely related to obesity.

Table (3):): Food intake, body weight gain, feed efficiency ratio (FER) of the control group and the different groups.

Parameters / Groups	FI (g)	BWG (g)	(FER)
Group1 = Control (-ve)	954.60 ± 40.54^{ab}	174.17± 14.89^{a}	0.183 ± 0.02^{a}
Group2 (Mean± SE)	914.63 ± 24.87^{a}	174.17 ± 11.48^{a}	0.191 ±0 .01^{a}
Group3 (Mean± SE)	932.17 ± 29.99^{a}	195.00 ± 13.02^{a}	0.210 ±0 .02^{a}
Group4 (Mean± SE)	943.10 ± 8.43^{ab}	178.50 ± 8.52^{a}	0.189 ± 0.01^{a}
Group5 (Mean± SE)	940.90 ± 15.09^{ab}	173.33 ± 8.86^{a}	0.179 ± 0.01^{a}
Group6 (Mean± SE)	950.37 ± 23.92^{ab}	185.67 ± 9.65^{a}	0.196 ±0 .01^{a}
Group7 (Mean± SE)	946.53 ± 18.27^{ab}	187.17 ± 6.13^{a}	0.198 ± 0.01^{a}
Group8 (Mean± SE)	1017.70 ± 4.61^{b}	174.50 ± 13.27^{a}	0.172 ± 0.01^{a}
Group9 (Mean± SE)	975.73 ± 33.86^{ab}	178.50 ± 12.72^{a}	0.183± 0.01^{a}

N.B.: Control (-ve) = Group1; Group 2: 15% buffalo milk; Group 3: 30% buffalo milk; Group 4: 15% cow milk; Group 5: 30% cow milk; Group 6: 15% buffalo yoghurt;Group7: 30% buffalo yoghurt; Group 8: 15% cow yoghurt & Group 9: 30% cow yoghurt.
*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Concentration of plasma cholesterol, triglycerides and total lipids

Plasma levels of cholesterol, triglycerides and total lipids of all groups are illustrated in table (4). As shown in the table, there is a non-significant change in either cholesterol or triglycerides or total lipids of all groups when being compared to the negative control group or when being compared to each other. Although the fat content of the diets that containing milk or yoghurt are more than that of the control diet, yet there was no change in any of the plasma lipid parameters. It was reported that not only the quantities of dietary fat but also its composition are very important in determining the relative risk to diseases and milk-derived fat may offer significant health benefits compared to some common sources of dietary fats (6). Also, milk fat is not a negative for heart health (36).

Individual fatty acids differ in their effects on serum cholesterol, whereas most saturated fatty acids (lauric, myristic and palmitic acids) caused a pronounced increase in low density lipoprotein (37). This led to the development of an atherogenic index which ranked foods based on their content of these three fatty acids (38).

On contrast to the previous studies, it was reported that lauric acid increases plasma cholesterol concentrations, but most of the increasing is in high density lipoproteins (HDL), with consequent increasing HDL/LDL ratio, which is considered to be a positive marker for cardiovascular health (39). The atherogenicity of palmitic acid has long been uncertain, perhaps because when included in diets that contain adequate quantities of unsaturated fatty acids, no negative effects of palmitic acid are observed (40).

Table (4): Concentration of plasma cholesterol, plasma triglycerides & plasma total lipids of the control group and all other groups.

Parameters / Groups	**Cholesterol** (mg/dl)	**Triglycerides** (mg/dl)	**Total lipids** (mg/dl)
Group1 = Control (-ve)	94.66 ± 3.87[a]	87.00 ± 3.40[a]	331.83 ± 33.59[a]
Group2 (Mean± SE)	92.85± 7.13[a]	84.84 ± 7.75[a]	359.93 ± 14.42[a]
Group3 (Mean± SE)	92.47 ± 3.76[a]	71.70 ± 4.57[a]	351.29 ± 26.57[a]
Group4 (Mean± SE)	90.12 ± 3.79[a]	83.32 ± 3.87[a]	342.04 ± 49.64[a]
Group5 (Mean± SE)	80.09± 4.31[a]	77.22 ± 7.14[a]	317.42 ± 8.59[a]
Group6 (Mean± SE)	85.24± 5.45[a]	81.38 ± 5.55[a]	324.55 ± 28.71[a]
Group7 (Mean± SE)	84.32 ± 2.82[a]	93.04 ± 11.33[a]	359.57 ± 28.72[a]
Group8 (Mean± SE)	85.39 ± 5.29[a]	82.00 ± 5.05[a]	362.96 ± 25.77[a]
Group9 (Mean± SE)	83.41 ± 4.01[a]	73.20 ± 6.09[a]	333.57 ± 5.84[a]

N.B.: Control (-ve) = Group1; Group 2: 15% buffalo milk; Group 3: 30% buffalo milk;Group 4: 15% cow milk; Group 5: 30% cow milk; Group 6: 15% buffalo yoghurt;Group 7: 30% buffalo yoghurt; Group 8: 15% cow yoghurt & Group 9: 30% cow yoghurt.
*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Concentration of plasma LDL-cholesterol, plasma HDL-cholesterol, plasma VLDL-cholesterol & atherogenic index (A. I.)

The concentration of HDL-C, LDL-C, VLDL-C & atherogenic index "AI" (log {triglycerides/ HDL-C}) of all groups are illustrated in table (5). As shown in the table, there is a non-significant change in each of HDL-C, VLDL-C and atherogenic index of all groups when being compared to the negative control group or between each other. On the other hand, a significant decrease in LDL-C was noticed in the group that was fed on 30% buffalo yoghurt, while LDL-C in all other groups showed a non-significant change. Buffalo yoghurt is known to contain conjugated linoleic acid (CLA) which is reported to modulate lipid metabolism in rodents and humans **(46)**. Also, numerous potential physiological effects have been attributed to CLA in buffalo yogurt including anti-adipogenic and anti-atherosclerotic effects (41).

Table (5): Concentration of plasma LDL-cholesterol, plasma HDL-cholesterol, plasma VLDL-cholesterol &atherogenic index (A.I.) of the control group and all other groups.

Parameters / Groups	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	A.I
Group1 = Control (-ve)	56.75± 5.43[a]	20.53 ± 2.33[a]	17.40 ± 0.67[a]	0.64 ± 0.06[a]
Group2 (Mean± SE)	55.99 ± 5.25[a]	19.91 ± 1.78[a]	16.95 ± 1.55[a]	0.63 ± 0.05[a]
Group3 (Mean± SE)	58.02 ± 4.80[a]	20.13 ± 1.68[a]	14.32 ± 0.92[a]	0.55 ± 0.04[a]
Group4 (Mean± SE)	53.08 ± 3.99[ab]	20.37 ± 2.07[a]	16.67 ± 0.77[a]	0.62 ± 0.05[a]
Group5 (Mean± SE)	43.40 ± 4.09[ab]	21.28 ± 3.12[a]	16.43 ± 1.02[a]	0.57 ± 0.08[a]
Group6 (Mean± SE)	45.38 ± 5.14[ab]	23.58 ± 1.17[a]	16.28 ± 1.12[a]	0.54 ± 0.04[a]
Group7 (Mean± SE)	40.73 ± 2.19[b]	24.98 ± 1.59[a]	16.50 ± 0.79[a]	0.56 ± 0.07[a]
Group8 (Mean± SE)	45.68 ± 4.78[ab]	23.32 ± 0.95[a]	16.41 ± 1.01[a]	0.54 ± 0.04[a]
Group9 (Mean± SE)	48.60 ± 4.22[ab]	20.17 ± 2.18[a]	14.63 ± 1.21[a]	0.57 ± 0.06[a]

N.B.: Control (-ve) = Group1; Group 2: 15% buffalo milk; Group 3: 30% buffalo milk; Group 4: 15% cow milk; Group 5: 30% cow milk; Group 6: 15% buffalo yoghurt; Group 7: 30% buffalo yoghurt; Group 8: 15% cow yoghurt & Group 9: 30% cow yoghurt.
*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The activities of AST and ALT for all groups were shown in table (6). As illustrated in the table, there was a non-significant change in AST and ALT for all groups either when compared to the control negative group or when being compared with each other. Consequently, it is obvious that the milk and yoghurt fat do not exert any adverse effect on liver. In this respect, high dairy fat intake was associated with a lower fat content in the liver and better insulin sensitivity. They attributed that to the milk phospholipids since dairy fat is a rich source of phospholipids which may act on the liver to lower liver fat content (35).

Table (6): Activities of aspartate aminotransferase (AST) & alanine aminotransferase (ALT) of the control group and other groups.

Parameters / Groups	Aspartate aminotransferase (AST) (U/ml)	Alanine aminotransferase (ALT) (U/ml)
Group1 = Control (-ve)	88.17 ± 2.69[a]	33.00 ± 0.78[a]
Group2 (Mean± SE)	82.77 ± 2.68[a]	29.17 ± 4.97[a]
Group3 (Mean± SE)	95.23 ± 6.37[a]	31.27 ± 5.53[a]
Group4 (Mean± SE)	90.50 ± 1.76[a]	34.50 ± 1.49[a]
Group5 (Mean± SE)	83.83 ± 6.11[a]	30.83 ± 4.39[a]
Group6 (Mean± SE)	80.50 ± 4.82[a]	30.97 ± 1.18[a]
Group7 (Mean± SE)	85.00 ± 6.68[a]	29.40 ± 3.03[a]
Group8 (Mean± SE)	80.50 ± 4.65[a]	30.83 ± 1.05[a]
Group9 (Mean± SE)	82.50 ± 7.78[a]	31.40 ± 2.76[a]

N.B.: Control (-ve) = Group1; Group 2: 15% buffalo milk; Group 3: 30% buffalo milk;Group4: 15% cow milk; Group5: 30% cow milk; Group6: 15% buffalo yoghurt;Group7: 30% buffalo yoghurt; Group 8: 15% cow yoghurt & Group 9: 30% cow yoghurt.
*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Concentration of plasma urea, creatinine and uric acid

The concentration of urea, creatinine and uric acid of all groups are illustrated in table (7). As shown in the table, there is no significant change in either urea or creatinine or uric acid in all groups when compared to the negative control group. The milk fat is a rich source of polyunsaturated fatty acids (PUFAs) which is

associated with improving kidney function. The relationship between total plasma PUFA levels and change in creatinine clearance was examined. The study showed that older adults with low total plasma PUFA levels have a greater decline in creatinine clearance. This finding suggested that a higher dietary intake of PUFA may be protective against progression to chronic kidney disease (42).

Table (7): Concentration of plasma urea, plasma creatinine & plasma uric acid of the control group and all other groups.

Parameters / Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group1 = Control (-ve)	30.695 ± 0.92[a]	0.551 ± 0.04[a]	1.47± 0.18[a]
Group2 (Mean± SE)	32.33 ± 0.69[a]	0.456 ± 0.07[a]	1.43 ± 0.18[a]
Group3 (Mean± SE)	33.03 ± 0.78[a]	0.552 ± 0.14[a]	1.67 ± 0.03[a]
Group4 (Mean± SE)	33.24 ± 0.90[b]	0.690 ± 0.02[a]	1.63 ± 0.08[a]
Group5 (Mean± SE)	31.94 ± 1.13[a]	0.593 ± 0.10[a]	1.37± 0.19[a]
Group6 (Mean± SE)	32.46 ± 0.71[a]	0.642 ± 0.04[a]	1.59 ± 0.17[a]
Group7 (Mean± SE)	32.41 ± 0.40[a]	0.627± 0.04[a]	1.45 ± 0.08[a]
Group8 (Mean± SE)	33.16 ± 0.82[a]	0.569± 0.13[a]	1.52 ± 0.17[a]
Group9 (Mean± SE)	30.51 ± 1.48[a]	0.549 ± 0.07[a]	1.47 ± 0.17[a]

N.B.: Control (-ve) = Group1; Group 2: 15% buffalo milk; Group 3: 30% buffalo milk;Group 4: 15% cow milk; Group 5: 30% cow milk; Group 6: 15% buffalo yoghurt;Group 7: 30% buffalo yoghurt; Group 8: 15% cow yoghurt & Group 9: 30% cow yoghurt.
*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Activity of plasma catalase and concentration of plasma malondialdhyde

Catalase activity and malondialdhyde (MDA) concentration of all groups are illustrated in table (8). As shown in the table, there was a non-significant change in the malondialdhyde and catalase of all groups when compared to the control group, except the group which was fed on 15 % cow yoghurt which showed a significant decrease in catalase activity compared to the control group. It was reported that individuals who consume a greater amount of milk and dairy products have a slightly better health advantages than those who do not consume milk and dairy products(9).

Table (8): Activity of plasma catalase & concentration of plasma malondialdhyde of the control group and all other groups.

Parameters / Groups	Catalase (U/L)	Malondialdhyde (n mol/ml)
Group1 = Control (-ve)	607.8 ± 46.85[bc]	8.7 ± 1.66[a]
Group2 (Mean± SE)	618.15 ± 16.25[bc]	7.09 ± 0.83[a]
Group3 (Mean± SE)	612.65 ± 11.32[bc]	8.18 ± 0.21[a]
Group4 (Mean± SE)	578.82 ± 35.57[b]	8.4 ± 0.90[a]
Group5 (Mean± SE)	566.13 ± 35.31[b]	9.07 ± 1.07[a]
Group6 (Mean± SE)	681.38 ± 05.73[c]	8.00 ± 0.68[a]
Group7 (Mean± SE)	563.97 ± 28.43[b]	8.83 ± 0.96[a]
Group8 (Mean± SE)	438.42 ± 13.74[a]	8.07 ± 0.79[a]
Group9 (Mean± SE)	623.42 ± 33.54[bc]	8.35 ± 1.32[a]

N.B.: Control (-ve) = Group1; Group 2: 15% buffalo milk; Group 3: 30% buffalo milk ;Group 4: 15% cow milk; Group 5: 30% cow milk; Group 6: 15% buffalo yoghurt; Group 7: 30% buffalo yoghurt; Group 8: 15% cow yoghurt & Group 9: 30% cow yoghurt.
*Values that share the same letter at the same column are not significant.
*Values that share different letter at the same column are significant.
*The mean difference is significant at the 0.05 level.

Antioxidants help to protect the body against free radicals. MDA is considered as an index of lipid peroxidation. Inclusion of dairy products rich in CLA in the hypercholesterolemic diet that was fed to rats decreased the serum MDA level as compared to the control(43).On the other hand, it was reported that CLA

reduced lipid peroxidation by increasing oxidative stability in serum of rats fed experimental diets. They noticed that supplementation with the mixture of CLA did not alter plasma malondialdehyde (MDA), while, plasma catalase (CAT) activity showed a significant increased reached to (3.7fold) (44).

Histopathology

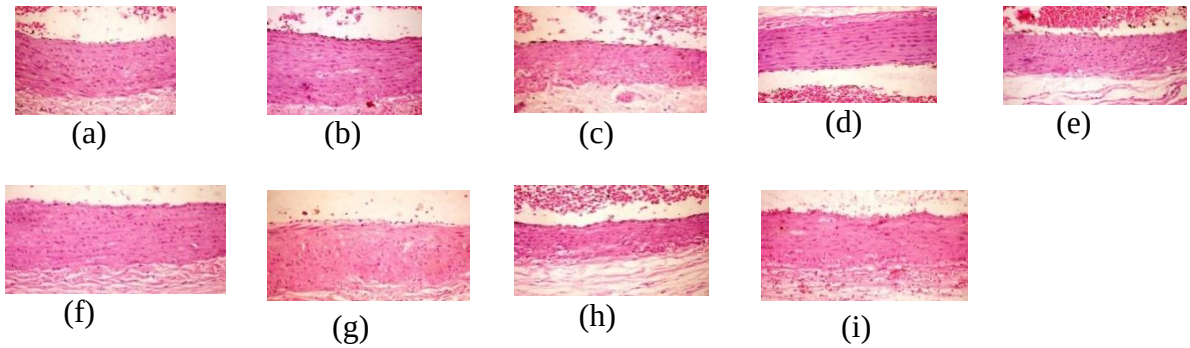

Fig. (1):(a)Aorta of rat from the control negative group showed no histopathological changes, figures (b), (c), (d), (e), (f), (g), (h), (i) showed aorta from groups 2, 3, 4, 5, 6, 7, 8, 9, respectively. No histopathological changes were noticed in aorta of any of these groups (H and E x200).

Aorta of rats

Histopathological examination of aorta from all groups, that were fed on either milk or yogurt by different ratios (15 % or 30 %) and different types (cow or buffalo), did not show any change compared to the aorta of the negative control group. In fact, these histopathological results confirm the obtained biochemical results.

In conclusion, our study showed that high consumption of milk and dairy products does not exert any adverse effect on cardiovascular health. In turn, it may be safely consumed.

References

1. Miller, G.; Jarvis,J. & McBean,L. (2007). "Handbook of Dairy Foods and Nutrition," 3rd ed. New York: CRC Press.
2. Crichton, G.E. & Elias, M.F. (2014). Dairy Food Intake and Cardiovascular Health: The Maine-Syracuse Study. Advances in Dairy Research 2 :112 pp:2-8.
   Dairy Sci. Technol. 89 (2009) 437–447.
3. Bauman, D. & Lock, A. (2010). Milk fatty acids composition: challenges and opportunities related to human health. World Buiatrics Congress, 278 – 289.
4. Demment, M. & Allen, L. (2003).Animal source foods to improve micronutrient nutrition and human function in developing countries. J Nutr 133:3875S–4062S.
5. Lock, A. (2009).Introduction to the Proceedings of the Symposium "Scientific Update on Dairy Fats and Cardiovascular Diseases" Journal of the American College of Nutrition, Vol. 27, No. 6, 720S–722S.
6. Bauman, D.; Lock, A.; Corl, B.; Salter, A. & Parodi, P. (2006). Milk fatty acids and human health: potential role of conjugated linoleic acid and trans fatty acids. In Sejrsen K, Hvelplund T, Nielsen MO, (eds): "Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress." Wageningen; The Netherlands: Wageningen Academic Publishers, pp 523–555.
7. Mensink, R.; Zock ,P.; Kester, A. & Katan, M. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a metaanalysis of 60 controlled trials. Am J ClinNutr 77:1146–1155.
8. Gibson, R.; Makrides, M.; Smithers, L.; Voevodin, M. & Sinclair, A. (2009). The effect of dairy foods on CHD: a systematic review of prospective cohort studies. Br. J. Nutr. 2009 Nov; 102 (9): 1267-57.
9. Kliem, K. & Givens, D. (2011). Dairy products in the food chain: their impact on health. 21-36.

10. Huth,P. &Park, K. (2012). Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence.
11. Subramonian, B.S. (2001).“Studies on preparation of dietetic milk powder with added bifidogenic properties.” Ph. D., Thesis submitted to Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India.
12. Soukoulis, C., Panagiotidis, P., Koureli, R., & Tzia, C. (2007). “Industrial Yogurt Manufacture: Monitoring of Fermentation Process and Improvement of Final Product Quality”. J. Dairy Sci., 90: 2641–2654.
13. A.O.A.C. (2007).Official Methods of Analysis of the Association of official Analytical chemists. 18thEd.Gaithersburg, Maryland, U.S.A.
14. FAO. (2003), Manual Of Food Quality Control, 14/8 , Page 8, IS 1479 Part1(1961).
15. Luddy, F.E.; Barford, R. A. & Riemenschneider, R.W. (1960). Direct conversion of lipid components to their fatty acid methyl esters. J. Amer. Oil Chem. Soc; 37: 447-451.
16. Reeves, P.; Rossow, K. &. Lindlauf, J. (1993). Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. J. Nutr, 123: 1923-1931.
17. NCEP Expert Panel (1988). Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Arch Intern Med.;148: 36-39.
18. Lopes, M.F. (1977). Cholesterol determination in high denisity lipoproteins separated by three different methods, Clin Chem.; 23(5): 882-884.
19. Warnick, G.R; Knopp, R.H.; Fitzpatrick, V. & Branson, L. (1990). Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cut points. Clin. Chem. 36 (1):15-9.
20. Goh, V.; Tain, C.; Terry, Y.; Mok, H. &Wong, M. (2004). Are BMI and other anthropometreic measures appropriate as indices for obesity ?- A study in Asian population .J. Lipid. Res. 45: 1892-1898.
21. Chowdhury, R. F.; Rodman, H. & Bleicher, S. J. (1971). Glycerol-like contamination of commercial blood sampling tubes, J. Clin Pathol. 12,, 116 .
22. ZÖllner, N. & Kirsch, K. (1962) Colorimetric method for determination of total lipids. Journal of Experimental Medicine, 135, 545-550.
23. Fawcett, J. K. and Soctt, J. E., (1960), "A rapid and precise method for the determination of urea.", J. Clin. Path.; 13: 156-159.
24. Fossati,P.; Prencipe,L. &Berti,G. (1980). Use of 3,5-dichloro-2-hydroxybenzene-sulfonic acid/4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clinical chemistry, 26: 227-231.
25. Bartels, H.; Bohemer, M. &Heirlic, A. (1972). "Serum creatinine determination without protein precipitation.",Clin. Chem Acta; 37: 193-7.
26. Reitman, S. & Frankel, S. (1957). "A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases.",Amer J. Clin. Path.; 28(1): 56-63.
27. Aebi, H. (1984).Aebi, Hugo. ed. "Catalase in vitro". Meth. Enzymol. Methods in Enzymology 105: 121–126.
28. Draper, H. H. & Hadley, M. (1990) Malondialdehyde Determination as an Index of Lipid Peroxidation. Methods in Enzymology, 186, 421-431.
29. Carleton, H. (1976).Histolgical Technique. 4 Th Ed. London, Oxford University Press, New York, Toronto. Chemists' Society, Chicago, v.37, p.447-451.
30. Mckinley, M.C., (2005).The nutrition and health benefits of yoghurt. International Journal of Dairy Technology, 58 (1) 1-12.
31. Ivanova, S.; Nacheva, I. ; Miteva, D.; Dimov, K. &Tsvetkov, T. (2010). Physicochemical composition, atherogenic index andprevantive lipid score of buffalo yoghurt after technological treatment and preservation. Bulgarian Journal of Agricultural Science, 16, 407-411.
32. Colomb, M.; Sollberger, H. & Butikofer, H. (2004). Impact of basal diet of hay and fodder beet supplemented with rapeseed, linseed and sunflower seed on the fatty acid composition of milk fat. In International Dairy journal, 14, 6, 549-559.
33. Cruz A.G., Faria, J. A.F. Van Dender, A.G. F. (2007). Packaging system and probiotic dairy foods Food Research International, 40,pp. 951–956.

34. Shingfield, K.; Chilliard, Y.; Toivonen, V.; Kairenius, P. & Givens, D. (2008). Trans fatty acids and bioactive lipids in ruminant milk. Adv. Exp. Med. Biol.606:3–65.
35. Gibson GR, Brummer RJ, Isolauri E, Lochs H, Morelli L, Ockhuizen T, Rowland IR, Schrezemmeir J, Stanton C, Verbeke K (2011) . The design of probiotic studies to substantiate health claims. Gut Microbes 2: 299- 305.
36. Kratz,M.;Marcovina,S.;Nelson,J.;Yeh,M.;Kowdley,K.;Callahan,H.;Song,X.&Utzschneider,K. (2014). Dairy fat intake is associated with glucose tolerance, hepatic and systemic insulin sensitivity, and liver fat but not β-cell function in humans. Am J Clin Nutr. 99(6):1385-1396.
37. PARODI, P.W. (2009) Review: has the association between saturated fatty acids, serum cholesterol and coronary heart disease been over emphasized? International Dairy Journal, v.19, p.345-361.
38. German, J.B. & Dillard, C.J. (2006). Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Critical Review Food Science Nutrition, 46, 57-92.
39. Chilliard,Y.; Ferlay, A.; Rouel, J. & Lambert, G. (2003). A review of nutritional and physiological factors afecting goat milk lipid synthesis and lipolysis. Journal Dairy Science, 86(5), 1752-1770. Retrieved from http://www.sciencedirect.com/science/article/
40. Fernandez, M. & Webb, D. (2008). Review: the LDL to HDL cholesterol ratio as a valuable tool to evaluate coronary heart disease risk. Journal of the American College of Nutrition, v.27, p.1-5,.
41. Clandinin, M.T.(2000).The effect of palmitic acid on lipoprotein cholesterol levels. International Journal of Food Sciences and Nutrition, v. 51, p.S61-S71.
42. Belury, M.A.(2002).Dietary conjugated linoleic acid in health: Physiological Effects and Mechanisms of Action1. Annu Rev Nutr; 22:505-531.
43. Lauretani, F.;Maggio, M. & Pizzarelli, F. (2009).Omega-3 and renal function in older adults. Curr Pharm Des. 2; 15(36):4149-56.
44. Chen Kung, F.; Jhih Chang, J. & Chien Yang, M. (2007). The reduction of oxidative stress, anticoagulation of platelets, and inhibition of lipopolysaccharide by conjugated linoleic acid bonded on a polysulfone membrane Polym. Adv. Technol, 18: 286-91.
45. Kim, H.K.; Kim, S.R.; Ahn, J.Y.; Cho, I.J.; Yoon, C.S. & Ha, T.Y. (2005). Dietary conjugated linoleic acid reduces lipid peroxidation by increasing oxidative stability in rats, J. Nutr. Sci. Vitaminol. 51 8–15.
46. Pariza, M.; Park, Y.; XU, X.; Nlambi, J.& Kang, K.(2003).Speculation on the mechanisms of action of conjugated linoleic acid. In: Sebedio, J. L., Chiristie, W.W. and Adolf, R., editors. Advances in conjugated linoleic acid research. Volume 2: 251-258. AOCS press, Champaign, lllinois, USA.

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