Journal

J. Biol. Chem. Environ. Sci., 2019, Vol. 14(1): 335-353
http://biochemv.sci.eg

EVALUATE THE INFLUENCES OF BUFFALO MILK ON HYPERCHOLESTEROLEMIA AND HYPERLIPIDEMIA IN MALE ALBINO RATS

Aly, H. E[(1)]; Rania S. Yousef[(2)], H. S El-Beltage, [(2)] and E. A. Abdel-.Rahim, [(2)]

(1) Agric. Res. Center (A.R.C.), Giza Egypt
(2) Biochem. Dept. Fac. Agric. Cairo Univ. Giza, Egypt

ABSTRACT

Hyperlipidemia is a condition of elevated lipid level in blood. Hyperlipidemia is a major cause of atherosclerosis which atherosclerosis related conditions like coronary heart disease (CHD), ischemic cerebrovascular disease, peripheral vascular disease and pancreatitis1-2. The increase in lipids like low density lipoproteins (LDL-c), cholesterol (esters derivatives) and triglycerides are mainly responsible for this condition. This present study was designed to explore the efficiency of the buffalo milk dose in controlling hyperlipidemia . 25 male albino rats were used in this investigation which divided into five groups, The first one was used as a normal health control group (G1), while the remaining four groups were feeding with high-fat diet in order to induce hyperlipidemia. The hyperlipidemic group was divided into hyperlipidemic control group (G2), groups of the treated buffalo milk according to the amount of buffalo milk divided into 1 ml (G 3), 1.5 ml (G 4) and 2 ml (G 5) for 100 g body weight, and the period of the study was for 7 weeks. Blood lipid profile, liver function and kidneys function was measured at the end of the experimental period.

This study revealed that there was a significant changes in blood parameters of treated groups in comparison with the other group without any treatment. The buffalo milk group exhibited a significant improvement in lipid profile compared to hyperlipidemic control animals. This study documented the efficiency of the buffalo milk in

controlling of hyperlipidemia and improvement of kidneys function and liver function. So an extensive research on the buffalo milk is still needed to demonstrate this mechanism.

Key words: Antitoxicants,Buffalomilk,Hypocholesterolemia, Hypolipidemia.

INTRODUCTION

Hyperlipidemia is accompanied by elevated serum total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (vLDL-c), and decreased high-density lipoprotein cholesterol (HDL-c) levels (**Rahaman *et.al.*,2013**).. It is associated with cardiovascular diseases (CVD) including coronary heart disease and stroke which is one of the leading causes of mortality in both developed and developing countries, accounting 30% of all worldwide deaths per year (**Helal *et.al.*, 2011**). Hyperlipidemia is considered as the primary mediator of a cascade of atherosclerosis (**Balakmar *et.al.*, 2007**). Atherosclerosis is a cardiovascular and fibro proliferative inflammatory disease commonly associated with age and "dietary-related factors" in humans. In animals, atherosclerosis is rarely noticed.

Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood. Cholesterol is a waxy, fat-like substance that is produced in the body and obtained from foods that come from animals (particularly egg yolks, meat, poultry, fish, and dairy products). The body needs this substance to build cell membranes, make certain hormones, and produce compounds that aid in fat digestion. Hypercholesterolemia has been associated with enhanced oxidative stress related to increased lipid peroxidation. Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach and efforts have been made to identify the antioxidative functions of various medicinal plants (**Adaramoye *et.al.*, 2008**).

High fat diet is the term used to denote raised serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol and triglycerides. Nowadays, the treatment of hyperlipidemia and

cardiovascular diseases with plants has increased in recent years, consumer demand for healthy, safe, natural, and fresh foods that require only a minimum effort and time for their preparation has increased, (**Ramos *et.al.*, 2012**).

Exogenous hypercholesterolemia causes fat deposition in the liver and depletion of the hepatocyte population; it can also cause malfunctioning of the liver, which apparently follows micro vesicular stenosis due to the intracellular accumulation of lipids (**Assy** *et.al.*, 2000). In addition, feeding cholesterol rich diets induces free radical production (ROS), followed by oxidative stress and hypercholesterolemia (**Bulur *et.al.*, 1995**).

Reactive oxygen species (ROS) are by products of normal cellular metabolism. Low and moderate amounts of ROS have beneficial effects on several physiological processes, including killing of invading pathogens, wound healing, and tissue repair processes. However, excessive levels of ROS lead to cell damage and apoptosis and play an important role in cancer, neurodegenerative disorders, and coronary heart and inflammatory bowel diseases (**Valko *et.al.*, 2007**).

The presence of antioxidant peptide segments in proteins may help to explain why dietary protein intake can promote animal and human health beyond the normal nutritional benefits exerted. During gastrointestinal digestion of parent proteins there is a slow and continuous release of antioxidant peptides and amino acids, which protect the gastrointestinal tract itself and prevent the onset of oxidative stress (**Tagliazucchi *et.al.*, 2016**) .

Different studies have suggested that bioactive peptides, released from dietary proteins during digestion, could exert metabolic and physiologic actions by acting on specific targets at the digestive level or after absorption(**Jahan *et.al.*, 2011**, **Shimizu** and **Son (2007**) and **González *et.al.*, 2018**). Many food proteins possess antioxidant peptide sequences which are released during the gastrointestinal digestion process. The digestion by gastrointestinal enzymes is a natural process for the release of antioxidant peptides, differing from oral administration of commercially available bioactive peptides which are subjected to degradation and, hence, inactivation, after oral intake. Furthermore, an important aspect is the low bioavailability of food-derived antioxidant peptides, which determine their accumulation in the gastrointestinal tract, suggesting that the major physiological effects could be locally explicated (**Tagliazucchi *et.al.*,**

2016) .

Several studies showed many bioactive peptides in different dairy species, such as bovine, ovine, and caprine milk respectivety reported by **Park** and **Nam**. (**2015**), **Capriotti *et.al.,*** (**2016**) and **Sánchez *et.al.,*** (**2014**). In spite of few studies have been conducted on buffalo-milk or dairy products. Buffalo milk contributes to 13% of the total milk production in the world and is produced abundantly, where the buffalo find a favorable environment (**Brescia *et.al.,* 2005**). Buffalo milk is highly suitable for the manufacturing of a wide range of value-added dairy products (**Ahmad, *et.al.,* 2014**).

Accordingly, the purpose of the present study was to investigate the effect of buffalo milk against high cholesterol diet induced hypercholesterolemia in rats. Also, to determine whether buffalo milk when administered to hypercholesterolemic induced-rats beneficial for prevention and treatment of hypercholesterolemia complications such as obesity.

The present studies were undertaken to investigate the effect of buffalo milk on their biological and nutritional evaluation through an animal experiment as following studies:

1- Chemical composition of buffalo milk.
2- The biological evaluations of buffalo milk by 1 ml, 1.5 ml and 2 ml as

antioxidants agents on blood analysis, i.e., glucose, lipid profile, liver function, kidney function and heart function (LDH activity).

MATERIALS AND METHODS

Preparation of samples :

Samples of the present study were collected from the hard form of the Faculty of Agriculture, Cairo University. The samples were used fresh warm milk at arrived in the morning as treatment doses of oral by ingestion using stomach tube which was the dose 1 ml, 1.5 ml and 2 ml milk / 100 g body weight / day after day in animal experiments.

General chemical analysis:

The determination of moisture, crude protein, total lipids and ash were done, nitrogen free extract was calculated by difference (as total carbohydrate), deducing the percentage of ash, crude protein and total lipids from 100 according to **A.O.A.C.** (**2000**).

Biological effects of investigated plans:

Experimental animals :

Spraque-Dawley albino male, rats weighing 130 – 160 g were used for the present study. The animals were obtained from Agriculture Research Centre (A.R.C.), Giza, Egypt. The animals were raised in the animal house of (A.R.C.). The rats were kept under normal laboratory conditions (temperature remain 25± 2°C) for 48 h before the initiation of experiment. During this period, the animals were allowed free access of water and basal diet. Food consumption and body weight were monitored daily for each animal.

Animal diet:

a. Health diet

The control diet is composed of 15% casein **Lane-Peter** and **Pearson** (**1971**), 10% corn oil, 5% cellulose, 4% salt mixture (**Schneeman *et.al.,* 1989**), 1% vitamins mixture (**Philip *et.al.,* 1993**) and starch 65%.

b. Hyperlipidemic diet

On the other hands, high fat diet was similar to the control diet but differ in fat content which was 20% sheep fat; 2% cholesterol; 0.25% bile salts and starch 42.75%.

Rats were given high fat with cholesterol diet for 4 weeks (20 animals). At the end of the experimental feeding period blood samples were taken from the suborbital vein to test for blood cholesterol level. A high level of serum cholesterol was considered as an indication to hypercholesterolemia. The hyperlipidemic rats of the second group were subdivided into 4 groups (5 rats/group):

Experimental design:

After a period of adaptation (48 h), 25 adult rats weighing between 130 - 160 g were divided into five groups :

Group (1): (Healthy control group) (G1) : Rats were given normal diet (5 rats) as health control**.**

Group (2): (Hyperlipidemic control group) (G2) : Rats were fed on high fat/high cholesterol diet without any treatment**.**

Group 3: (Buffalo Milk., Dose 1 ml). (G3) : Rats were fed on high fat/high cholesterol diet next to dose of treatment 1 ml /100g body weight / day after day from buffalo milk**.**

Group 4: (Buffalo Milk., Dose 1.5 ml). (G4) : Rats were fed on high fat/high cholesterol diet next to dose of treatment 1.5 ml /100g body weight / day after day from buffalo milk**.**

Group 5: (Buffalo Milk., Dose 2 ml). (G5) : Rats were fed on high fat/high cholesterol diet next to dose of treatment 2 ml /100g body weight / day after day from buffalo milk**.**

During the experimental period (7 weeks) food intake, body weight were determined and feed efficiency was calculated for individual rat of the present experiment tell to the end of studies.

At the end of 7 weeks interval, rats were fasted overnight and then the animals were killed by decapitation and blood samples were collected from each rat and subjected to centrifugation tube at 3000 xg to obtain the serum which was kept in the deep freezer for the subsequent investigation.

Blood biochemical analysis:

Determination of serum glucose:

Enzymatic determination of serum glucose was carried out calorimetrically according to the method of **Trinder** (**1969**).

Liver function:

For liver function GOT (AST) and GPT (ALT) activities in the serum were determined calorimetrically according to the method of **Reitman** and **Frankel** (**1957**).

Kidneys function:

For kidneys function urea contents in serum were determined colorimetrically according to the methods described by **Caraway** (**1975**) and the determination of serum creatinine content was carried out colorimetrically according to the methods described by **Faulkner** and **King** (**1976**).

Serum lipid and lipoprotein profile:

For serum lipid profile, such as total lipids, total triglycerides and total cholesterol levels were determined colorimetrically according to the methods of **Knight *et.al*.,** (**1972**), Fossati and **Prencipe** (**1982**) and **Allain *et.al*.,** (**1974**) respectively. But for lipoprotein profile such as HDL-cholesterol and LDL-cholesterol levels were determined according to **Warnick *et.al*.,** (**1983**) and **Bergmenyer (1985)** respectively, vLDL-cholesterol was calculated by using the equation (Total triglycerides\5) which was described by **Fiedewaid *et.al*.,** (**1972**).

Statistical analysis:

All data pooled through this study were proceeded by General Linear Model procedure (GLM) of the statistical analysis system described in SAS User's Guide (**SAS Institute**, **2000**)., The

significance of the differences among treatment) groups were tested using Waller-Duncan k-ratio (**Waller** and **Duncan**, **1969**). All statements of significance were based on probability of $P<0.05$

RESULTS AND DISCUSSION

Chemical composition of buffalo milk:

Buffalo milk considered from famous Dairy in Egypt. The chemical composition of buffalo milk are shown in **Table** (1). It could be observed that buffalo milk are contains protein, fat, elements and carbohydrates, the buffalo milk contain amount large of vitamin A, Potassium (K), Iron (Fe), Zinc (Zn) and Copper (Cu). Recently, a special interest was given to milk vitamins and elements as good antioxidants, antilipidimic, anticholesterolemic agents and for detoxification as antitoxins. For that the present studies analyzed the elements, vitamins, etc. compounds of buffalo milk. The chemical analysis of buffalo milk for their chemical compounds (**Table** 1) showed the presence of 17 compounds in a good amounts which considered as a beneficial diet.

Table 1: Nutritious Values in Buffalo Milk

No.	Components	Buffalo	
1	Water	82.5	%
2	Ash	0.8	%
3	Protein	4.2	%
4	Lactose	5.4	%
5	Fat	7.1	%
6	Total solids	16.19	%
7	Vitamin A	0.23	mg/100 ml
8	Vitamin B1 (Thiamin)	0.1	mg/100 ml
9	Vitamin B2 (Riboflavin)	0.35	mg/100 ml
10	Vitamin C	3	mg/100 ml
12	Sodium (Na)	52	mg/100 ml
13	Potassium (K)	142	mg/100 ml
14	Calcium (Ca)	180	mg/100 ml
15	Phosphors (p)	86	mg/100 ml
16	Magnesium (Mg)	17	mg/100 ml
17	Iron (Fe)	0.2	mg/100 ml
18	Zinc (Zn)	0.22	mg/100 ml
19	Copper (Cu)	0.01	mg/100 ml

Effects of different therapeutic doses of buffalo milk on body weight gain and feed efficiency of the experimental rats:

The data presented in **Table (2)** showed that the hypercholesterolemic diets caused significant increase in food intake , body weight gain and feed efficiency of male albino rats. The feeding on different therapeutic doses of buffalo milk for hypercholesterolemic rats resulted insignificant changes in food intake at that of normal control and hypercholesterolemic groups which ranged between 755 to 762 g, but body weight gain and feed efficiency showed significant improvement at the diseased normal controls.

The gain in body weight at the end of the experimental period (7 week) for the normal control was 70 g, while for the diseased control rats was 95 g. Feeding on buffalo milk by dose 1 ml, 1.5 ml and 2 ml. showed body weight gain of 89, 88 and 86 g. This means that treatments with doses 2, 1.5 ml showed the lowest body weight gain which had the highest effect against the hyperlipidemia. These values were still more than that of normal health control.

Table 2. Body weight gain ,food intake and food efficiency of the experimental animals

Treatment	Initial B.W (g)		Final B.W (g)		B.W gain (g)		feed intake (g)		feed efficiency %100	% at control
N.control health group (G1)	160	± 8^{a}	230	± 16^{b}	70	± 3.8^{b}	758	± 53^{a}	9.23^{b}	100
Intoxicated Hyper lipidimic control group (G2)	164	± 6^{a}	259	± 16^{a}	95	± 10.2^{a}	756	± 47^{a}	12.57^{a}	136
Buffalo 1 ml group (G3)	163	± 7^{a}	252	± 15^{a}	89	± 5.6^{ab}	762	± 55^{a}	11.68^{ab}	126
Buffalo 1.5 ml group (G4)	159	± 9^{a}	247	± 12^{ab}	88	± 5.5^{ab}	756	± 42^{a}	11.64^{ab}	126
Buffalo 2 ml group (G5)	161	± 6^{a}	247	± 13^{ab}	86	± 3.8^{ab}	755	± 54^{a}	11.39^{ab}	123

Each value represents the mean of 5 rats (Mean ± SE) after7 weeks experimental period.
Means in the same column followed by the same letter are not significantly different at (P<0.05).

Effects of different therapeutic doses of buffalo milk on lipid profile of the experimental rats:

Results of the evaluation of five studied samples as hypocholesterolemic, hypolipidemic agents detoxification in albino rats were statistically analyzed **Tables (3 and 4)** presented the lipid and lipoprotein profiles of the five groups at the end of the experimental period to treatments of hyperlipidemia as a toxic agent

(7 weeks). The data pointed out a significant increase in total lipids, cholesterol and triglycerides when rats fed on the high fat/cholesterol diet, the values amounted 214, 283 and 186 % respectively relative to that of normal control. The results presented in the same tables show the effect of ingestions with different doses of buffalo milk treatment on lipid profile of hyperlipidemic animals. In case of hypolipidemic animals, the ingestions of different dose of buffalo milk treatments exhibited different effects on the blood lipid profiles the three treatments significantly alleviated the harmful of hypolipidemia where the dose of buffalo milk 2 and 1.5 ml treatments were the most effective as hypolipidemic and hypocholesterolemic agents than that of I ml dose. On the other hands, the doses of buffalo milk 2 and 1.5 ml treatments possessed remarkable hypolipidemic and hypocholesterolemic activity but the levels of total lipids, cholesterol and triglycerides in blood which were still higher than that of the control. The results are in agreement of those data of blood lipoproteins content of the present study **Table (4)** except the HDL-cholesterol (HDL-c).

The effects of different doses of buffalo milk treatments on hyperlipidemic animals was also different. Hyperlipidemia increased blood LDL-c and vLDL-c contents, but the increase of HDL-c. This drastic effect of hyperlipidimia was improved by the present treatments whereas, increased HDL-c content of blood of hyperlipidemic animals observed after treatments by feeding on different doses of buffalo milk treatment. In contrast, LDL-c and vLDL-c contents in blood of hyperlipidemic rats were significantly alleviated by the present treatments which were reduced but the levels were still higher than that of control animals. From the present results the hypolipidemic and hypocholesterolemic effects of the different treatments can be arranged in the following increasing order:

buffalo milk (1 ml) < buffalo milk (1.5 ml) < buffalo milk (2 ml)

Table 3. Lipid fraction of the experimental animals

Treatment	Total Lipid			Total Cholesterol			Tri Gylciraide		
	mg / dL		% at control	mg / dL		% at control	mg / dL		% at control
N.control health group (G1)	268	± 14^{c}	100	129	± 11^{d}	100	134	± 18^{d}	100
Intoxicated Hyper lipidimic control group (G2)	574	± 59^{a}	214	365	± 39^{a}	283	249	± 23^{a}	186
Buffalo 1 ml group (G3)	557	± 32^{a}	208	310	± 22^{b}	240	222	± 13^{b}	166
Buffalo 1.5 ml group (G4)	515	± 21^{ab}	192	294	± 14^{bc}	228	218	± 17^{b}	163
Buffalo 2 ml group (G5)	464	± 26^{b}	173	280	± 16^{c}	217	208	± 12^{c}	155

Table 4. Lipoprotein profile of the experimental animals

Treatment	HDL-C			LDL-C			vLDL-C		
	mg / dL		% at control	mg / dL		% at control	mg / dL		% at control
N.control health group (G1)	38	± 2.1^{a}	100	72	± 3^{d}	100	26	± 2.3^{d}	100
Intoxicated Hyper lipidimic control group (G2)	18	± 1.3^{c}	48	282	± 23^{a}	392	53	± 2.4^{a}	205
Buffalo 1 ml group (G3)	20	± 1.2^{ab}	52	233	± 8^{b}	324	51	± 2.8^{b}	195
Buffalo 1.5 ml group (G4)	24	± 1.4^{b}	64	208	± 12^{c}	290	50	± 3.4^{b}	191
Buffalo 2 ml group (G5)	24	± 1.5^{b}	64	203	± 7^{c}	283	46	± 2.4^{c}	178

- Each value represents the mean of 5 rats (Mean ± SE) after7 weeks experimental period.
- Means in the same column followed by the same letter are not significantly different at (P<0.05).

Effects of different therapeutic doses of buffalo milk on liver function and kidneys function of the experimental rats:

The effects of the present antilipidemic agents on liver and kidneys function of hyperlipidemic rats were statistically analyzed and illustrated in **Tables (5 and 6)**.The results showed that hyperlipidemia and hypercholesterolemia significantly stimulated AST and ALT activity. relative to healthy control. These stimulations of AST activity indicated slight liver cell necrosis and the magnitude of increase correlated with the extent necrosis (**Murray *et.al.*, 2006**). The all different dose of buffalo milk treatments (as hypolipidemic and antitoxins agents) into diseased animals were characterized by an alleviation and normalization in the both transaminases activity of serum. These are conflicting report on the changes in the blood alterations which showed that the transaminases content in serum have been thought to be significant in the pathogenesis of lipidemia and

cholesterolemia. The increases in serum transaminases activity is unlikely to be due to damage in liver (**Murray *et.al.*, 2012**). The treatment with the present antilipidemic agents (buffalo milk) was characterized by attenuation in both transaminases activity content of seurm in diseased animals.

The effects of buffalo milk treatments on kidneys functions of hyperlipidemic animals was done by the determinations of blood urea and creatinine which were statistically analyzed. The data in **Table (6)** showed that hyperlipidemia and hypercholesterolemia caused a significant increase at control in urea and creatinine contents of the diseased animal blood. The different dose of buffalo milk treatments as lipotropic factors for present diseased rats produced a significant improvements in the both parameters of kidneys function. The highest treatment effect on kidneys function was detected by the dose of 2 and 1.5 ml buffalo milk. The feeding of buffalo milk produced lower improved effects in the same respect. These effects were similar to those of the liver functions.

Table 5. Liver function of the experimental animals

Treatment	AST			ALT			Ratio AST/ALT		
	U/L		% at control	U/L		% at control			% at control
N.control health group (G1)	117	± 9^{d}	100	60	± 4^{c}	100	1.95	± 0.12^{b}	100
Intoxicated Hyper lipidimic control group (G2)	229	± 16^{a}	196	90	± 5^{a}	150	2.55	± 0.21^{a}	131
Buffalo 1 ml group (G3)	216	± 15^{b}	185	86	± 4^{ab}	144	2.51	± 0.20^{a}	128
Buffalo 1.5 ml group (G4)	188	± 18^{c}	161	77	± 3^{b}	129	2.43	± 0.16^{a}	125
Buffalo 2 ml group (G5)	181	± 13^{c}	155	76	± 5^{b}	126	2.40	± 0.14^{a}	123

Table 6. Kidneys function of the experimental animals

Treatment	Urea			Creatinine		
	mg / dL		% at control	mg / dL		% at control
N.control health group (G1)	58	± 7^{b}	100	0.9	± 0.04^{c}	100
Intoxicated Hyper lipidimic control group (G2)	66	± 4^{a}	113	1.6	± 0.02^{a}	173
Buffalo 1 ml group (G3)	65	± 5^{a}	112	1.4	± 0.02^{b}	151
Buffalo 1.5 ml group (G4)	63	± 4^{ab}	109	1.3	± 0.03^{b}	145
Buffalo 2 ml group (G5)	63	± 4^{ab}	109	1.3	± 0.04^{b}	145

- Each value represents the mean of 5 rats (Mean ± SE) after7 weeks experimental period.
- Means in the same column followed by the same letter are not significantly different at (P<0.05).

Effects of different therapeutic doses of buffalo milk on glucose function and heart function of the experimental rats:

The results presented in **Table (7)** indicated that the hyperlipidemia effects on blood glucose and heart function (LDH activity) in blood which were statistically analyzed. These results showed that hyperlipidemia significantly changed the values of blood glucose level relative to healthy control animals. Also, the present data showed that the LDH activity was significantly increased by lipidemia or cholesterolemia. In contract the data observed that there occurred a significant increase in seurm heart function value in the diseased animals. Also, the results of **Table (7)** pointed out that the feeding antioxidant diets buffalo milk caused an improvement in the blood glucose and heart function (LDH activity) values.

It can reported that buffalo milk treatment using as antioxidant diets alleviated the harmful effects of hyperlipidemia on heart function. These values were still far from that of normal healthy control animals.

Table 7. Blood glucose and L D H activity of the experimental animals

Treatment	Glucose			L D H		
	mg / dL		% at control	U / L		% at control
N.control health group (G1)	70	± 3 ^{c}	100	1787	± 104 ^{b}	100
Intoxicated Hyper lipidimic control group (G2)	126	± 6 ^{a}	180	2698	± 114 ^{a}	151
Buffalo 1 ml group (G3)	100	± 4 ^{b}	143	2591	± 185 ^{b}	145
Buffalo 1.5 ml group (G4)	97	± 5 ^{b}	138	2573	± 131 ^{b}	144
Buffalo 2 ml group (G5)	96	± 4 ^{b}	137	2538	± 197 ^{b}	142

- Each value represents the mean of 5 rats (Mean ± SE) after7 weeks experimental period.
- Means in the same column followed by the same letter are not significantly different at (P<0.05).

Dairy products play an important role in human nutrition. Chemotherapy has been shown to be a source of proteins, soluble dietary fiber, calories, certain minerals, vitamins and antioxidants such as vitamin A and vitamin E iron, copper, zinc and potassium. Current results can be reported using buffalo milk as a food remedy against lipidemia and cholesterolemia as well as toxins which had significantly improved effects on lipid profile, heart enzyme activity, kidneys function and liver function of blood. Also, either improved doses high of buffalo milk treatments have alleviated the harmful effects of hyperlipidemia and hypercholesterolemia. Major

contributors to the accumulation of cholesterol in the cell arteries during the development of atherosclerosis include several factors such as the high level of serum cholesterol (**Joanne *et.al.*, 2005**), inhibit paraoxinase blood (**Lee *et.al.*, 2008**), increase serum cholesterol level (**Aviram *et.al.*, 2000**). With regard to the most effective factor is the increase of oxidative stress (**Van Lieshout *et.al.*, 2003**). This has been presented in a number of studies to look for feeding on food rich in protein and antioxidant that can be preventive the atherosclerosis among exposed individuals. The improvement in lipid profile of blood could have been referred to a multi factors near the role of amino acids of protein, dietary fibers and antioxidants may play a good part in this action. The beneficial treatment of buffalo milk on cholesterol-bearing rats has shown that this milk fiber has the potential to lower total cholesterol levels and LDL-c in serum. Absorption of bile salts by (soluble dietary fibres) SDF results led to changes in cholesterol metabolism, (loss of cholesterol and lack of bile salts in the intestines to form micelle) which results in absorption of fat and cholesterol. Increased fecal bolts dilutes bile acid in the lower intestine, producing short-chain fatty acids. In particular, propionate has been suggested to inhibit the synthesis of cholesterol in the liver (**Tharanathan** and **Mahadevamma**, **2003**). Also, amino acids of the present dietary food used as precursors of lipoprotein, including HDL-c which has 50% of its structure protein (**Elliott** and **Elliott**, **2001**), that stimulates via protein biosynthesis and also the three hypophyseal peptides the fatty acids β-oxidation (catabolism) (**Chatterjea** and **Shinde**, **2002**). In addition, these amino acids are considered as lipotropic factors used in the protein biosynthesis of β-oxidation enzymes, glucose oxidase, antioxidant enzymes (SOD and catalase) and other protein factors. These enzymes stimulate fat oxidation rate, and antioxidative power, suggesting that dietary protein outputs its hypolipidemic effects by stimulating β-oxidation of fatty acids through their esterification (**Wang and Jones**, **2004**),which is believed to possess a strong antioxidant property (**Kaplan *et.al.*, 2001 and Li *et.al.*, 2006**). Also, it showed that buffalo's milk contained an antioxidant strength appreciated. This means that the effects of buffalo milk associated with the lack of blood lipids are mainly related to the antioxidant elements, but they are related to protein for buffalo's milk. The chemical analysis pattern (**Table** 1) which seems to agree with **Li *et al***., (**2006**). It is clear that these chemical compounds are detected in

buffalo's milk responsible for the antioxidant effect and cholesterol reduction. The effect of hypoglycaemia may be either due to the effect of inhibitory on lipid absorption or increase in receptors LDL-c.

The present work of antioxidants for buffalo milk, suggested that its effect was due to the ability of antioxidants to transfer electron anion free radicals, metal catalyst reaction, enzymes active antioxidants (**Spencer *et.al.*, 2001**).

Therefore, the peripheral mechanism of action of current treatments and especially the high doses of buffalo's milk may be the main activity responsible for the subsequent activity of inflammation of protein and antioxidants, although other target organs (liver and kidneys) can't be eliminated. This may be due to the fact that the milk is composed of an optimal combination agent such as protein and antioxidant agent like as vitamin A, C, E and compounds B beside to iron, copper, zinc, potassium etc.

This was confirmed by the present observations of results, which showed that biological activity has been returned to several factors of buffalo milk. Current results are in harmony with each other. Thus stimulating the transaminases activity content in the blood of rats studied, which has been used largely as an indicator of liver function. Also, urea and creatinine in blood, confirmed each other as well as previous results. Therefore, further studies are needed to assess the biochemical effects and mechanism of the factors studied for lipids and antioxidants as agents antilipidemic and anticholesterolemic which may be used in edible foods to recommend their use as hypodermic food additives.

REFERENCES

Adaramoye, O.A., O.Akintayo, , J.Achem, and M.A. Fafunso, (2008). Lipid-lowering effects of methanolic extract of vernonia amygdalina leaves in rats fed on high cholesterol diet. Vascular health and Risk Management. 4: 235-- 241.

Ahmad, S.; F.M.Anjum,; N.Huma,; A.Sameen, and T.Zahoor, (2014). Composition and physico-chemical characteristics of buffalo milk with particular emphasis on lipids, proteins, minerals, enzymes and vitamins. J. Anim. Plant Sci., 23: 62–74.

Allain, C.C.; L.S.Poon,; C.S.Chan,; W. Richamand, and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. Clin. Chem., 20 (4): 470.

A.O.A.C. (2000). Official Methods of Analysis of Association of Official Analytical Chemists, 17 th ed. Washington D.C., USA.

Assy, N., K.Kaita, , D.Mymin, , C.Levy, , B.Rosser, and G.Minuk, (2000). Fatty infiltration of liver in hyperlipidemic patients. Dig. Dis. Sci. 45: 1929–1934.

Aviram, M.; L. Dorenfeld,; M.Rosenblat,; N.Volkova,; M.Kaplan, and T.Hayek, (2000). Pomegranate juice consumption reduces oxidative stress, low density lipoprotein modifications and platelet aggregation: studies in the atherosclerotic apolipoprotein E deficient mice and in humans. Am. J. Clin. Nutr., 71: 1062–1076.

Balakumar, P., S.Jindal, , D.I. Shah, and M.Singh, (2007). Experimental models for vascular endothelial dysfunction. Trends Med. Res., 2(1): 12-20.

Bergmenyer, H.U. (1985). Methods of Enzymatic Analysis, vol. VIII (third ed.), Verlag Chemie, Weinheim – Deerfield Beach – Basel 154–160.

Brescia, M.A.; M.Monfreda,; A.Buccolieri,; C.Carrino, (2005). Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. Food Chem., 89: 139–147.

Bulur, H., G.Ozdemirler, , B.Oz, , G.Toker, , M.Ozturk, and M.Uysal, (1995). High cholesterol diet supplemented with sunflower seed oil but not olive oil stimulates lipid peroxidation in plasma, liver, and aorta of rats. J. Nut. Biochem. 6: 547–550.

Capriotti,A.L.; C.Cavaliere,; S.Piovesana,; R.Samperi,; A.Laganà, (2016). Recent trends in the analysis of bioactive peptides in milk and dairy products. Anal. Bioanal. Chem., 408 : 2677–2685.

Caraway, W.T. (1975). Standard methods of clinical chemistry. Academic press, New York and London, 4: 239.

Chatterjea, M.N. and R.Shinde, (2002). Text Book of Medical Biochemistry. Jaypee Brothers Medical Publishers, New Delhi.

Elliott, W.H. and Elliott, D.C. (2001). Biochemistry and Molecular Biology 2nd (ed.), Oxford University Press, Oxford.

Faulkner, N.R. and J.W. King, (1976). Fundamental of clinical chemistry, 2nd ed., Tietz (ed.), Sannders Philadelphia, 994-998.

Fiedewald, W.T.; R.I. Levy, and D.S. Fredrickson, (1972). Estimation of concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 18: 499–502.

Fossati, P. and L.Prencipe, (1982). The determination of triglyceride using enzymatic methods. Clin. Chem., 28: 2077.

González-Montoya, M.; B.Hernández-Ledesma,; J.M.Silván,; R.Mora-Escobedo, and C.Martínez-Villaluenga, (2018). Peptides derived from in vitro gastrointestinal digestion of germinated soybean proteins inhibit human colon cancer cells proliferation and inflammation. Food Chem., 242 : 75–82.

Helal, E.G.E., F.A.Eid, , M.Amira, and S.E.A. El-Wahsh, (2011). Effect of fennel (Foeniculum vulgare) on hyperlipidemic rats. Egypt. J. Hosp. Med., 31(754):1-28.

Jahan-Mihan, A.; B.L.Luhovyy,; D.El Khoury, and G.H. Anderson, (2011). Dietary proteins as determinants of metabolic and physiologic functions of the gastrointestinal tract. Nutrients, 3 : 574–603.

Joanne M.; M.D.Murabito,; J.Michael; P. Pencina,; H.Byung,; P.Nam,; B.Ralph, Sr.D'Agostino,; J.Thomas; M.D.Wang,; M.D.Donald Lloyd-Jones,; W.F.Peter,; M.D.Wilson,; J.Christopher, and M.D. O'Donnell, (2005). Sibling Cardiovascular Disease as a Risk Factor for Cardiovascular Disease in Middle-aged Adults. J. Am. Med. Asso. 294: 3117-3123.

Kaplan, M.; T.Hayek,; A.Raz,; R.Coleman,; L.Dornfeld,; J.Vaya, and M.Aviram, (2001). Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. J. Nutr., 131(8): 2082 - 2089.

Knight, J.A.; S.J. Anderson, and J.M. Rawle, (1972). Chemical basis of the sulfo-phosphovanillin reaction for estimating serum total lipids. Clin. Chem., 18 (3): 723.

Lane-Peter, W. and A.E. Pearson, (1971). Dietary require In The laboratory animal principles and practice". Academic press. London and New York.

Lee, M. S.; J. Y.Pairk,; H.Freake,; I. S. Kwun, and Y.Kim, (2008). Green tea catechin enhances cholesterol-7-alpha-hydroxylase gene expression in Hep.G2. Cells. Sr. J. Nutr., 99(6): I 182- 1185.

Li, Y.; J.Yang,; J.Wei,; J.Xu,; and S.Cheng, (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem., 96(2): 254-260.

Murray, R.K.; D.K. Granner, and V.W. Rodwell, (2006). Harper's Illustrated Biochemistry, 27th ed. McGraw-Hil Education Casia.

Murray, R.K.; D.A.Bender, , R. M.Bothan,; P. J. Kennel, V.W.Rodwell, , and P. A. Weil (2012). Harper's Illustrated Biochemistry, 29th ed. Large Medical Book.

Park, Y.W. and M.S. Nam, (2015). Bioactive Peptides in Milk and Dairy Products: A Review. Korean J. Food. Sci. Anim. Resour., 35: 831–840.

Philip G.R.; H.N. Forrest and C.F. George, (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1935-1951.

Rahaman, M.A., D.Vel, , B.Janardhan, , K.N. Pragathi, and R. Deep, (2013). Evaluation of the antihyperlipidemic and anti-atherosclerotic activities of ethanolic extract of Cissus pallida in atherogenic diet fed rat. Int. J. Pharm. Res. Sch., 2(2): 1-3.

Ramos, M., A. Moreno, G. Cevallos, M. Navarro, L. Siciliano, H. Mondragón and M. Ortega, (2012). Hypolipidemic Effect of Avocado (*Persea americana* Mill) Seed in a Hypercholesterolemic Mouse Model. Plant Foods Hum Nutr, 67: 10-16.

Reitman, S. and S.Frankel, (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Anal. J. Clin. Path., 28 (56): 56.

Sánchez-Rivera, L.; D.Martínez-Maqueda,; E.Cruz-Huerta,; B.Miralles, and I.Recio, (2014). Peptidomics for discovery, bioavailability and monitoring of dairy bioactive peptides. Food Res. Int., 63: 170–181.

SAS institute (2000). SAS/STAT User's Guide. SAS Institute Inc., Cary, NC.

Schneeman, B.O.; R.Rice, and B.D. Richter, (1989). Reduction of plasma and hepatic triacylglycerides with whole milk containing diets in rats. J. Nutr., 119: 965-970.

Shimizu, M. and D.O. Son, (2007). Food-derived peptides and intestinal functions. Curr. Pharm. Des., 13 : 885–895.

Spencer, J.P.; H.Schroeter,; A.J.Crossthwaithe,; G.Kuhnle,; R.J. Williams, and C.Rice-Evans, (2001). Contrasting influences of glucuronidation and O-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts. Free Radic. Biol. Med., 31(9):1139-1146.

Tagliazucchi, D.; A.Helal,; E.Verzelloni, and Conte, (2016). A. Bovine milk antioxidant properties: Effect of *in vitro* digestion and identification of antioxidant compounds. Dairy Sci. Technol., 96: 657–676.

Tharanathan R.N. and S.Mahadevamma, (2003). Grain legumes- a boon to human nutrition. Trends Food Sci. Technol., 14: 507-518.

Trinder, P. (1969). Determination of blood glucose using an oxidation peroxidase system with a non carcinogenic chromogene. Ann. Clin. Biochem., 6: 24.

Valko, M.; D.Leibfritz,; J.Moncol,; M.T.D.Cronin,; M.Mazur, and J. Telser, (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39 : 44–84.

Van Lieshout, M.; C. E. West, and van R.B. Breemen, (2003). Isotopic tracer techniques for studying the bioavailability and bioefficacy of dietary carotenoids, particularly {beta}-carotene, in humans: a review. Am. J. Clin. Nutr., 77(1): 12 - 28.

Waller, R. A. and D. B. Duncan, (1969). A Bayes rule for symmetric multiple comparisons problems. J. Am. Stat. Asso., 64: 1484-1503.

Wang, Y. and P.Jones, (2004). Dietary conjugated linoleic acid and body composition. Am. J. Clin. Nutr., 79 (6): 1153-1158.

Warnick, G.R.; J.Benderson, and J.J.Albers, (1983). Selected Methods. Clin. Chem, 10: 91-99.

تقييم تأثير لبن الجاموس على زيادة الدهون والكوليسترول في ذكور الجرزان البيضاء

هيثم عماد الدين علي [1]– رانيا صابر يوسف [2]– حسام سعد البلتاجي [2]
إمام عبد المبدئ عبد الرحيم[2]

(1) مركز البحوث الزراعية الجيزة (2) كلية الزراعة جامعة القاهرة

زيادة الدهون في الدم هو حالة ارتفاع مستوى الدهون. وفرط شحميات الدم هو أحد الأسباب الرئيسية لتصلب الشرايين والظروف المرتبطة بتصلب الشرايين مثل أمراض القلب التاجية ، وأمراض الأوعية الدموية الدماغية، وأمراض الأوعية الدموية الطرفية والتهاب البنكرياس 1-2. والزيادة في الدهون مثل البروتينات الدهنية منخفضة الكثافة (LDL-c) والكوليسترول (مشتقات استرات) والدهون الثلاثية هي المسؤولة بشكل أساسي عن هذه الحالة. وقد تم تصميم الدراسة الحالية لاستكشاف كفاءة حليب الجاموس في السيطرة على فرط شحميات الدم. تم استخدام 25 جرذ ذكور بيضاء في هذا التقييم الذي تم تقسيمها إلى خمس مجموعات ، استخدمت المجموعة الأولى كمجموعة مراقبة صحية طبيعية (G1) ، بينما كانت المجموعات الأربع الباقية تتغذى على نظام غذائي غني بالدهون من أجل تحفيز فرط شحميات الدم. حيث تم تقسيم مجموعة فرط شحميات الدم إلى مجموعة مراقبة لفرط شحميات الدم (G2) ، مجموعات من حليب الجاموس المعالج وفقًا لكمية حليب الجاموس المعالج به 1 مل (G 3) ، 1.5 مل (G 4) و 2 مل (G 5) تأخذ عن طريق الفم، وكانت فترة الدراسة لمدة 7 أسابيع. تم قياس صورة الدهون في الدم ، وظائف الكبد ووظائف الكلى في الأسبوع السابع.

كشفت هذه الدراسة أن هناك تحسين كبير في قياسات الدم في المجموعات المريضة بالسمنة المعالجة مقارنة مع المجموعة الأخرى دون أي علاج. أظهرت مجموعة حليب الجاموس تحسنًا ملحوظًا في مستوى الدهون مقارنةً بالكنترول (G2) لارتفاع الدهون في الدم. هذه الدراسة وثقت كفاءة حليب الجاموس في التحكم في ارتفاع نسبة الدهون في الدم وتحسين وظائف الكلى ووظائف الكبد. آلية هذا التأثير لا تزال تحتاج لدراسة ، لذلك لا تزال هناك حاجة إلى إجراء بحث واسع حول حليب الجاموس لإثبات هذه الآلية.