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Effect of Some Fermented Milk on Blood Picture of Hypercholesterolemic Rats

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ABSTRACT

This study is designed to determine the effect of some fermented milk (Yakult, Actimel and kefir) on the level of red blood cells (RBCs), packed cell volume (PCV), hemoglobin concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Volume hemoglobin concentration (MCHC) and White Blood Cell Differential Count (WBCs) in adult healthy and hypercholesterolemic male rats (*Sprague- Dawley*). The rats were separated into five groups with each group containing six rats. The first group was control negative (healthy rate), the second was control positive (hypercholesterolemic male), and the other three groups were given fermented milk (Yakult milk, Actimel milk and kefir milk) for 28 days. The result of the current study showed a significant increase ($P<0.05$) in the RBCs count in the groups fed on fermented milk (Yakult milk, Actimel milk and kefir milk) which recorded as (8.59, 7.41, 7.45)×10^6 cell/ mm^3 respectively compared with the positive control group which recorded as 6.35 ×10^6 cell/mm^3. The result also showed an increase in PCV and Hb in groups fed on fermented milk in compared to the control groups. WBCs count increased significantly in groups fed on fermented milk (12.40, 10.90, 12.10) ×10^3cell/ mm^3 compared with the positive control group (9.45 ×10^3cell/ mm^3).

Keywords: Fermented milk, Blood pictures, Hypercholesterolemia.

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INTRODUCTION

Fermented dairy products are some of the oldest and most accepted healthy products in the world; because of the cryopreservation method used in them, they are considered to be sources for therapeutic organisms to perform therapeutic action [(1)]. Fermented milk is a good source of beneficial microorganisms' humans such as *Lactobacillus* and *Bifidobacterium* [(2)]. Previous studies revered to about 65% of functional food is a milk product and more than a million bottles are sold daily in Europe which contain *Lb.bulgaricus, Str. theromophilus* and *Lb. casei* [(3)]. In Japan, millions of bottles of Yakult mero-mero and Yakult are sold daily which contain *Lb.casei*, Middle Eastern countries, especially some Gulf countries, began to use Bifidobacterium in camel milk production[(4)]. In some regions, the increasing demand for dairy products, is evident from the increase in the number of types of products, so the concept of functional or therapeutic foods among other names has become common in the markets, Milk and fermented products are the appropriate nutritional means for eating therapeutic foods such as therapeutic milk and therapeutic cheese. Products containing therapeutic bacteria are being introduced under many names[(5)].

MATERIALS AND METHODS

Yakut milk: prepared according to Tamime and Robinson[(6)] Begin by filtering of milk with a clean gauze then gradually temperature raising so access to 90° C for 30mints then cooling to 42-45° C. then inoculated with milk starter which added in rate of 3% (Prepared by 1:1 mixing of *Lactobacillus casei* and *Bifidobacterium longum*) for 2mints. Mixture put in bottles and incubated at 42-45°C until ferment completed than cooling to 5±2°C.

Actimel milk: milk filtered by a clean gauze then temperature raising gradually to 90 °C for 30 mints, then milk cooling to 42 o -45 °C, and inoculated in rate of 3% with milk starter (prepared by 1: 1 mixing of *Lb. casie* and *Str. thermophiles*) for 2 minutes, mixture put in bottles and incubation at a temperature of 42 -45°C until the milk fermentation complete. Then cooling to 5±2°C pending tests [(6)].

kefir milk: Kefir milk of Turkish origin was obtained from local markets packed in sealed bottles and kept in the refrigerator at a temperature of 5±2°C.

Chemical tests: protein ratio was estimated according to the Kelldal method as described by Hool et al.,[(7)]. Fat ratio estimated according to Kerber method as reported by Min and Ellefson [(8)]. Humidity and Ash percentage estimated according to the method A.O.A.C. [(9)]. pH value was calculated as Hool *et al*.,[(7)].

Preparation of animals: Thirty albino wister rats were used, 2-3 months age and their weights ranged between 180-200 g, were obtained from the College of Veterinary Medicine/Tikrit University. the rats were haused in a plastic cages 6 rats/cage under constant environmental condition (22-25 °C), 12 h/ dark/light cycle and free access to drink water *ad libitum*.

Weighted food preparation: Weighted food was prepared as mentioned in NAS / NRC, [(10)] to contain cellulose 50g /kg, oil 100 g /kg, casein 150.5g /kg, minerals 50g /kg, vitamins 5g/kg, starch 544.5g /kg, glucose 100g /kg.

Preparing a high-calorie diet: The high-calorie diet was prepared using De Meijer *et al*,[(11)] to contain carbohydrates, fat, beef, protein, cholesterol, and a mixture of vitamins and minerals in the amounts of 17, 58, 13, 25, 1 and 0.6%, respectively.

Experimental design: animals randomly distributed into five groups of six animals in each group. Each group-administered daily by oral tubal with 2ml/animal milk depending on the type of treatment. The dose was divided twice by 1ml/animal /12 hours for 28 days. The control group was administered with 2 ml of distilled water.

1- The first group (negative control): that were fed normal nutrition.

2- The second group (positive control): Rats fed on a high-calorie diet (a fatty diet) for the duration of the experiment.
3- The third group: Rats fed on a fat diet with 2 ml from Yakult milk.
4- Fourth group: It was fed on a fat diet with 2 ml from Actimel milk.
5- Fifth group: It was fed on a fat diet with 2 ml from kefir milk.

Biochemical tests: After the end of the experiment period, the rats were prevented from food for approximately 12 hours Fasting, the animals were then drugged with chloroform, then blood was drawn directly from the heart and placed in test tubes that contain the substance (EDTA) for blood tests that included estimating RBCs, Hb, MCV, MCHC, WBCs count [12].

Statistical analysis: The experiment was carried out under Complete Randomized Design (CRD) An analyzed of variance was performed using the Linear Model General Within the ready statistical program SAS [13] In the case of significant differences, use the Duncan test [14] to determine the significance of the differences between the different averages at the probability level (0.05).

RESULTS AND DISCUSSION

Chemical composition of fermented milk: from a table (1) showed that the chemical composition of Yakult, Actimel, and kefir milk used in the study was a protein ratio were 3.77%. 4.21% and 2.98% respectively. Fat ratio were 2.93%, 3.65% and 3.0% respectively. Ash ratio were 0.68%, 0.72% and 0.69% respectively while the pH 4.56, 4.42 and 4.50 respectively. This result agreement with result of AL-Jobouri, [15] who recorded ratio protein, fat, humidity, ash and total acidity in Yakult milk were 3.37%, 3.00%, 88.08%, 0.60% and 0.94% respectively while pH 4.35. also, the result of current study agreement with result of AL-Jashe, [16] who recorded protein ratio, fat ratio, humidity, ash and total acidity in (Actimel milk) were 4.27%, 3.68%, 87.29%, 0.60% and 0.88%, respectively. and results showed by Al-Krenawie, [17] who recorded that the ratio of protein, fat and, humidity in (kifer milk) were 2.98, 3.00 and 89.81% respectively while pH 4.45.

Table 1: Chemical composition of fermented milk

Fermented milk	percentage (%)					pH
	Protein	Fat	Humidity	Ash	Total acidity	
Yakult	3.77^{b}	2.93^{c}	87.95^{b}	0.68^{b}	0.90^{a}	4.56^{a}
Actimel	4.21^{a}	3.65^{a}	87.34^{b}	0.83^{a}	0.83^{b}	4.42^{c}
Kefir	2.98^{c}	3.00^{b}	89.81^{a}	0.69^{b}	0.87^{a}	4.45^{b}

Each value is an average of three experimental samples analyzed in triplicate.

Red blood cells: The results of the statistical analysis showed a probability level ($P \leq 0.05$) for some blood picture parameters of experimental animals dosed for 28 days as in table (2) There was a significant difference in the total number of RBCs and all groups compared to the control group (6.35×10^{6}) cells/mm^3. also, the result showed significant increasing in PCV value in group treated with Yakult, aktimel and kifer fermented milk (41.78, 39.09 and 40.12)% respectively compare with positive control group (32.16%). As for the concentration of (Hb), the results showed that there was a significant difference in the Hb for all groups dosed with fermented milk compared to the positive control group that amounted (10.02)g/dL. The result of current study refers to significant increasing in MCV in treated group (67.58, 64.86 and 66.27) μm^3 in yakult, aktimel and kifer fermented milk compared to the positive control group which is 65.08μm^3. The (MCHC) significant increase fermented feeding in groups in compared to the positive control group which reach to 32.15g/dL. as in table (2).

The decrease in value of Hb, PCV and RBCs in hypercholestromic group occur when distraction more than production [18]. Decrease in RBCs count lead to Decrease in Hb value, Because of the relationship between RBCs, Hb and PCV it decreases with gather in current study, RBCs maybe lysis by cholesterol oxidative stress which lead to precipitation of Heinz bodies in RBSc [19]. The results of the current study showed that the treatment of rats with yakult, actimel and kefir led to a significant increase in The increasing in the value of RBCs, PCV and Hb, The reason is that it contains some antioxidants, including a group that works to prevent the formation of free radicals such as zinc, which is included in the superoxide desmutase enzyme synthesis which in turn prevent oxidative stress and Selenium which is included in the synthesis of Glutathione peroxidase (GSH_PX) which in turn prevent oxidative stress, Others remove the free radicals formed before entering the chain reaction, such as vitamins A and E [20].

Table 2: effect of fermented milk feeding on the complete blood count

Parameters / Groups	**RBCs ($X10^6 mm^3$)**	**PCV (%)**	**Hb (g/dl)**	**MCV (μm^3)**	**MCH (Pg/cell)**	**MCHC (g/dl)**
negative control	6.35 ±0.15 ^{bc}	34.82 ±1.28^{d}	11.14 ±0.76 ^{b}	65.08 ±1.14 ^{b}	20.82 ±0.23 ^{c}	31.99 ±0.86 ^{a}
positive control	5.21	32.16	10.02	74.01	23.80	32.15

	±0.43 [c]	±0.35 [e]	±0.82 [c]	±1.29 [c]	±0.57 [d]	±1.67 [c]
Yakult milk	8.59 ±0.45 [a]	41.78 ±1.32 [a]	14.63 ±0.94 [a]	67.58 ±0.87 [a]	22.59 ±0.67 [a]	33.43 ±1.09 [a]
Actimel milk	7.41 ±0.16 [b]	39.09 ±1.56 [c]	13.38 ±0.82 [b]	64.86 ±1.18 [b]	21.03 ±0.49 [b]	32.43 ±1.90 [b]
kifer milk	7.45 ± 0.65 [b]	40.12 ±1.44 [b]	13.70 ±0.51 [b]	66.27 ±1.72 [b]	21.46 ±0.68 [b]	32.39 ±1.77 [b]

- Values are expressed as mean ± SD for six animals in each group.
- Level of significance $p < 0.05$.

or due to the role of microorganism which increase readiness of Fe, Cu, and folic acid for absorption [21]. increase number of platelets is a healthy sign due to bioactive components of whey proteins [22].

White Blood Cell Differential WBCs Count: The result showed significant increasing in the total number of white blood cells (WBC_S) for all fermented dairy groups, reaching (12,40, 10.90 and 12.10) $\times 10^3$ cells /mm^3 for yakult, aktimel and kifer fermented milk respectively compared with positive control group which 8.20×10^3cells/mm^3. The results showed a significant increase in the number of Lymphocytes in all treated group compared with positive control group which reach to 30.75%. The percentage of granulocytes were decreased significantly in treated groups compared with positive control group reach to 68.78%. as in table (3). This result agreement with Al-shaikh and Doosh, [23] who recorded decrease in WBCs count in animal was fed on high cholesterol diet. While increase in WBCs occur in animal fed on fermented milk. That's due to ability of microorganism to increase diagestibilty, feed metabolism, growing rate, and preservation WBCs from oxidative stress [24]. Researcher Alzubaidi et al, [25] noted fermented milk is a popular food that contains various probiotic lactic acid bacteria (LAB). This study was conducted to evaluate the effect of fermented milk on some physiological parameters which have benefits to health of human. 75 volunteers were selected and divided into 3 groups the volunteers were given fermented milk every morning as a breakfast meal for (21) days. Blood samples were collected at the end of the experiment. The results in this study exhibited a significant decrease ($P<0.05$) in in Hb, PCV, and WBCs.

Table 3: effect of fermented milk on White Blood Cell Differential WBCs Count.

Parameters / Groups	**WBC_S ($\times10^3$/mm^3)**	**Lymphocyte %**	**Monocyte %**	**Granulocyte %**
negative control	9.45 ±1.13[c]	32.54 ±1.36[b]	5.10 ±1.21[c]	62.78 ±1.89[b]
positive control	8.20 ±1.93[d]	30.75 ±1.52[c]	4.30 ±1.76[d]	68.78 ±2.11[a]
Yakult milk	12.40 ±1.21[a]	35.97 ±0.98[a]	5.90 ±1.17[a]	54.03 ±1.06[d]
Actimel milk	10.90 ±1.86[b]	35.35 ±1.16[a]	5.40 ±1.67[b]	57.05 ±1.63[c]
kifer milk	12.10 ±1.24[a]	35.30 ±1.68[a]	5.30 ±1.04[b]	57.20 ±1.15[c]

-Values are expressed as mean ± SD for six animals in each group.
- Level of significance $p < 0.05$.

CONCLUSION

Fermented milk is crucial to keep the people healthy. As per the study It can therefore be concluded that Yakult milk, Actimel milk and kefir milk played a positive role in improving the efficiency of the immune system in hypercholesterolemic rats.

Ethical Clearance: Ethical clearance from the institutional ethical committee obtained for the study.
Conflict of Intrest: Nil
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