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Effect of Some Strains of Lactic Acid Bacteria and Their Mixture on the Level of Fats and Cholesterol in Albino Rats (*Rattus norvegicus*) Male with Hypothyroidism Induced Using Carbimazole

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Abstract: Fortified milk containing *Lactobacillus plantarum, L. casei,* and *L. acidophilus* isolates and their mixture were used in dosing the male albino rats at an age of 9-12 weeks at an average of 23 g with induced hypothyroidism at a concentration of 0.6 $g.kg^{-1}$ of carbimazole. Total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) were estimated. The results showed a significant increase in the level of triglycerides (TG), cholesterol and triglycerides. Low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), with a significant decrease in the level of high-density lipoprotein (HDL) in infected male mice, compared to the control sample, and upon dosing with liquid milk fortified, it returned to its normal level without significant differences from the control group.

Keywords: Carbimazole, Lactic acid bacteria, Hypothyroidism.

Introduction

The World Health Organization (WHO) indicated that cardiovascular diseases would affect about 3.3 million people by the year 2030 by all over the world (Saxena *et al*., 2013) and confirmed that 17.5 million people lost their lives due to cardiovascular diseases and this represents about 31% of all global deaths. The volume of cardiovascular disease continues worldwide, and heart disease affects three times more in people with high cholesterol levels compared to normal people (Nichols *et al.*, 2013). Medicines and chemical treatments have been used as a means to control the level of cholesterol in the blood but may cause potential and unwanted side effects such as digestive discomfort (Wang *et al.*, 2010; Kumar *et al.*, 2012). Functional foods and nutrients have recently received more attention in lowering cholesterol level in the blood (Bartley *et al.*, 2010) and in the past decade, lactic acid bacteria have been confirmed (Wang *et al.*, 2010), where they have been carried out *in vivo* and laboratory experiments. To verify the effect of lactic acid bacteria on lowering the level of fats and cholesterol (Ding *et al.*, 2017; Pavli *et al.,* 2018). Where lactic acid bacteria reduce cholesterol (Gaudana *et al*., 2010).

Therefore, this study aimed to find out the effect of using Fortified milk by some strains of *Lactobacillus* (*L. planetarium*, *L. casei* and *L. acidophilus*) and their mixturein reducing the level of fats and cholesterol in male rats with hypothyroidism induced by carbimazole.

Materials & Methods

Design the experiment

The experiment was carried out in the laboratory of the Department of Food Science in the Faculty of Agriculture at the University of Basrah for the period During April 2019, including a 14-day acclimatization period.

Experiment animals

In this study, using 117 male rats. Their ages ranged from 9 to 12 weeks and the weight ranged from 23 to 25 g. The animals were distributed in plastic cages covered with metal sheets and furnished with sawdust, as the mulch was changed every four days. The cages were placed in the laboratory at a temperature from 22 to 28 °C and a lighting system 12 hours from lighting. The animals were provided with water and feed as in the table (1) manufactured according to the Subcommittee of Laboratory Animal Nutrition (AIN93) Reeves (1996) method. This procedure continued for up to two weeks as an acclimatization period.

Table (1): The rat's food ingredients

Materials	Quantities ($g.kg^{-1}$)
Skim Milk	200
Corn Starch	670
Vitamins and minerals	30
Corn oil	50 ml
Cellulose	50

Experience design

The experimental animals were divided into 9 groups, with 13 rats per group:

Negative control group: (T1) A control group was given water and diet only.

The second group (T2): Positive control group: the diet and water containing

carbimazole 0.6 $g.kg^{-1}$ were given to induce thyroid atrophy.

The third group (T3): were given feed and water containing carbimazole and milk products containing *L. acidophilus*.

The fourth group (T4) were given feed and water containing carbimazole and milk product containing *L. plantarum* bacteria.

The fifth group (T5) were given feed and water containing carbimazole and milk product containing *L. casei* bacteria.

Sixth group (T6) were given feed and water containing carbimazole, *L. acidophillus*, and *L. plantarum*.

The seventh group (T7) were given feed and water containing carbimazole and milk product containing *L. plantarum* and *L. casei*.

The eighth group (T8) were given feed and water containing carbimazole and milk product containing *L. acidophillus* and *L. casei*.

Group IX (T9) were given feed and water containing carbimazole and lactic product containing *L. acidophillus*, *L. casei*, and *L. plantrum*.

Collect blood samples

Blood was drawn from the heart using 5 ml medical syringes in plastic anticoagulant-free tubes for biochemical tests. The serum was separated with a centrifuge at 4000 rpm for 10 minutes and then the serum was separated by a micropipette. Divide the serum in several clean and sterile tubes and kept it frozen at -18°C until the biochemical tests are performed.

Estimation of total cholesterol concentration

The serum cholesterol concentration was estimated using a kit equipped by the French company. Biolab technical support and analyzes were performed according to the instructions provided by the supplied company by reading the samples with a spectrophotometer at a wavelength of 505 nm and according to the following formula:

$$\text{cholesterol mg}.100.\text{cm}^{3^{-1}} = \frac{\text{sample read}}{\text{standard solution read}} \times 200$$

Determination of triglycerides in serum

The concentration of triglycerides in the blood serum was estimated according to the method attached with the kit prepared by the Tunisian Biomagaghreb company, which depends on the enzymatic degradation of the triglyceride and based on the formation of the quinonemine compound as a result of the hydrolysis of the TC in an enzymatic form with a red pigment, the intensity of which increases with the increase in the concentration of the triglycerides according to the following equation:

$$\text{Concentration of triglycerides (mg}.100\text{ml}^{-1}) = \frac{\text{sample read}}{\text{standard solution read}} \times a$$

a =Concentration of standard solution 200 mg.100^{-1} ml

Determination of HDL concentration for cholesterol in serum

The HDL concentration was estimated using the ready-made kit mentioned in the determination of cholesterol and was measured using an optical spectrometer at a wavelength of 500 nm and according to the following formula:

$$\text{HDL concentration (mg}.100\text{ml}^{-1}) = \frac{\text{Sample absorbance}}{\text{standard solution absorbency}} \times \text{ n}$$

n = concentration of the standard solution 200 mg.dL^{-1}

Determination of the concentration of the low-density lipoprotein level for cholesterol in serum

Estimate according to the following equation:

$$\text{LDL} - \text{C mg}.\text{dl}^{-1} = \text{Total cholesterol } - (\text{HDL} + \text{ VLDL})$$

Determination of the very low-density lipoprotein concentration of cholesterol in serum

The value of VLDL-C was calculated by the following equation and by the method Burtis *et al.* (2012).

$$\text{VLDL mg.dl}^{-1} = \text{Triglycerides} / 5$$

Statistical analysis

The data were analyzed statistically according to the complete random design (CRD), and the lowest significant difference was used to compare the averages using the available statistical analysis program (SAS) (SAS, 1990).

Results & Discussion

Cholesterol:

The results of the table (2) showed a significant increase in the level of cholesterol in the group of rats with hypothyroidism induced by the action of caramidazole, as the average reached 87.6 $mg.kg^{-1}$ compared with the control group whose average cholesterol in the serum of its individuals reached 57.5 $mg.kg^{-1}$. Hepatocytes are the reason for reducing cholesterol release in the duodenum or the reason may be due to the blockage of the hepatic duct due to the effect of carbimazole and this caused the suspension or reduction of cholesterol release to the duodenum which confirmed Kalender et al. (2010) an increase in cholesterol concentration in rats with thyroid images as a result of administering malathion at a concentration of 1.28 mg.kg-1 of body weight with drinking water for four weeks. The results of the study were consistent with that confirmed by Ismail (2013) who observed elevated cholesterol levels in groups of hypothyroid rats. The reason is attributed to the production of lactic acid bacteria, hydroxyl menthyl glutarate during fermentation, which reduces cholesterol synthesis by inhibiting this compound Hydroxyl menthyl glutarate CoA-reductase necessary in the synthesis process or the ability of the bacteria to convert cholesterol into non-absorbable coprostanol and cholestanols and excrete them with feces. Hence lowering total cholesterol levels (Kumar *et al*., 2012).

Table (2) Effect of lactic product fortified with lactic acid bacteria on cholesterol in male rats with carbimizole-induced hypothyroidism (mean ± standard error).

Treatments	Cholesterol concentration ($mg.100cm^{-1}$)
T1	57.50^{a} ± 0.760
T2	87.60^{b} ± 0.858
T3	76.01^{c} ± 0.634
T4	60.00^{d} ± 1.320
T5	59.70^{d} ± 0.801
T6	70.00^{e} ± 0.853
T7	58.11^{d} ± 0.551
T8	56.50^{d} ± 0.591
T9	55.92^{ad} ± 0.998

Means in the same vertically with different letters show significant differences (P<0.05).

Triglycerides:

The results of the table (3) exhibited a significant increase in the number of triglycerides in T2 group rats with an average of 94.8 compared to the control group in which the average was 62.2. The cause of elevated triglyceride levels may be due to the low level of secretion of thyroid hormones that perform the function of lipolysis and then put it in bile; or it may be due to hypothyroidism leading to a loss of the efficacy of the lipoprotein lipase enzyme located in the lining of the capillary blood vessels, which works to break down triglycerides, so the ineffectiveness of this enzyme prevents the removal of TG; this increases its concentration in the blood (Abbas *et al.*, 2008). It is also noted from the results of the table that a significant decrease (P <0.05) was observed in the number of triglycerides when dosing experimental rats with the milk product containing lactic acid bacteria compared to the infected rats with a significant superiority of *L. casei* and *L. planetarum* and their mixture as averages 64, 65 and 65 respectively. The reason for the decline may be attributed to the fact that the lactic acid bacteria act to stimulate the lipase enzyme responsible for the decomposition of triglycerides and thus their quantity; or bacteria can stimulate the secretion of bile acid from liver cells, which works to inhibit the absorption of cholesterol and triglycerides from the intestine and thus excrete it with excreta (Abdullah *et al.*, 2013).

Table (3): Effect of lactic product fortified with lactic acid bacteria on triglycerides in male rats with carbimazole-induced hypothyroidism (mean ± standard error).

Treatments	Triglycerides concentration (mg.100cm^{-1})
T1	62.20^{a} ± 0.665
T2	94.80^{b} ± 1.029
T3	81.75^{c} ± 0.750
T4	64.33^{a} ± 0.712
T5	63.91^{a} ± 0.566
T6	72.38^{d} ± 0.545
T7	63.8 2^{a} ± 0.530
T8	71.05^{c} ± 0.238
T9	70.00^{c} ± 0.364

Means in the same vertically with different letters show significant differences (P<0.05).

High-density lipoprotein:

The results of table (4) displayed that the average amount of high-density lipoprotein in the serum of the negative control group rats was 48.2 mg.kg^{-1} decreased to 28.1 mg.kg^{-1} in the serum of the rats of the positive control group with hypothyroidism due to the effect of carbimazole on the permeability of membranes. Hepatic cells or bile duct obstruction, which causes cholesterol to be reduced or released into the duodenum, or due to inhibition of the enzyme Butyrlcholinessterase, which performs the function of metabolism of fats and lipoproteins (Kalender *et al.*, 2010).

Table (4): Effect of lactic product fortified with lactic acid bacteria on HDL in male rats with carbimazole -induced hypothyroidism (mean ± standard error).

Treatments	High Density Lipoprotein concentration (mg.100cm^{-1})
T1	48.20^{a} ± 0.234
T2	28.10^{b} ± 0.188
T3	32.42^{c} ± 0.556
T4	44.45^{d} ± 0.841
T5	45.02^{d} ± 0.612
T6	36.66^{e} ± 0.575
T7	46.20^{d} ± 0.402
T8	30.00^{f} ± 0.777
T9	33.81^{c} ±1.056

Means in the same vertically with different letters show significant differences (P<0.05)

HDL increased again when the rats were dosed with the lactic product containing *L. acidophilus*, *L. casei* and *L. planetarium*, to reach 32, 44 and 45 mg.kg^{-1} respectively, and reached the highest average of 46 mg. kg^{-1} when the rats were dosed with the Fortified milk with a mixture of lactic acid bacteria.

The high level of HDL may be caused by the bacteria *L. plantarum* and *L. casei*, which reduce cholesterol absorption. It is a positive sign for preventing atherosclerosis and reducing the risk of coronary heart disease (El-Shafie *et al*., 2009).

Low-density lipoprotein:

It is noted from the results of the table (5) that the mean amount of LDL in the serum of control group rats was 35.4 $mg.kg^{-1}$, the level increased to 63.1 $mg.kg^{-1}$ in the group T2 and the reason may be attributed to that it is also noted from the table that the level of LDL decreased again to 55 $mg.kg^{-1}$ when the rats were dosed with the lactic product containing *L. acidophilus* and the effect of the addition of bacteria was *L. plantarium* and *L. casie* were most effective and at a significant level ($P < 0.05$) in reducing the level of LDL in the rat blood serum where the average was 38 and 37 $mg.kg^{-1}$, respectively, and the mean amount of LDL 50 and 51 $mg.kg^{-1}$ when dosings the milky product rats containing a mixture of *L. plantarium*, and *L. casei* and *L. acidophilus*, respectively. The lowest mean LDL was 36 $mg.kg^{-1}$ when administered with the mixture of *L. casei* and *L. plantarium*. The cause of the harmful LDL level decline may be attributed to the effect of the decrease in the lactic acid bacteria in the fatty acid metabolism, thereby reducing VLDL-C in the liver and a decrease in the formation of LDL in the bloodstream. (Abdullah *et al*., 2013).

Table (5): Effect of lactic product fortified with lactic acid bacteria on LDL in male rats with carbimazole-induced hypothyroidism (mean ± standard error).

Treatments	Low-density lipoprotein concentration ($mg.dl^{-1}$)
T1	$35.40^{a} \pm 0.389$
T2	$63.10^{b} \pm 0.645$
T3	$56.06^{c} \pm 0.858$
T4	$38.07^{d} \pm 0.638$
T5	$37.82^{d} \pm 0.564$
T6	$50.00^{e} \pm 0.811$
T7	$36.18^{d} \pm 0.477$
T8	$50.98^{e} \pm 0.425$
T9	$40.00^{d} \pm 0.778$

Means in the same vertically with different letters show significant differences (P<0.05).

Very Low-Density Lipoprotein

The results of the table (6) revealed a significant increase ($P < 0.05$) in the amount of very-low-density protein in the serum of rats with hypothyroidism, reaching a level of 18.9 $mg.kg^{-1}$ compared to control group rats with an average of 12.4 $mg.kg^{-1}$, and the reason may be attributed to that. It is also noted from the results of the table that the dosing of rats with the milk product containing lactic acid bacteria and its mixture led to a significant decrease in the amount of VLDL in the blood serum of the rats to reach the lowest average when the doses of the rats with the milk product containing the bacteria *L. plantarium* and acetic *L. plantarium*

Table (6): Effect of lactic product enhanced with lactic acid bacteria on VLDL in male rats with carbimazole-induced hypothyroidism. (mean ± standard error).

Treatments	very low-density lipoprotein concentration ($mg.dl^{-1}$)
T1	$12.40^{a} \pm 0.593$
T2	$18.90^{b} \pm 0.518$
T3	$17.03^{c} \pm 0.422$
T4	$13.00^{a} \pm 0.547$
T5	$12.95^{a} \pm 0.219$
T6	$14.94^{ad} \pm 0.511$
T7	$13.11^{ad} \pm 0.222$
T8	$14.23^{a} \pm 0.548$
T9	$14.00^{a} \pm 0.056$

Means in the same vertically with different letters show significant differences (P<0.05).

and *L. casei*, the effect was significant (P <0.05), with an average of 13 $mg.kg^{-1}$ each. This may be attributed to the role of lactic acid bacteria in metabolism (Abdullah *et al.*, 2013; Ma *et al*., 2019).

Conclusions

Lactic acid bacteria have an inhibitory efficacy of the negative effects of carbimazole and the occurrence of hypothyroidism and the possibility of its safe use in regulating the work of the thyroid gland and Significant decrease in the levels of total cholesterol, triglycerides, LDL, and VLDL with a significant increase in the level of HDL in the blood serum of rats with hypothyroidism induced by carbimazole when dosing with fortified milk in lactic acid bacteria compared to the control sample. These results are encouraging to use this. product by people with heart disease and arterial hypertension.

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Conflict of interest: The authors declare that they have no conflict of interest.

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تأثير بعض سلالات بكتيريا حامض اللاكتيك وخليطها على مستوى الدهون والكوليسترول في ذكور الجرذان (*Rattus norvegicus*) المصابة بقصور الغدة الدرقية المستحث باستخدام Carbimazole

المستخلص: تم استخدام الحليب المدعم الحاوي على العزلات البكتيرية *L. plantarum* و *L. casei* و *L. acidophilus* وخليطهما في تجريع ذكور الجرذ الابيض Albino rats بعمر يتروواح بين 9-12 اسبوع وبمعدل وزن 23 غم المصابة بقصور الدرقية المستحث بتركيز 0.6 غم.كغم$^{-1}$ من مادة الكاربيمازول ، تم تقدير الكولسترول الكلي ، الكليسيرايدات الثلاثية، البروتين الدهني عالي الكثافة (HDL)، البروتين الدهني واطيء الكثافة (LDL)، البروتين الدهني واطيء الكثافة جدا (VLDL)، واوضحت النتائج ارتفاع معنوي في مستوى الكولسترول والكليسيرايدات الثلاثية .T.G واللايبوربروتين واطيء الكثافة LDL وواطيء الكثافة جدا VLDL مع انخفاض معنوي في مستوى اللايبوبروتين العالي الكثافة HDL في ذكور الفئران المصابة مقارنة بعينة السيطرة وعند تجريعها بالحليب السائل المدعم عاودت الى الوصول الى مستواها الطبيعي وبدون فروقات معنوية عن مجموعة السيطرة.

الكلمات المفتاحية: كاربيمازول، بكتيريا حمض اللاكتيك، قصور الغدة الدرقية.