



Physiological effects of lactic acid bacteria against melamine induced toxicity in female albino rats

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Abstract

The aim of this study was to investigate the ameliorative effect of two type of lactic acid bacteria *Lactobacillus casei* and *Lactobacillus acidophilus* against melamine toxicity by some physiological indicators in mature female rats after 21 days. In this study using 35 of female mature rats and divided randomly into seven groups each group contain five animals. The results showed that melamine caused a significant decrease in the organs weights liver and spleen and increase in kidney weight with increase of melamine concentration. Also showed to decrease in value of hemoglobin, red blood cells, white blood cells, lymphocyte and platelets, while the values of granules were increasing with increase of melamine concentration as compared with control group. Also found that the addition of melamine led to increase in cholesterol, low density lipoproteins and blood glucose, while the values of triglyceride and high density lipoproteins was decreased with increase of melamine concentration. The addition of two types of lactic acid bacteria *L. casei* and *L. acidophilus* led to decreasing the negative effect of melamine on the values of all the parameters determined.

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Introduction

Melamine chemical formula $[C_3N_3(NH_3)_3]$ (triazine-2,4,6-triamine 1,2,5) chemical compound have white color widely diffused contained 66.7 % of its weight nitrogen and melamine related to triazine family (1). Its molecular weight 126 Dalton, and considered of illegal milk additives and protein products lead to melamine toxicity in pet animals and its death in America and renal failure in children and death of high number of them in china (2). High nitrogen content of melamine and low cost make them used in many countries have low quality control to resist cheating in nutritional products and animal feed stuff (3). And the aim of this additives to increase nitrogen concentration to increase appeared protein value in nutritional products (4). The addition of one gram of melamine in one liter of milk lead to increase protein content with percentage 0.4%, and the melamine can transferee to food with low concentration do not cause dangerous on humans and considered nontoxic

when use in industry of cans that use to save the food that attached to food directly, and the daily limit that approved of melamine and cyanuric acid at 0.2 and 1.5 mg/kg Body weight respectively (5). Melamine considered low toxic compound if present lonely, but present of cyanuric acid that formed as byproducts of formation of melamine and usually be associated with it that increase dangerous of melamine, and make low concentration of melamine source of dangerous on human and animal health (6).

Gossner (7) mentioned that consuming of melamine of cyanuric acid lead to renal calculi formation. And cause clinical sign of vomiting, fever, hematuria, hypotension and pain at the site of kidney and not all children have renal calculi showed same clinical signs (8). The European food organization advice in European Union that all products that contain melamine more than or equal to 2.5 mg/kg should destructed (9). Also American food and drug agency (FDA) and European food organization mentioned that the daily limit approves of melamine that did not cause dangerous on

human its 0.63 mg/kg (10). Al-Seeni (11) Recorded decrease in total leukocyte count, lymphocyte, platelets, MCV and MCH and increase in red blood cell count, hemoglobin, MCHC and monocyte with increase concentration of melamine in feed of rats. Hameed *et al.* (12) Mentioned to probiotic supplementation to quail ration improved the hormonal status and enhance reproduction. On other hand Carr *et al.* (13) mentioned this microorganism have the ability to suppress growth of wide spectrum of pathogenic bacteria, and have rule in increase production of immunoglobulin (IgA) that have main rule in immune response of mucus membrane.

The aim of the study is to know the effect of lactobacillus bacteria in decrease negative effect of melamine in some growth, blood and lipid parameters in rats.

Materials and methods

This study was done in animal house and laboratories of Agriculture College, Tikrit University by using 35 of female mature rats obtained from college of veterinary medicine/ Mosul University and it's from Albino Sprague-Dawley weanling species at age 8-9 weeks and 140-155 g body weight, divided randomly into 7 groups each group contain 5 animals and included as following: 1st group: control, 2nd group: animal that given melamine at dose 10 mg/kg body weight, 3rd group: animal that given melamine at dose 20 mg/kg body weight, 4th group: animal that given melamine at dose 10 mg/kg body weights with *L. casei*, 5th group: animal that given melamine at dose 20 mg/kg body weights with *L. casei*, 6th group: animal that given melamine at dose 10 mg/kg body weights with *L. acidophilus*, 7th group: animal that given melamine at dose 20 mg/kg body weights with *L. acidophilus* animal/day.

Lactic acid bacteria obtained from Gazi Culture Collection (GCC) Gazi University/Ankara. Melamine was obtained from Chemicals Ltd (Poole, UK). The melamine given to animals after mixing with sterile water (14) and after dissolve the concentration that mentioned in groups above. And addition of bacterial suspension of lactobacillus of each type *L. casei* and *L. acidophilus* with drinking water at number 1.5×10^8 colony/ml of peptone water depending on Macfarland solution. The beginning weight recorded after one day of feeding animal lonely and the temperature was 20 - 25 °C and light period more than 12 hours a day, and feed ad libitum and prepared according to (15) throughout period of experiment that extended for 21 days. After ending of period of experiment directly the animals fasted for 20 hours the animals anesthetized by using chloroform, anatomized to do the tests, the blood collected at 8 - 10 ml at two groups on contain anticoagulant Ethylene diamine tetra acetic acid (EDTA) to do blood tests the other tubes without anticoagulant that centrifuged by using

Centrifuge at 3000 round/ minute for 15 minute to obtained the serum that stored at temperature -20 °C until analysis, Moreover, it has been taking the weights of internal organs, which included the liver, spleen and kidney by using a sensitive balance (11).

Blood that collected by tube with anticoagulant EDTA used for measuring the red blood cells count (RBC) ($10^6/\text{mm}^3$), total leukocyte count ($10^6/\text{mm}^3$), hemoglobin concentration, platelets, granulocytes and lymphocyte by using complete blood picture system.

The serum was used for measuring Glucose, total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) (mg/100ml) according to (16) by using Biolabo France kit and the analysis done by using Japanese spectrophotometer type Shimadzu and according to the wave length advised by kit company of each analysis and the concentration calculated according to the equation according to the leaflet of the kit company.

Statistical analysis

The results analyzed by using Linear Model General by SAS ready program (17) to study the effect of factors according to complete random design (CRD) also Duncan test was done (18) to defining the significant differences between means of effected factors on parameters at levels $P < 0.05$.

Results

Table 1 showed effect of addition of melamine with two concentration 10 and 20 mg/kg with drinking water on internal organ weight of female rats, the results showed presence of significant decrease in weight of liver and spleen at level $P < 0.05$ for both concentrations 10 and 20 mg/kg of melamine that was at 7.81 and 7.32, 1.05 and 0.92 in compare with control group 8.89 and 1.47 g respectively, also presence of significant increase in weight of kidney that reached 1.78 and 1.82 g of both concentration in compare with control group 1.74 g. Also noticed there are significant increases in weight of liver and spleen and decrease weight of kidney with addition of both type of lactobacillus bacteria *L. casei* and *L. acidophilus* with melamine in compare with animals groups of melamine addition lonely.

Table 2 showed effect of melamine with two concentrations 10 and 20 mg/kg with drinking water in some blood value of female rats, the results showed presence of significant decrease in leukocyte number and lymphocyte percentage at $P < 0.05$ for both concentrations 10 and 20 microgram/ kg of melamine that was 5.32 and 4.97 in compare with control group 6.53 and 73.5 respectively, also presence significant increase in monocyte percentage that reached 6.53 and 7.43 for both concentrations in compare with control group 5.62. Also noticed there are significant increases in total leukocyte

count and percentage of lymphocyte and decrease percentage of granulocytes at the addition of both type of lactobacillus bacteria *L. casei* and *L. acidophilus* with melamine in compare with melamine lonely.

Table 3 showed effect of addition of melamine with two concentrations 10 and 20 mg/ kg with drinking water on blood values of female rats, the results showed presence significant decrease number of red blood cells and hemoglobin concentration and platelets count at $P<0.05$ that was the number of erythrocyte 5.96 and 4.85 and

hemoglobin concentrations 11.21 and 9.44 and platelets count 317.3 and 305.2 in compare with control group 13.86, 7.97 and 341.7 respectively, also noticed significant increase number of erythrocyte and hemoglobin concentrations and platelets count at the addition of both type of lactobacillus bacteria *L. casei* and *L. acidophilus* with melamine in control with animal groups of addition of melamine lonely.

Table 1. Effect of adding two types of lactic acid bacteria and melamine effect in organs weight (g) of female rats

Groups	Concentration (mg/kg)	Kidney (g)	Spleen (g)	Liver (g)
G1	0	1.74± 0.029 ^d	1.47± 0.032 ^a	8.89±0.015 ^a
G2	10	1.82±0.054 ^b	1.05±0.024 ^c	7.81±0.020 ^c
G3	20	1.89±0.015 ^a	0.92±0.043 ^d	7.32±0.012 ^d
G4	10	1.78±0.026 ^c	1.24±0.017 ^b	8.35±0.058 ^b
G5	20	1.83±0.018 ^b	1.08±0.088 ^c	7.85±0.029 ^c
G6	10	1.77±0.005 ^c	1.30±0.037 ^b	8.22±0.020 ^b
G7	20	1.79±0.011 ^c	1.11±0.167 ^{bc}	7.91±0.085 ^c

Different letters in column refer to significant differences at level $P<0.05$.

Table 2: Effect of adding two type of lactic acid bacteria and melamine effect in some parameters of WBC in female rats

Group	Concentration (mg/kg)	WBC ($10^3/\text{mm}^3$)	Lymphocytes (%)	Granulocytes (%)
G1	0	6.53±0.032 ^a	73.5±0.057 ^a	5.62±0.051 ^d
G2	10	5.32±0.004 ^d	70.6±0.176 ^c	6.53±0.055 ^b
G3	20	4.97±0.068 ^e	68.7±0.328 ^e	7.43±0.005 ^a
G4	10	5.72±0.007 ^c	71.9±0.261 ^b	5.92±0.073 ^c
G5	20	5.61±0.096 ^{cd}	70.8±0.196 ^c	6.30±0.378 ^b
G6	10	5.83±0.021 ^b	71.6±0.140 ^b	5.84±0.035 ^c
G7	20	5.85±0.043 ^b	69.9±0.043 ^d	6.32±0.029 ^b

Different letters in column refer to significant differences at level $P<0.05$.

Table 3: Effect of adding two types of lactic acid bacteria and melamine in some parameters blood in female rats

Groups	Concentration (mg/kg)	Hb (g/dl)	RBC ($10^6/\text{mm}^3$)	Platelets ($10^3/\text{mm}^3$)	MCV (μm^3)
G1	0	12.86±0.043 ^a	7.27±0.034 ^a	341.7±0.953 ^a	69.8±0.503 ^a
G2	10	11.21±0.006 ^c	5.96±0.016 ^c	317.3±0.266 ^d	67.7±0.405 ^c
G3	20	9.44±0.007 ^e	4.85±0.037 ^d	305.2±0.321 ^f	64.9±0.841 ^e
G4	10	11.92±0.032 ^b	6.14±0.113 ^b	328.6±0.761 ^c	68.5±0.776 ^b
G5	20	10.84±0.056 ^d	5.64±0.102 ^c	321.3±0.888 ^d	66.1±0.581 ^d
G6	10	11.86±0.045 ^b	6.27±0.043 ^b	330.2±0.712 ^b	68.7±0.288 ^b
G7	20	10.79±0.088 ^d	5.72±0.002 ^c	323.8±1.096 ^e	66.3±0.123 ^d

Different letters in column refer to significant differences at level $P<0.05$.

Table 4 showed effect of addition of melamine with both concentrations 10 and 20 mg/kg with drinking water on lipid profile of female rats, the results showed presence of significant increase in cholesterol value and LDL and blood sugar at $P<0.05$ for both concentrations 10 and 20 microgram\ kg of melamine that was cholesterol 98.76 and

102.41 and LDL 31.82 and 33.93 in compare with control group 72.43 and 19.64 respectively, also the results showed presence of significant decrease in value of triglyceride and HDL that was 73.11 and 70.58, 22.65 and 20.31 respectively in compare with control group. Also noticed there are significant decrease in cholesterol value and LDL and blood

sugar and significant increase in value of triglyceride and HDL at the addition of both type of lactobacillus bacteria *L.*

casei and *L. acidophilus* with melamine in compare with animals groups of melamine addition lonely.

Table 4: Effect of adding two types of lactic acid bacteria and melamine effect in lipid profile in female rats

Groups	Concentration (mg/kg)	Cholesterol (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Glucose (mg/dl)
G1	0	72.43±0.058 ^c	83.55±0.056 ^a	19.64±0.029 ^d	31.52±0.015 ^a	115.53±0.023 ^d
G2	10	98.76±0.061 ^b	73.11±0.031 ^c	31.82±0.034 ^b	22.65±0.037 ^d	133.63±0.006 ^b
G3	20	102.41±0.125 ^a	70.58±0.056 ^d	33.93±0.023 ^a	20.31±0.026 ^e	148.58±0.049 ^a
G4	10	83.46±0.007 ^d	77.33±0.149 ^b	26.84±0.040 ^c	25.86±0.109 ^b	125.71±0.084 ^c
G5	20	95.38±0.032 ^c	72.96±0.022 ^c	30.45±0.036 ^b	23.12±0.043 ^c	133.26±0.026 ^b
G6	10	85.72±0.097 ^d	76.92±0.132 ^b	27.94±0.049 ^c	26.34±0.002 ^b	127.45±0.049 ^c
G7	20	95.13±0.124 ^c	73.28±0.028 ^c	29.91±0.051 ^b	25.23±0.055 ^b	135.01±0.006 ^b

Different letters in column refer to significant differences at level P<0.05.

Discussion

The results were agreeing with the results of and also agree with the results mentioned by (19) that recorded significant decrease in weight of liver and spleen and increase weight of kidney in female rats with the addition of melamine in the feed in compare with control group. Also the results were agreeing with Son *et al.* (20) that mentioned to significant decrease in weight of liver and increase weight of kidney at the addition of melamine to the feed in compare with control groups. Dobson *et al.* (21) Mentioned to increase weight of kidneys in rats due to addition of melamine and cyanuric acid to the feed of rats. Hameed *et al.* (12) mentioned presence of decrease of weight of spleen at the addition of melamine to the feed of rats with concentration of 50 mg/kg body weight. Also mentioned by (11,22) to presence of significant increase of kidney weight at the addition of melamine to the feed of rats due to the damage of the renal tissue and necrosis of renal tubules and dilation of Bowman's capsule and swelling of the epithelial cells of tubules (6). Also the reason of increase of kidney weight is may be due to formation of calculi in the kidney also because obstruction of tubules in the kidney that leads to renal damage and acute renal failure (23).

The results were agreeing with the results that reached by Chan and Lai (11) that mentioned presence of significant decrease in number of leukocyte and lymphocyte and presence increase of monocyte at the addition of melamine to the feed of female rats. And the results agree with the result that mentioned by Hameed *et al.* (12) that indicate to significant decrease in total leukocyte count and lymphocyte in elevation of monocyte value at the addition of melamine to the feed of mice in compare with control group. Also the results agree with the results of Chen *et al.* (22) in presence of decrease in total leukocyte count and lymphocyte at the addition of melamine to the feed of rats.

The results were agreeing with the results that reached by (24) that mentioned presence of significant decrease in

number of red blood cells and hemoglobin concentration and platelets count at the addition of melamine to the feed of female rats. And agree with what mentioned by (12) that recorded significant decrease red blood cell number and hemoglobin concentration and mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume at the addition of melamine to the feed of mice in compare with control group, because of formation insoluble compound of Melamine cyanurate during metabolism of melamine (25). Ismail and Al-Hamdani (26) showed there were significantly increase in hemoglobin concentration in three experiments, and also the packed cell volume was significantly increase in three experiments in common carp.

The consumption this toxic compound lead to loss of iron from hemoglobin molecule of blood (27) and this reason that responsible for anemia happening. And the reason of increase of number of erythrocyte and hemoglobin concentration and the platelets count due to the rule of probiotics of production of vitamin B, also its effect by enhance immune system, and promote absorption of vitamins and minerals, and stimulate production of organic and amino acids (28). And Kim *et al.* (29) mentioned that lipoteichoic acid that presence in wall of lactobacillus bacteria has anti-inflammatory effect by suppression of receptor of platelets activation, due to the rule of platelets in activation part of immune response, this explain increase its number as response of immune activation due to consumption of probiotics that mentioned by researchers as immune enhancer.

The results were agreeing with that mentioned by (19) in presence of significant increase of value of cholesterol and triglyceride at the addition of melamine to the feed. And (9) mentioned that presence of significant increase of cholesterol value at the addition of melamine and cyanuric acid to the rat feed. Also agree with (30) that mentioned that the consumption of lactobacillus bacteria by the humans as

probiotics lead to decrease significantly total cholesterol concentration and triglyceride and low density lipoproteins LDL. Also find that number of lactobacillus type has the ability to decrease concentration of total cholesterol and triglyceride in rats (6,31).

The researches mentioned to presence of many mechanisms used by lactobacillus bacteria to decrease cholesterol level in blood, include theory of enzymes by release conjugation of bile salt by Bile salt hydrolase (BSH) enzyme of probiotic, with the release of conjugation the bile salts be less soluble and absorb by intestine and this leads to excrete with feces (32). The use of cholesterol in formation of new bile acid for homogenizing leads to decrease of cholesterol in serum (33). Also suppression formation of cholesterol in liver may be occur through production of short chain fatty acids (SCFA) like propionic acid and other by types of lactobacillus bacteria or redistribution of cholesterol from plasma to the liver (34). The case of capacitation of cholesterol done be precipitation with unconjugated bile salts cause reabsorption inside the body or by suppression cholesterol forming enzyme (35), also way of converting of cholesterol to coprostanol that excrete directly in feces (36). And may the decrease of total cholesterol and triglyceride and low density lipoproteins returned to production of glucan sugar by lactobacillus bacteria that work on increase viscosity of small intestine lead to increase excretions of bile acid lead to decrease the converting of these acids to formation of cholesterol then decrease its level in blood plasma (37).

The decrease of blood sugar in control group resulted from burning of saccharide completely burn inside the body to produce energy to utilized by different activity, while the elevation in glucose sugar returned to ability of melamine to attack beta cells of pancreas that specialized by excrete insulin hormone and destruction by accumulation of free radicals that be toxic in beta cells lead to arrest the process of glucose lyses and activation process of glucose formation and glycogen lyses, and explained the ability of bacteria to decrease glucose level through delay or suppress absorption of glucose from intestine or stimulation of insulin secretion by pancreas, or through conjugation of glucan compound that produced by lactobacillus bacteria in the intestine lead to reabsorption to the blood then decrease its concentration by excretion outside with feces (38).

This significant increase in concentrations of blood biochemical picture may be returned to increase nitrogen value in composition of melamine and reached 66.7%, that the molecule of melamine can lysed by effect of some enzyme and increase nitrogen in blood and occur disturb in blood content equilibrium lead to physiological disturbance of cells and transmitted nutrient and increase blood viscosity caused formation of calculi in kidney and disturbance in secretory organs like liver, also hepatocyte can effected to

disturbance due to lysed nitrogen more than nature demand or cells capacity (16).

The ability of types of lactobacillus in decreases the negative effect of harmful compound through its ability to conjugate the compound and removed from intestine (39).

This bacteria work as probiotics cause decrease cholesterol in blood, many studies that showed was many types of lactobacillus have the ability to decrease cholesterol, the cholesterol levels elevated in blood a result of consumption of animal fat in food and the increase its level over normal limit leads to precipitation in blood vessels and lead to obstruction, and can decrease that levels by using probiotics as lactobacillus bacteria, also its ability to metabolite saturated fatty acids and production short chain fatty acids in the intestine by its production of enzyme that lyse fat and some of them distract cholesterol and utilize them as carbon source in its metabolism, also its able to suppress formation of cholesterol in the liver (40).

Conclusion

The results of present study showed that the using of melamine produces nephrotoxicity and the potential use lactic acid bacteria considered have the ability to decrease negative effects of melamine in some growth, blood and lipid parameters in rats, and have rule in increase production of immunoglobulin.

Conflict of interests

The authors declare no conflict of interest.

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التأثيرات الفسلجية لبكتريا حامض اللاكتيك على السمية المحدثه بالميلامين في إناث الجرذان البالغة

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الخلاصة

تهدف الدراسة الحالية إلى معرفة تأثير نوعي بكتريا حامض اللاكتيك *Lactobacillus acidophilus* و *Lactobacillus casei* على السمية

والصفائح الدموية لمجاميع الجرذان، وارتفاع قيم الخلايا الحبيبية مقارنة مع مجموعة السيطرة. وبينت النتائج ارتفاع قيم الكوليسترول والدهون البروتينية منخفضة الكثافة وسكر الدم وانخفاض معنوي في قيم الكليسيريدات الثلاثية والدهون البروتينية مرتفعة الكثافة مع زيادة تركيز الميلايين. وكان لإضافة نوعي بكتريا حامض اللاكتيك *L. acidophilus* و *L. casei* مع الميلايين تأثيراً معنوياً في خفض التأثير السلبي للميلايين على جميع المعايير المقاسة أعلاه.

المحدثة بالميلامين بواسطة بعض المعايير الفسلجية في إناث الجرذان المختبرية بعد مدة ٢١ يوماً. استعملت في هذه الدراسة ٣٥ من إناث الجرذان البالغة والتي قسمت إلى سبعة مجاميع كل مجموعة تضم خمسة حيوانات. بينت النتائج إن إضافة الميلايين سبب في انخفاض معنوي في أوزان الكبد والطحال وارتفاع وزن الكلى مع زيادة تركيز الميلايين مقارنة مع مجاميع السيطرة. وكذلك انخفاض قيم الهيموكلوبين والعدد الكلي لكريات الدم الحمراء وكريات الدم البيضاء والخلايا اللمفية