

THE ROLE OF *LACTOBACILLUS CASEI* AND *LACTOBACILLUS ACIDOPHILLUS* TO DECREASE THE BIOLOGICAL EFFECTS OF POTASSIUM BROMATE IN RATS

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ABSTRACT

This study was conducted to investigate the ameliorative effect of lactic acid bacteria *Lactobacillus casei* and *Lactobacillus acidophilus* against Potassium bromate (25, 50) mg / kg toxicity by some physiological indicators in 35 of female rats after 21 days. The animals were divided into 7 groups within each group 5 animals weighted 140 – 155 g. The results showed a significant decrease ($P<0.05$) in value of Red blood cells (RBC), hemoglobin (Hb), White blood cells (WBC), Lymphocyte (LYM) and Platelets (PLT), While increasing the values of Granules (GRN). Also found that the addition of Potassium bromate led to increase in cholesterol, triglyceride (TG), Low Density Lipoprotein (LDL) and blood glucose, while decreased the values of High Density Lipoprotein (HDL) for rats groups with increasing the concentration of Potassium bromate compared with control group. The addition of two types of lactic acid bacteria *L. casei* and *L. acidophilus* with Potassium bromate showed a positive effect to reducing the negative effect of Potassium bromate on blood and lipid profile parameters compared with the control group and Potassium bromate group. It is concluded that the lactic acid bacteria has protective effects and reduces the effects that Potassium bromate.

Key words: lactic acid bacteria, Potassium bromate, physiological parameters.

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دور بكتريا *Lactobacillus casei* و *Lactobacillus acidophilus* في تقليل التأثيرات البايولوجية لبرومات

البوتاسيوم في الجرذان

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المستخلص

تهدف الدراسة الحالية الى معرفة تأثير نوعي بكتريا حامض اللاكتيك *Lactobacillus casei* و *Lactobacillus acidophilus* على السمية المحدثة ببرومات البوتاسيوم بتركيز (25 و 50) ملغم / كغم بواسطة بعض المعايير الفسلجية في اناث الجرذان المختبرية بعد مدة 21 يوما. استخدمت في التجربة 35 من اناث الجرذان البالغة قسمت الى 7 مجاميع وكل مجموعة تضمنت 5 حيوانات. بينت النتائج ان إضافة برومات البوتاسيوم سبب انخفاض معنوي عند ($P<0.05$) في العدد الكلي لكريات الدم الحمراء وقيم الهيموكلوبين وكريات الدم البيضاء واللمفوسايت والصفائح الدموية. وارتفاع قيم الـ Granules. وبينت النتائج ارتفاع قيم الكوليستيرول والكليسيريدات الثلاثية والبروتين الدهني منخفض الكثافة LDL وسكر الدم مع زيادة تركيز برومات البوتاسيوم وانخفاض معنوي في قيم البروتين الدهني عالي الكثافة HDL لمجاميع الجرذان مع زيادة تركيز برومات البوتاسيوم مقارنة مع مجموعة السيطرة لكل منها. وكان لأضافة نوعي بكتريا حامض اللاكتيك *L. casei* و *L. acidophilus* مع برومات البوتاسيوم تأثيراً معنوياً في خفض التأثير السلبي لبرومات البوتاسيوم على معايير الدم والدهون المقاسة اعلاه مقارنة مع مجموعة السيطرة ومجاميع برومات البوتاسيوم.

الكلمات المفتاحية: بكتريا حامض اللاكتيك، برومات البوتاسيوم، المعايير الفسلجية.

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INTRODUCTION

Potassium bromate (KBrO_3) is a white crystal, granules or powder, it has no medicinal value but is added to treat flour for bread and bakery product (12). Potassium bromate is a potential oxidizing agent and also acts as a bleaching agent that imparts bond strength by improving the elasticity that results in soft, fluffy and White bread (38). Potassium bromate is widely used as improving additive for bread making (4). Bromate decomposes in distilled water at (350°C - 400°C) but in bread decomposes within the range of 150°C - 250°C . The decomposition of bromate ions in bread at low temperatures may be due to the presence of metal ions in flour, which acts as a catalyze(3). (30) analyzed fifteen different bread brands produced in Zaria metropolis of Northern Nigeria to determine potassium bromate content using the iodometric titration method. The study revealed the presence of potassium bromate in all the samples analyzed, the concentrations ranged from 2.46 to 13.60mg/kg, all the values were above the allowed limit by FDA.. International Agency for Research on Cancer (IARC) has classified KBrO_3 as a possible human carcinogen (group 2B) and can induce multiple organ toxicity in humans and rats (27). (15) showed that all bread and flour samples contain bromate residue with concentrations higher than the permissible guide line values. It degrades Vit. A1, B1, B2, E and niacin, which are the main vitamins available in the bread (24 , 37). The Joint Food and Agricultural Organization (FAO)/ World Health Organization Expert Committee on Food Additives (JECFA) has temporarily recommended a maximum level of 75 ppm of KBrO_3 for treating flour, provided that baking products prepared from such treated flour contain negligible residues of KBrO_3 (54). In Japan, the level has been set at 30 ppm under the same conditions as for JECFA (46). Potassium bromate (KBrO_3) is classified as mutagenic and carcinogenic although used in food and cosmetics industries, and its ability to cross the blood-brain barrier lead to the investigation of its effect on the cerebral cortex (2). WHO also banned the use of potassium bromate in

1993, It has been shown to be nephrotoxic in human and experimental animals (48). Lactic acid bacteria have been found to have the ability to produce vitamin B; stimulate immune response, cholesterol reduction, and anti-oxidant potential; and prevent ulcers, GIT tract infections, diabetes, and heart disorders (9). A supplement of highly active lactic acid bacteria strains provided significant potential to enhance host's immunity, offering prevention from many diseases including some cancers (39). The gastrointestinal tract (GIT) microflora plays a crucial role in maintaining the health status of humans and animals. The importance of GIT microflora in the health of their host was first postulated by Metchnikoff at the beginning of the twentieth century (49). The probiotics are the microbes that induced benefit on the health of host by modifying gut microbiota. The goal of the supplement of these probiotics is mainly to enhance the immunity of their hosts (52). Currently, these probiotic bacteria have been widely used as important sources for the development of practical foods. The healthy gut microflora has also proved to be capable of preventing diseases caused by gastrointestinal flora (8).

MATERIALS AND METHODS

Experimental design

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\ AL 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: (1) Control group (2) animal group that gave Potassium bromate at dose 25 mg/kg body weight (3) animal group that gave Potassium bromate at dose 50 mg/kg body weight (4) animal group that gave Potassium bromate at dose 25 mg/kg body weights with *L. casei* (5) animal group that gave Potassium bromate at dose 50 mg/kg body weights with *L. casei* (6) animal group that gave Potassium bromate at dose 25 mg/kg body weights with *L. acidophilus* (7) animal group that gave Potassium bromate at dose 50

mg/kg body weights with *L. acidophilus* animal/day.

Collection and preparation of bacteria and chemical

Lactic acid bacteria obtained from Gazi Culture Collection (GCC) Gazi University/Ankara. Potassium bromate was obtained from Chemicals Ltd (Poole, UK). The Potassium bromate gave to animals after mixing with sterile water (43) 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 (33) 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 (45).

Blood samples

The blood samples collected at 6 – 8 ml at two groups on contain anticoagulant Ethylene diamine tetraacetic 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 to do the analysis (47). 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, monocyte % 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 (47) 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 (42) to study the effect of factors according to complete random design (CRD) also Duncan test was done (14) to defining the significant differences between means of effected factors on parameters at levels (0.05).

RESULTS AND DISCUSSION

Table 1 showed effect of Potassium bromate with two concentrations (25 and 50) 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 (25 and 50) microgram\ kg of Potassium bromate that was (5.25 and 4.85) (69.8 and 68.2) in compare with control group (6.33 and 71.3) respectively, also presence significant increase in GRN percentage that reached (6.47 and 7.11) for both concentrations in compare with control group 5.58. Also noticed there are significant increases in total leukocyte count and percentage of lymphocyte and decrease percentage of monocyte at the addition of both type of lactobacillus bacteria *L. casei* and *L. acidophilus* with Potassium bromate in compare with Potassium bromate lonely. The results was agree with the results that reached by (51) that mentioned presence of significant decrease in number of WBC and lymphocyte at the addition of Potassium bromate to the feed of rats. And the results agree with the results that mentioned by (34) that indicate to significant decrease in total WBC count and lymphocyte at the addition 10 mg/kg (KBrO_3) to the feed of mice in compare with control group. Also the results agree with the results of (31) in presence of decrease in total leukocyte count and lymphocyte at the addition of Potassium bromate. *L. acidophilus*, *L. casei* and *L. delbrueckii subsp. bulgaricus* are promising to create highly effective immunobiotics, that are able to increase the innate immunity in cases of infections (28). One of the mechanisms of probiotics as immune enhancer is promote lymphocyte and phagocyte activity and increase production of

immunoglobulin and interferon, its associated with activation of dendritic cells DC, macrophages, epithelial cells, T regulatory cells, effector lymphocytes, B-lymphocytes and natural killer cells NKC (16).

Table 1. Effect of adding lactic acid bacteria to decrease of Potassium bromate effect in some parameters of white blood cells in female rats

Treatment	Concentration	WBC($10^3/mm^3$)	Lymph.(%)	GRN(%)
T1	0	6.33±0.029 a	71.3±0.015 a	5.58±0.024 d
T2	25	5.25±0.054 d	69.8±0.020 c	6.47±0.085 b
T3	50	4.85±0.005 e	68.2±0.012d	7.11±0.043 a
T4	25	5.57±0.018 cb	70.4±0.017 b	5.85±0.029 c
T5	50	5.44±0.011 c	69.8±0.037 c	6.44±0.020 b
T6	25	5.62±0.026 b	70.6±0.167 b	5.82±0.058 c
T7	50	5.42±0.015 c	69.5±0.088 c	6.43± 0.032 b

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

Table 2 showed effect of addition of Potassium bromate with two concentrations (25 and 50) 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, hemoglobin, platelets and MCV (5.87 and

4.92) (11.18 and 9.37) (324.8 and 307.8) and (67.55 and 64.37) in compare with control group 12.91, 7.42, 362.5 and 70.54 respectively, also noticed significant increase number of all parameters at the addition of both types of lactobacillus bacteria *L. casei* and *L. acidophilus* with Potassium bromate in control with animal groups of addition of Potassium bromate lonely.

Table 2. Effect of adding lactic acid bacteria to decrease of Potassium bromate effect in some parameters of Red blood cells in female rats

Treat.	Concent. (mg/kg)	RBC ($10^6/mm^3$)	Hb (g/dl)	PLT ($10^3/mm^3$)	MCV (μm) ³
T1	0	7.42±0.034 a	12.91±0.751a	362.5±1.096 a	70.54±0.776 a
T2	25	5.87±0.016 c	11.18±0.097b	324.8±0.043 d	67.55±0.288 c
T3	50	4.92±0.035 d	9.37±0.482 e	307.8±0.503 e	64.37±0.123 e
T4	25	6.26±0.953 b	11.98±0.462b	335.2±0.405 b	68.92±0.032 b
T5	50	5.77±0.261 c	10.90±0.381d	321.3±0.712 d	65.82±0.581 d
T6	25	6.31±0.102 b	11.86±0.032b	328.8±0.841 c	69.33±0.007 b
T7	50	5.82±0.118 c	10.83±0.154d	325.1±0.002 d	66.11±0.085 d

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

The results was agree with the results that reached by (51) that mentioned presence of significant decrease in number of red blood cells and hemoglobin concentration and PCV count at the addition of Potassium bromate to the feed of female rats. And agree with what mentioned by (34) that recorded significant decrease red blood cell number, hemoglobin and Platelet concentration at the addition 10 mg/kg of Potassium bromate to the feed of rats in compare with control group. (31) Showed to decrease in RBC, Hb, HCT, MCV, Platelet and MCH when added $KBrO_3$ at 100 mg/kg. (28) Showed to Decrease in RBC, Hb, PCV at the addition of Potassium bromate to the feed of rats. Probiotics are 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 (13, 17). (25) mentioned that Lipoteichoic acid that presence in wall of lactobacillus bacteria has anti-inflammatory effect by suppuration 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. Similar results were reported by (30, 53) who claimed that potassium bromate was known to induce methaemoglobinaemia and cyanosis that resulted from oxidation of ferrous ion to ferric by reactive oxygen species (ROS) generated from potassium bromate. The platelet count showed statistically significant decreased values in post administration rats when compared with the control. These reductions in the leucocyte and platelet count

could be due to the DNA strand breakage in these cells induced by the oxidative stress associated with potassium bromate (10 , 40 , 36), on the other hand it could be that potassium bromate has a direct damaging effect on the platelets. The blood picture showed moderate lymphocytosis in rats administered with potassium bromate, which could be due to the depletion of the intracellular GSH by diethylmaleate in lymphocytes, which decreases the amount of strand breakage induced by potassium bromate (36). Table 3 showed effect of addition of Potassium bromate with both concentrations (25 and 50) mg/kg with drinking water on lipid profile of female rats, the results showed presence of significant increase in cholesterol, triglycerides, LDL

and blood sugar at ($P < 0.05$) for both concentrations (25 and 50) microgram/kg of Potassium bromate that was (111.25 and 127.38) (94.04 and 98.27) (31.97 and 33.88) and (135.18 and 146.37) in compare with control group 75.12, 85.42, 22.47 and 117.18 respectively, also the results showed presence of significant decrease in HDL value that was (23.72 and 20.15) in compare with control group. Also noticed there are significant decrease in cholesterol, triglyceride value, LDL and blood sugar and significant increase in value of HDL at the addition of both type of lactobacillus bacteria *L. casei* and *L. acidophilus* with Potassium bromate in compare with animals groups of Potassium bromate addition lonely.

Table 3. Effect of adding lactic acid bacteria to decrease of Potassium bromate effect in some parameters of lipid profile in female rats

Tret.	Concen.	Chol.	TG	LDL	HDL	Glucose
(mg / dl)						
T1	0	75.12±0.006e	85.42±0.037e	22.47±0.055d	30.89±0.236a	117.18±0.058d
T2	25	111.25±0.084b	94.04±0.026b	31.97±0.026b	23.72±0.112d	135.14±0.114b
T3	50	127.38±0.026a	98.27±0.109a	33.88±0.006a	20.15±0.248e	146.37±0.097a
T4	25	94.85±0.006d	90.25±0.162b	26.67±0.040c	26.47±0.011b	125.98±0.032c
T5	50	112.16±0.042b	94.76±0.043b	30.65±0.149b	24.95±0.007c	135.16±0.007b
T6	25	89.19±0.015d	89.64±0.021d	27.91±0.109c	26.79±0.072b	127.82±0.125c
T7	50	108.87±0.023c	92.85±0.002c	29.87±0.029b	24.51±0.002c	136.54±0.061b

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

The results was agree with that mentioned by (51) in presence of significant increase of value of cholesterol and triglyceride at the addition of Potassium bromate to the feed. (35) mentioned that presence of significant increase of Total cholesterol, triglycerides value and decrease in HDL at the addition of Potassium bromate to the rat feed. (5) showed a significant increase in cholesterol, triglycerirde (TG) and LDL at $p < 0.05$ but showed significant decrease in (HDL). Also agree with (55) that mentioned that the consumption of lactobacillus bacteria by the humans as probiotics led 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 (50 , 6). The researches mentioned to presence of many mechanism 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 (7). Some lactic acid bacteria (LAB) have been reported to have cholesterol-lowering effects in animals and humans (23). Moreover, several mechanisms associated with serum cholesterol reduction by LAB have been proposed, including assimilation, surface binding, incorporation into cellular membranes, coprecipitation with deconjugated bile, enzymatic deconjugation of bile acids, conversion of cholesterol, and production of short-chain fatty acids (11). (56) showed that the use of *L. plantarum* EM as a functional starter culture for juice fermentation exerts microbial control, enhances sanitary safety, and provides beneficial food effects against hypercholesterolemia, also conjugation of cholesterol with cell wall of probiotics during growth stages. (41) found that probiotics can activate both the innate and adaptive immune

systems, and thus provide better protection against pathogens. also way of converting of cholesterol to Coprostanol that excrete directly in feces (29). 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 (18). 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 Potassium bromate 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 or reduce of glucose removal rate from blood to tissues (22) 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 (1). Probiotic lactic acid bacteria (LAB) have shown enormous potential in the treatment of various diseases, including diabetes and obesity (19). The remove of these materials by decrease absorption by intestine with ability to prevent conjugation with intestinal villi lead to decrease absorption then decrease its toxic effect (20). Many of studies was showed that many types of lactobacillus have the ability to decrease levels of cholesterol and triglyceride (26, 21, 44). The addition of two types of lactic acid bacteria *Lb. casei* and *Lb. acidophilus* led to decreasing the negative effect on the values of all the parameters determined (32).

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