Effect of Lactic Acid Bacteria against Heavy Metals Toxicity in Rats

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Abstract: Cadmium and lead are highly toxic metals; people are exposed to them primarily through food and water. Therefore the study aimed to estimate the effect of lactic acid bacteria against toxicity induced by contaminated diet with lead and cadmium mixture in rats. Forty two Albino male rats (Sprague Dowely strain) of an average weight 130 ± 10 g were divided into 6 groups each group contains 7 rats. G1**:** fed on basal diet (negative control); G2**:** fed on contaminated food with 0.025mg lead acetate/kg diet + 0.025mg cadmium chloride /kg diet (positive control); G3**:** fed on basal diet supplemented with strain 1 of lactic acid bacteria (*Streptococcus thermophilus*); G4: fed on basal diet supplemented with strain 2 of lactic acid bacteria (*Lactobacillus bulgaricus*). The other two groups received heavy metals contaminated diet supplemented with strain1and strain2 lactic acid bacteria for 6 weeks. The results revealed that positive control gave a highly significant increased in liver functions (alanine aminotransferase (ALT) and aspartate minotransferase (AST) activities), kidney functions (creatinine and urea); significantly decreased in glutathione peroxidase (GPX), blood hemoglobin, body weight and feed efficiency ratio. However lactic acid strains supplemented to heavy metals treated group significantly improved the in glutathione peroxydase, blood hemoglobin, body weight and feed efficiency ratio and the elevation of ALT, AST, creatinine and urea. The results also showed that the group received basal diet supplemented with strain 1 (*Streptococcus thermophilus*) and strain 2 *(Lactobacillus bulgaricus)* has beneficial health effects on animals. It was noticed that the group received strain 1 (*Streptococcus thermophilus*) showed better results than strain 2 (*Lactobacillus bulgaricus*). The results of histopathology obtained also indicate that tested lactic acid bacteria strains have an effective role against the toxicity induced by lead and cadmium. These results indicated the potential protective action of tested lactic acid strains against lead and cadmium toxicity as well as their beneficial health effects. This may be due the ability of lactic acid strains to bind heavy metals, the DNA protective effect of LAB and thought to have several presumably beneficial effects on immune function. In addition LAB decreased the amount of administered carcinogens reaching the blood.
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Key Words*:* lactic acid bacteria, Heavy Metals, lead, cadmium.

1. Introduction:

Heavy metals are undegradable compounds that may exist in number of different inorganic and organic forms. Some heavy metals such as Fe, Cu and Zn are essential trace elements but others such as Cd and Pb have no advantageous biological function and are toxic even in very small amounts. Cd, Pb and Hg are regarded as the most toxic heavy metals (Halttunen, 2007).

Lead and cadmium are now recognized to be two of most contaminants in the environment. They released into the environment from natural and anthropogenic sources contaminating food and water. Chronic oral ingestion of cadmium and lead is associated with adverse effects in the skin, internal organs and nervous system. Lead and cadmium are known to produce various adverse effects on reproduction. Pregnancy causes many physiological and biochemical changes that may affect the metabolism of trace elements in the dam. Chronic toxicity symptoms are renal malfunction, anemia, brain and liver damage, cancer (Santos *et al*., 2004; Chandra and Banerjee, 2004).

To protect man from the harmful effect of lead, the intake of the metal should not exceed 300 µg/60Kg body weight (b.w.) and it should not exceed 25µg/Kg b.w. for young children (WHO, 1987). Based on the renal toxicity of cadmium, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA, 2003) has set a provisional tolerable weekly intake (PTWI) of 7 µg Cd/kg b.w./week. However, recent reports have challenged this guideline as too high, since according to a recent meta-analysis of available data, an increased concentration of beta-2-microglobulin, a biomarker for proteinuria, was detected at an exposure level comparable to a PTWI of only 3 µg Cd/kg b.w.(Omarova and Phillips, 2007).

Lactic acid bacteria (LAB) are ubiquitous in fermented and non-fermented foods and are common components of the human commensal microflora.

They are a group of bacteria characterized by their ability to synthesize lactic acid and are widely used in food manufacturing for their beneficial technological properties and positive effects on health. Many of their beneficial properties are related to their capacity to adhere or bind to different targets (Nybom *et al*. 2007). The LAB could be comprised of about 20 genera. *Lactobacillus* is largest of these genera, comprising around 80 recognized species (Axelsson, 2004).

Numerous investigations indicated that LAB have beneficial health effects in humans (Saxelin *et al*., 2005). One of the effects identified is the protection against toxins contained in foods such as heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, mycotoxins and reactive oxygen species (Stidl *et al*., 2007).

Lactic acid bacteria have been reported to remove heavy metals (Halttunen, 2007), cyanotoxins (Meriluoto *et al*. 2005 and Nybom *et al*., 2007) and mycotoxins (Haskard *et al*., 2001 and Turbic *et al*., 2002) from aqueous solution in-vitro. The removal of heavy metals, cyanotoxins and mycotoxins from aqueous solution by LAB has been observed to be strain dependent, and the most efficient strains in the removal of these compounds vary between toxins (Halttunen 2007 and Nybom *et al*. 2007). Heavy metals and aflatoxin B1 (AFB1) have been reported to passively bind to the bacterial surface by electrostatic and hydrophobic interactions (Lahtinen *et al*., 2004 and Halttunen, 2007) respectively, whereas microcystins may also be metabolized (Nybom *et al*., 2007).

The reported metal removal by different inactivated biomasses, and the toxin removal capacity of lactic acid bacteria in vitro, inspired us to assess the ability of lactic acid bacteria to remove or reduce the toxicity of cadmium, and lead in vivo. Therefore this study was conducted to investigate the effect of lactic acid bacteria on the toxicity induced by lead and cadmium mixture.

2. Materials and methods

1. Chemicals

Cadmium chloride, lead acetate and other chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, USA).

2. Diagnostic Kits

Different Commercial diagnostic kits used were purchased from BioMerieux Company (L'Etoile/France and Eagle Diagnostics (Dollas, TX, USA).

3. Media

MRS Broth and MRS Agar were obtained from Oxoid Ltd., Wade Road, Basingstoke, U.K.

4. Organisms

Two strains of probiotic bacteria obtained from the agent of Chr. Hansens Laboratory Denmark A/S were used in this study: Strain 1 *(Streptococcus thermophilus* CH-1) and Strain 2 (*Lactobacillus delbrekrii ss. bulgaricus* CH-2)

5. Animals

Forty two albino male rats (Sprague Dowely strain) with an average weight 130 ± 10 g were obtained from animal house of National Research Center. The experiment was carried out in the experimental animal house of NRC. Rats were divided into 6 equal groups and housed in galvanized metal cages. Food and water were supplied and libtum for 6 weeks. All rats were adapted for three days on the control diet before the beginning of the experiment.

6. Activation of tested bacterial strains

Streptococcus thermophilus CH-1and *Lactobacillus delbrekrii ss. bulgaricus* CH-2 were activated according to DeMan, *et al*., (1960). *Streptococcus thermophilus* CH-1and anaerobically incubated at 37°C for 24h.

7. Preparation of bacterial strains

Strain1 and 2 were prepared at National Research Centre (NRC) in vitro as the following: 5.0 ml of the activated tested bacteria was added to 500ml of MRS broth. After that it was incubated at the optimum temperature (37°C anaerobic conditions) to 24 hrs then it was centrifugated at (3000 r.p.m at 4°C for 20 min) to harvest the cells. Dehydration was obtained by addition 50 g of defatted soy protein (soy protein without fat) to cells in big Petri dishes and the cells were incubated under vacuum incubator at 40°C overnight until it seemed like as thin slice or skins. The viability of the cells was tested on MRS agar plates then, the strain was chopped and made as a powder containing 10^9 of bacteria/g.

8. Preparation of contaminated diet

Mixture of 0.025mg lead acetate plus 0.025mg cadmium chloride was added to every kilogram diet.

9. Experimental animal design

The forty two rats were divided to 6 groups as following: Group1 (G1): fed on basal diet as negative control, which was prepared according to the method described by Campbell, (1963). Group 2 (G2): fed on contaminated diet with 0.025mg lead acetate/kg

diet plus0.025mg cadmium chloride/kg diet (Positive control). Group3 (G3): fed on basal diet plus strain 1 of lactic acid bacteria (*streptococcus thermophilus*).Group 4 (G4): fed on basal diet + strain 2 of lactic acid bacteria (*Lactobacillus bulgaricus*).Group 5 (G5): fed on contaminated diet with (0.025mg lead acetate/kg diet plus 0.025mg cadmium chloride/kg diet) plus strain1 *streptococcus thermophilas*. Group 6 (G6): fed on contaminated diet with (0.025mg lead acetate/kg diet plus 0.025mg cadmium chloride/kg diet) plus strain 2 *Lactobacillus bulgaricus.*

10. Biological evaluation

During the experimental period (6 weeks) the consumed diet was recorded every day (Food Intake), and body weight was recorded every week. Biological evaluation of different groups was carried out by determination of body weight gain (BWG) and food efficiency ratio (FER) according to Chapman *et al*., (1959).

11. Biochemical analyses

At the end of the experiment, rats were fasted overnight (about 12 hrs) and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from hepatic portal vein. All blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. Serum was carefully separated and transferred into dry clean eppendorf tubes and kept frozen at (-20°C) till analysis, according to Jacobs *et al*. (2001). Blood samples were used for determination the following parameters: assayed serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities (liver functions) according to method of Henry (1974); Glutathione peroxydase was determined as mu/ml according to Paglia and Valentine, (1967). Kidney functions were determined as serum urea according to Carawy, (1955) and serum creatinine according to Larsen, (1972). Blood hemoglobin was estimated according to Jacobs *et al*. (2001).

12. Determination of lead and cadmium level in blood of rats

Lead and cadmium concentrations were determined according to the method described by Davis *et al*. (2003) using atomic absorption spectrometry (Solaar M6 Dual Zeeman AAS Spectrometer, Thermo Electron Spectroscopy Ltd., Cambridge, England) either by flame or graphite furnace method depending on the metal concentration. In each analysis, samples spiked with lead and cadmium as quality control samples.

13. Organs weight:

After taking retro orbital blood samples, each rat was rapidly opened, the liver and kidney were removed cleaned in saline solution and dried then weighted and kept in a formalin solution (10%v/v) according to the method described by Drury and Wallington, (1980).

14. Histopathological Examination:

At the end of the experiment, rats from each group were anesthetized with light ether then sacrificed by decapitation. After animal dissection, the liver, kidneys, heart, spleen and brain were removed, thoroughly washed with a physiological saline (0.9% NaCl) solution and blotted on filter paper. Organs specimens were rapidly fixed in Bruin's solution for 4h then retained in 70% alcohol until processing**.** The fixed specimens were processed using a conventional paraffin embedding technique. From the prepared paraffin blocks,5 mm thick sections were obtained and stained with hematoxylin and eosin (HE) for light microscopic examination (Culling, 1983). Specimens from liver and kidney were collected after kept in formalin then embedded in paraffin 4/6 thin sections were prepared and stained with hemetolxin and eosin according to Carleton, (1978).

15. Statistical analysis

Statistical analysis was performed by using computer program COSTATE and compared with each other using the suitable tests (Armitage and Berry, 1987). One way ANOVA was used and results were reported as

1-mean ± SD

2- P value differences were considered to be significant

$p \leq 0.05$ significant; $p \leq 0.001$ highly significant

3. Results and Discussion

Effect of lactic acid bacteria on body weight gain, food intake and feed efficiency ratio in rats fed contaminated diet with lead &cadmium mixture:

The obtained data of body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) in different treatment groups of rats are shown in Table (1). The results demonstrated that group fed on contaminated diet with lead and cadmium mixture showed highly significant decreased ($p<0.001$) on BWG, FI, and FER as compared to basal diet. The affected body weight by lead and cadmium is similar to those reported by Mahaffey *et al*., (1981) who showed that cadmium and lead administered in combination may depress weight gain more than either metal alone. However the results illustrated health benefits and the efficiency of lactic acid bacteria strains *Streptococcus thermophilas* and

Lactobacillus bulgaricus against toxicity induced by lead and cadmium mixture.

Table (1): Effect of lactic acid bacteria on body weight gain (gm), food intake (g) and feed efficiency ratio in rats fed lead and cadmium mixture contaminated diet.

Parameter / Group	Body Weight Gain (g)	Food Intake (g)	Feed efficiency Ratio
Negative control	$24.61^{a} \pm 4.46$	$455.16^{b} \pm 27.8$	$0.05^{a} \pm 0.01$
Negative control +St1	$21.39^{a} \pm 7.4$	$453.33^{b} \pm 21.34$	$0.04^{a} \pm 0.01$
Negative control +St2	$20.04^{a} \pm 4.5$	$455.66^{b} \pm 6.31$	$0.03^{a} \pm 0.004$
positive control	-13.56 ± 6.36	$241.16^{c} \pm 9.41$	$-0.15^{b} \pm 0.02$
positive control +St1	$8.34^{c} \pm 1.73$	$365.83^{a} \pm 22.26$	$0.02^{a} \pm 0.005$
positive control +St2	$12.23^{c} \pm 15.62$	$371.5^{a} \pm 10.41$	$0.05^{a} \pm 0.07$
LSD	6.05	30.57	0.01
P value	≤ 0.05	≤ 0.05	≤ 0.05

*Values are expressed as mean ± SD (P ≤ 0.05). Different superscripts are indicating significant between the mentioned values within formula groups.
Negative control = group fed on basal diet
Positive control= group fed on lead &cadmium mixture contaminated diet
ST1 (*streptococcus thermophilus*). ST2 (*Lactobacillus bulgaricus*).
In the same raw different letters means significant variation

Effect of lactic acid bacteria on liver functions in rats fed lead and cadmium contaminated diet:

The results in Fig1 demonstrate the effect of different treatments on some serum liver function enzymes alanine aminotransferase (AST) and aspartate aminotransferase (ALT). Positive control showed significant increased ($p< 0.05$) in enzyme activities as compared to negative control. The affected liver functions by lead and cadmium is similar to those reported by Sauer *et al*., (1997), they found that the damage effect of cadmium on the liver is manifested by an increased of AST and of the most specific marker of liver cell damage ALT. Also, agree with Othman *et al.*, (2004) who found significantly increased in activities of ALP, AST and ALP of lead treated rats and with Al-Wabel *et al*., (2007) who showed a significant increased in the activities of ALT and AST in serum of rats received lead acetate compared with the negative control. On the other hand the intake lactic acid bacteria strain1 (*streptococcus thermophilas*) and strain 2 (*Lactobacillus bulgaricus)* significantly alleviated the elevation of enzyme activity ($P\leq0.05$) in Pb and Cd mixture -treated rats. Also AST and ALT levels of lactic acid bacteria strain1 and 2 supplemented to basal diet were around control negative.

Effect of lactic acid bacteria on glutathione peroxidase activity in rats fed heavy metals contaminated diet:

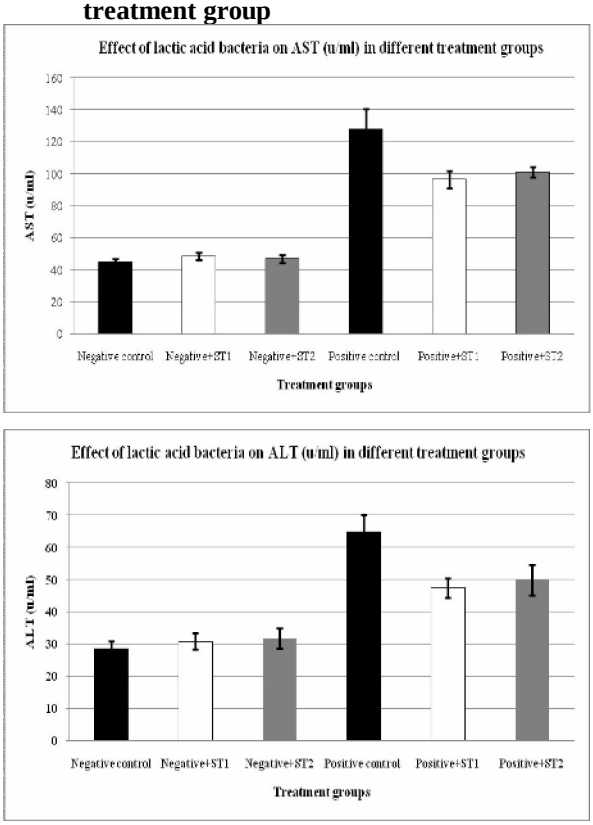
As shown in Fig 2, heavy metals treatment was highly significant depleted glutathione peroxidase (GPX) activity (used as marker of oxidative stress in liver) as compared to negative control ($p\leq0.001$). The depletion of GPX activity was observed by Amara *et al*., (2008). They found that cadmium exposure significantly decreased the GPx. The decrease in GPX due to Pb and Cd treatment was significantly reduced ($p\leq0.05$) when diet supplemented with strain1 (*Streptococcus thermophilas*) and strain2 (*Lactobacillus bulgaricus),* the former strain was better than the later.

Effect of lactic acid bacteria on kidney functions in rats fed heavy metals contaminated diet:

As shown in Fig 3, the effect of different treatments on some kidney function test (urea and creatinine) were investigated. There was high significant increased in urea and creatinine of positive control as compared to negative control

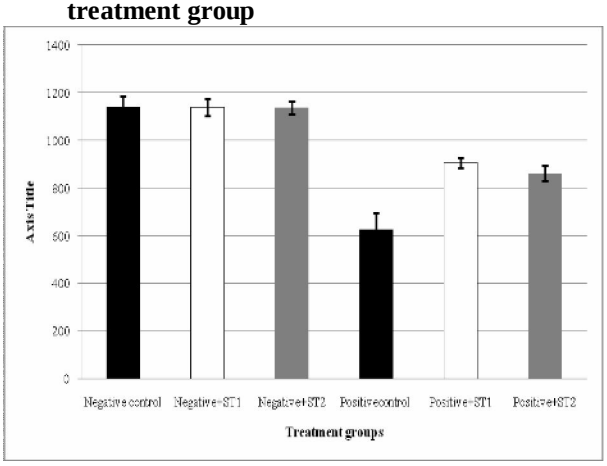
which improved after adding lactic acid bacteria *streptococcus thermophilas* and *Lactobacillus bulgaricus* to Pd and Cd contaminated diet.

Fig (1). Effect of lactic acid bacteria on liver function (AST &ALT) in different treatment group



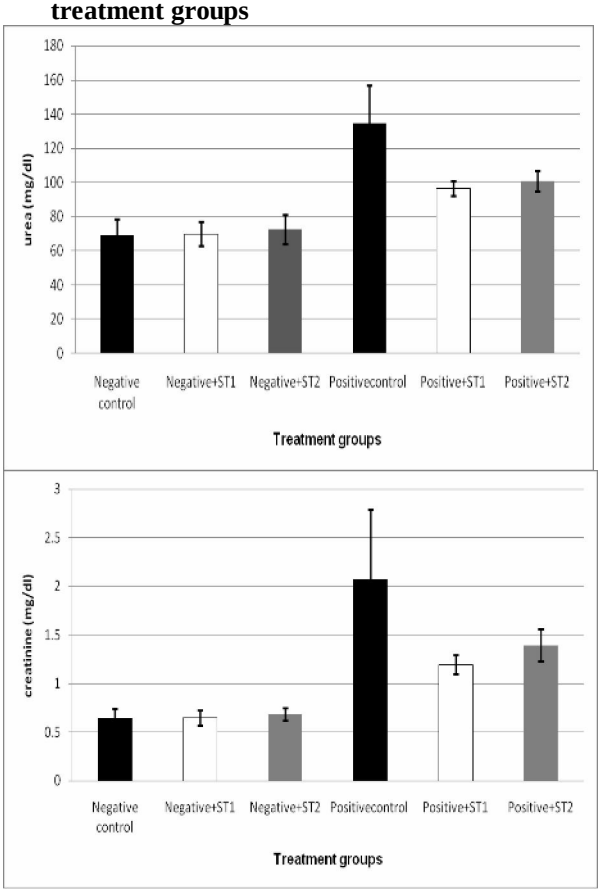
ST1 (*streptococcus thermophilus*).
ST2 (*Lactobacillus bulgaricus*).

Fig (2). Effect of lactic acid bacteria on Glutathione Peroxidase (GPX) in different treatment group



The toxicity of lead and cadmium on kidney functions in agree with Fels *et al*., (1998) who found that kidney function can be comprised due to chronic lead exposure. This may account for the increased of urea concentration in the animals received cadmium chloride. Also dietary exposure to cadmium has been reported to cause adverse health effects in the kidneys, liver, bone, peripheral vascular tissues, mammary gland, placenta, prostate, breast, pancreas and colon (Satarug and Moore 2004 and Satarug *et al*., 2006). In addition Haouema *et al*., (2007) reported that there are increased in urea and creatinine levels in groups fed on contaminated diet with cadmium and lead, also Adeyemi *et al*., (2009) concluded that lead contaminated water can possibly cause renal dysfunction as portrayed by the elevated serum concentration of urea and creatinine. Moreover Al-Hashem *et al*., (2009) found that highly significant increased in serum urea concentration through the experimental period of rats under administration of cadmium chloride compared with the control group.

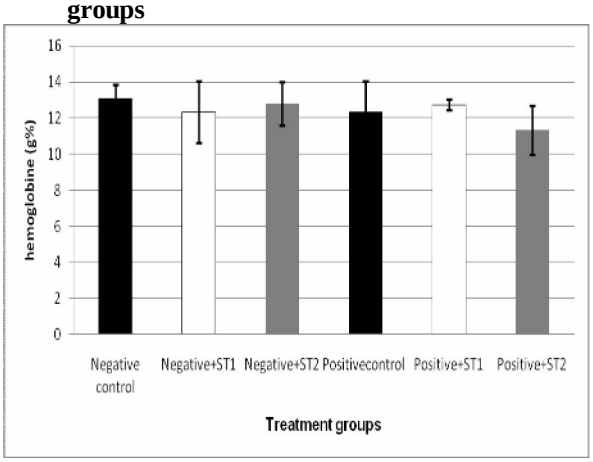
Fig (3). Effect of lactic acid bacteria on kidney function (urea & creatinine) in different treatment groups



Effect of lactic acid bacteria on blood hemoglobin in rats fed heavy metals contaminated diet:

As shown in Fig 4, level of blood hemoglobin was significant ($p \leq 0.05$) decreased in positive control as compared to negative control. These results indicated that, anemia caused by lead and cadmium mixture. The decreased in blood hemoglobin was significantly ($p \leq 0.05$) improved by strain1 (*Streptococcus thermophilas*) and strain2 (*Lactobacillus bulgaricus)* supplemented to lead and cadmium mixture treated group. The results are agree with those reported by (Piomelli *et al*., 1980) who concluded that Pb effects heme synthesis primarily by the inhibition of the s-aminolevulinic acid dehydrase (ALAD) and the enzyme synthesis (ferrochelatase) controlling the incorporation of an iron in to the heme molecule resulting in an iron deficiency anemia. Moreover Al-Hashem *et al*., (2009) found that exposure of rats to cadmium chloride resulted in highly significant decreased in blood hemoglobin levels compared with its levels in control group.

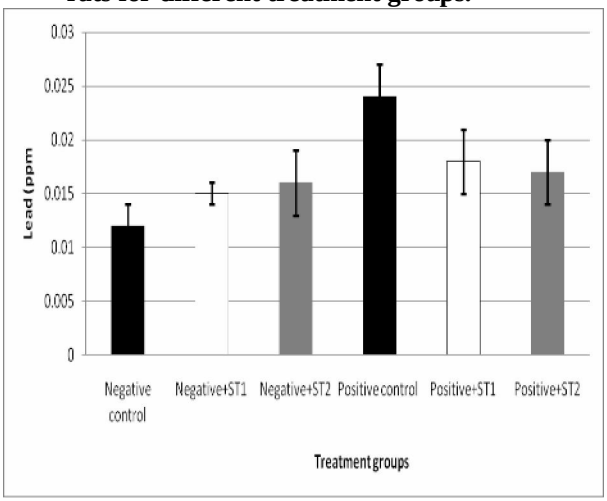
Fig (4). Effect of lactic acid bacteria on blood hemoglobin (g%) in different treatment groups

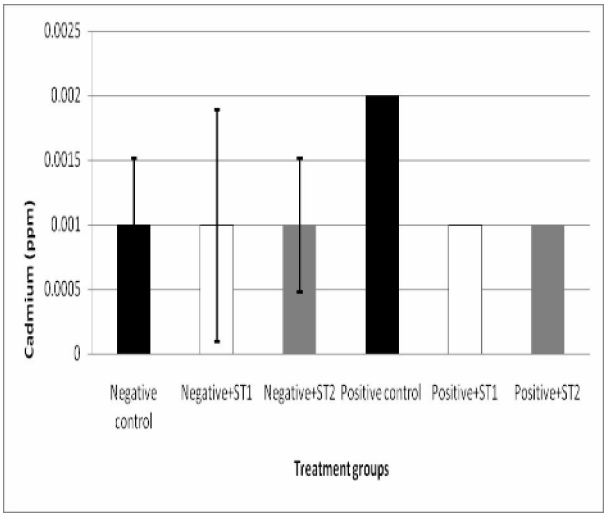


Effect of lactic acid bacteria on heavy metals level in blood of rats fed heavy metals contaminated diet:

The results in Fig 5 illustrated the effect of different treatment on lead and cadmium levels in blood of rats after exposure to Pb and Cd mixture. There was significant ($p \leq 0.05$) increased in positive control as compared to negative control. And significantly ($p \leq 0.05$) decrease in the groups received lead and cadmium plus lactic acid bacteria strain1 (*Streptococcus thermophilas*) and strain 2 (*Lactobacillus bulgaricus)* as compared to positive control. Groups received lactic acid bacteria strain1 and strain 2 gave concentrations around negative control. The presence of low levels of lead and cadmium in negative control and basal diet supplemented with lactic acid bacteria may be attributed to their level in drinking water. In general, natural concentration of cadmium and lead rarely exceed the guideline values of 3 and 10µg/L, respectively (WHO, 2006). The improved in heavy metal blood levels after adding lactic acid bacteria may be due to their ability to bind with lead and cadmium; this agrees with Rowland and Gangolli, (1999) who concluded that there was some experimental evidence that administered LAB decreased the amount of administered carcinogens reaching the blood in rats.

Fig (5). Levels of lead and cadmium in blood of rats for different treatment groups.



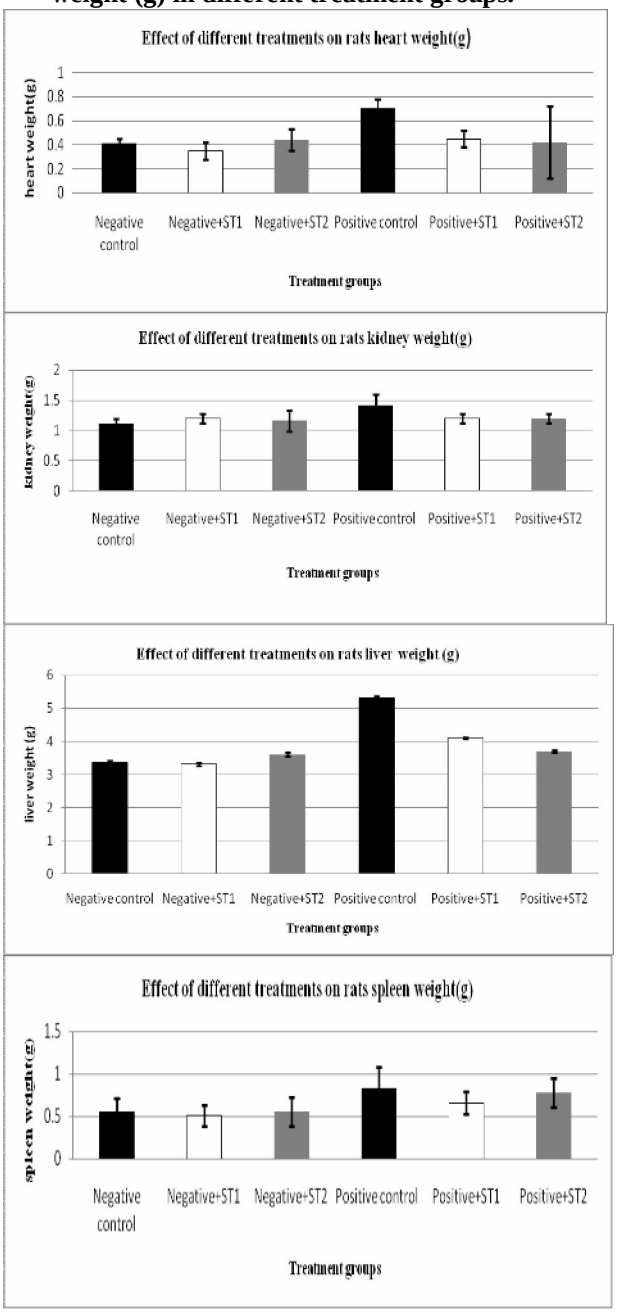


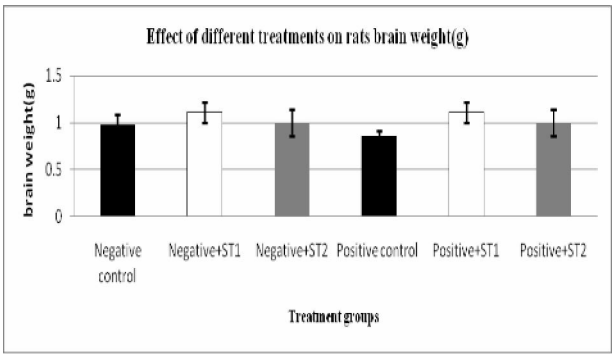
Effect of lactic acid bacteria on organs weight of rats fed heavy metals contaminated diet:

Data in Fig 6 showed the effect of tested lactic acid bacteria on organs weight (heart, kidney, liver, spleen and brain) for all treatments. It could be noticed that the group received contaminated diet with Cd and Pb mixture showed significantly

increased ($p \leq 0.05$) in organs weight as compared to basal diet group. The intake of lactic acid bacteria showed significantly lower ($p \leq 0.05$) and improved organs weight in heavy treated rats as compared to positive control. Simonyte *et al.*, (2006) found that a long term exposure to heavy metals there was a significant increased in spleen and liver weight.

Fig (6). Effect of lactic acid bacteria on organs weight (g) in different treatment groups.





Results of Histopathology:

1. Kidneys.

Kidneys of rat from group 1 which was fed on basal diet for 6 weeks showed no histopathological changes (photo1). Examined sections from group 2 which was fed on contaminated food with 0.025mg lead acetate and 0.025mg cadmium chloride /Kg diet for 6 weeks revealed focal interstitial nephritis associated with cystic dilatation of renal tubules (photo 2). However, kidneys of rats from groups 5 which was fed on contaminated food with 0.025mg lead acetate and 0.025mg cadmium chloride /Kg diet for 6 weeks + strain 1 (*streptococcus thermophilus*) and group 6 which was fed on contaminated food with 0.025mg lead acetate +0.025mg cadmium chloride /Kg diet for 6 weeks + strain 2 (*Lactobacillus bulgaricus),* showed no histopathological changes (photos 3 and 4).

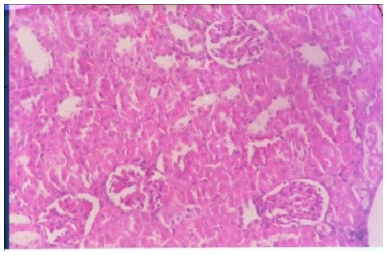


Photo (1). kidney of rat from group 1 showed no histopathological changes

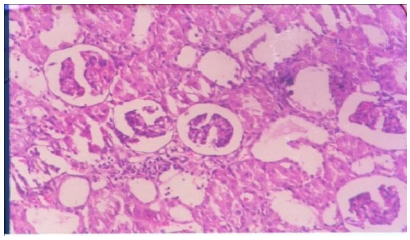


Photo (2). kidney of rat from group 2 showed focal interstitial nephritis associated with cystic dilatation of renal tubules.

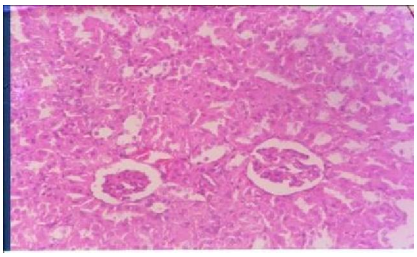


Photo (3). kidney of rat from group 5 showed no histopathological changes.

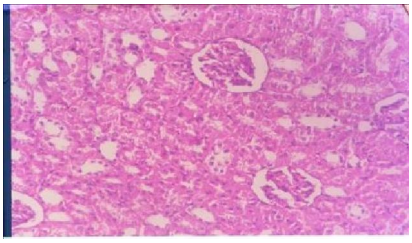


Photo (4). kidney of rat from group 6 showed no histopathological changes

2. Liver

Liver of rat from group 1which was fed on basal diet for 6 weeks revealed the normal histological structure of hepatic lobule (Photo 5). Mean while, liver of rat from group2 which was fed on contaminated food with 0.025mg lead acetate + 0.025mg cadmium chloride /Kg diet for 6 weeks showed vacuolation of Centro lobular hepatocytes and fibrosis in the portal triad (Photo 6). Slight congestion of central vein was the only change observed in liver of rat from group 5 which was fed on contaminated food with 0.025mg lead acetate + 0.025mg cadmium chloride /Kg diet for 6 weeks + lactic acid bacteria strain 1 (*streptococcus thermophilus*) and some examined sections from group 6 which was fed on contaminated food with 0.025mg lead acetate + 0.025mg cadmium chloride /Kg diet for 6 weeks + lactic acid bacteria strain 2 (*Lactobacillus bulgaricus)* (Photos 7 and 8). Other sections from group 6 revealed no histopathological changes (Photo 9).

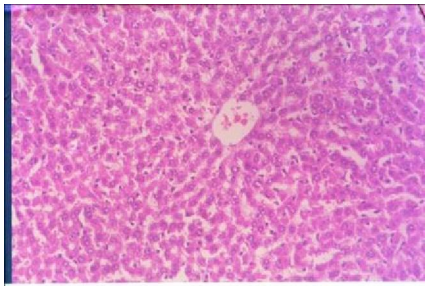


Photo (5). liver of rat from group 1 showed the normal histological structure of hepatic lobule.

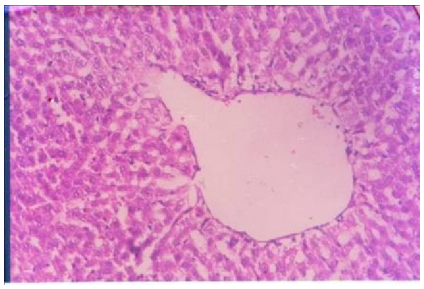


Photo (6). liver of rat from group 2 showed vacuolation of Centro lobular hepatocyte.

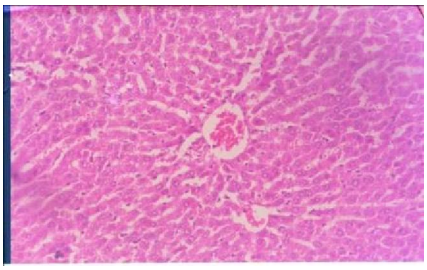


Photo (7). Liver of rat from group 5 showed no histopathological changes except slight congestion of central vein

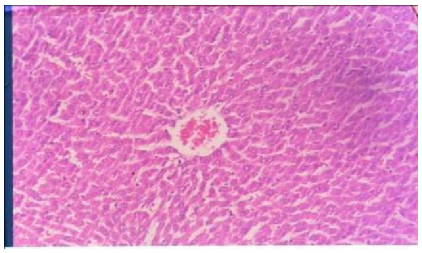


Photo (8). Liver of rat from group 6 showed no histopathological changes except slight congestion of central vein

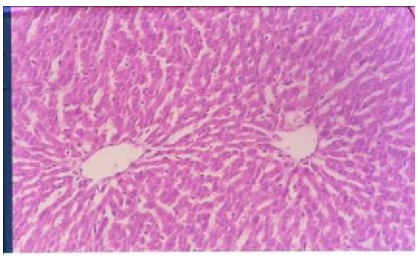


Photo (9). Liver of rat from group 6 showed no histopathological changes.

3. Brain

Brain of rat from group 1 which was fed on basal diet for 6 weeks revealed no histopathological changes (Photo 10). Meanwhile, brain of rat from group 2 which was fed on contaminated food with 0.025mg lead acetate + 0.025mg cadmium chloride /Kg diet for 6 weeks showed pylenosis of neurons (Photo11), focal cereloral hemorrhage and necrosis of

purkinge cells of cerebellum (Photos 12 and 13). No histopathological changes except Pylenosis of neurons was noticed in brain of rats from G5 which was fed on contaminated food with 0.025mg lead acetate + 0.025mg cadmium chloride /Kg diet for 6 weeks + lactic acid bacteria strain 1 (*streptococcus thermophilus*) (Photo 14). However, brain of rat from group 6 which was fed on contaminated food with 0.025mg lead acetate + 0.025mg cadmium chloride /Kg diet for 6 weeks + lactic acid bacteria strain 2 (*Lactobacillus bulgaricus)* revealed no histopathological changes (Photo 15).

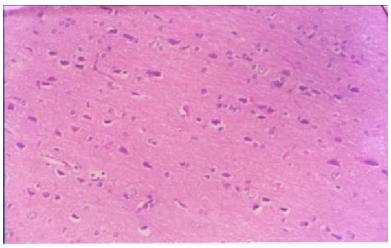


Photo (10). Brain of rat from group 1 showed no histopathological changes.

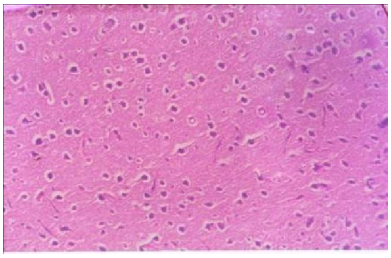


Photo (11). Brain of rat from group 2 showed pylenosis of neurons.

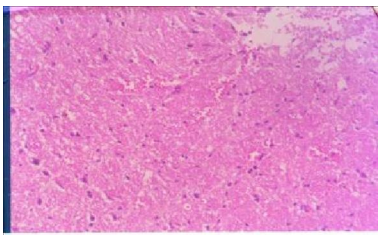


Photo (12). Brain of rat from group 2 showed focal cerebral hemorrhage .

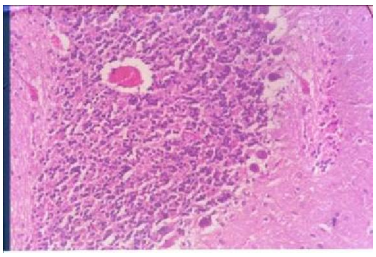


Photo (13). Brain of rat from group 2 showed necrosis of purkinge cells of cerebellum.

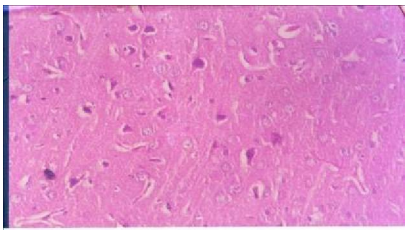


Photo (14). Brain of rat from group 5 showed no histopathological except pylenosis of some neurons.

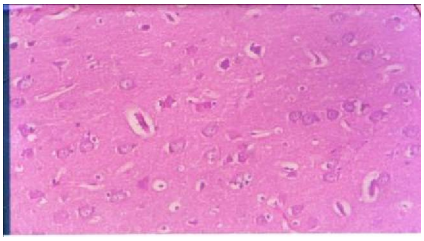


Photo (15). Brain of rat from group 6 showed no histopathological changes.

The results of histopathology obtained indicated that tested LAB strains have an effective role against the toxicity induced by contamination with lead and cadmium. The results illustrated that examined section for negative control showed no histopathological alternation. Meanwhile rats fed on contaminated diet showed marked focal interstitial nephritis associated with cytic dilatation of renal tubules in kidney and vacuolation of centro lobular hepatocyte and fibrosis in the portal triad in liver also pylenosis of neurons and necrosis of purkinge cells of cerebellum in brain. These results are agreed with El-Sokkary *et al*., (2005) who found severe histopathological damage in liver and kidney in lead-treated rats. Also Koyu *et al*., (2006) evaluated histipathologic changes included vacuolar and granular degeneration in hepatocytes, heterochromatic nucleuses and sinusoidal and portal widening in liver of rats treated with Cd. However the intake of strain1 (*Streptococcus thermophilas*) and strain2 (*Lactobacillus bulgaricus)* in the liver sections and showed no histopathological changes in kidneys also showed improved in the brain section in liver treated heavy metals.

It could be concluded that protective action of lactic acid bacteria strains especially the strains *streptococcus thermophilus* and *Lactobacillus bulgaricus* as a potential protective agent against lead and cadmium toxicity as well as their beneficial health effects this may be due to the ability to bind with lead and cadmium and remove the toxicity of lead and cadmium on rats. Numerous investigations indicated that LAB have beneficial health effects and thought to have several presumably beneficial effects on immune function (Ouwehand *et al*., 2002; Reid *et*

al., 2003 and Saxelin *et al*., 2005). Heavy metals have been reported to passively bind to the bacterial surface of LAB by electrostatic interactions (Haskard *et al*., 2000). Also Halttunen, (2007) found that Cd and Pb biniding by lyophilized lactic acid bacteria and bifidobacteria. In addition, Teemu, *et al*., (2008) concluded that specific lactic acid bacteria were observed to have a strain-specific capacity to bind the toxic cationic heavy metals, cadmium and lead, from water.

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