BEST: International Journal of Humanities, Arts, Medicine and Sciences (BEST: IJHAMS)
ISSN (P): 2348-0521, ISSN (E): 2454-4728
Vol. 3, Issue 11, Nov 2015, 131-148

POTENTIAL PROTECTIVE EFFECT OF FERMENTED CAMEL MILK CONTAINING PROBIOTIC, PREBIOTIC AND SYNBIOTIC AGAINST LEAD AND CADMIUM - INTOXICATED MALE RATS

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ABSTRACT

Potential protective effect of fermented camel milk containing probiotic, prebiotic and Synbiotic against lead and cadmium was studied in intoxicated male rats. The contents of the biologically active ingredients were investigated first and was found that the highest phenolic contents were recorded when the fermented camel milk was mixed with extracts of dandelion & spirulina (18.02 mg GAE/g), followed by Spirulina extract and then fermented camel milk with spirulina extract but no phenolic compounds were detected in fermented camel milk. The scavenging activity (%) of the experimental materials followed the same trend of the levels of the total phenolics except that analysis of fermented camel milk alone indicated a high scavenging activity (84.03 %) and this scavenging activity was further improved by addition of extract of dandelion & spirulina (98.32 %).

Treatment with lead acetate or Cd-chloride resulted in variable changes in body weight, liver and kidneys weight; the positive control groups scored the lowest gain in body weight and weight of organs. Activities of ALP, AST and ALT increased significantly following treatment with lead or cadmium. Administration of experimental preparations decreased significantly the elevated activities of these enzymes.

Administration of lead and cadmium elevated significantly the levels of both urea and creatinine. Administration of fermented camel milk with spirulina or preparation of fermented camel milk with spirulina and dandelion reduced significantly the concentration of urea and creatinine to almost normal values. Livers of rats treated with lead or cadmium showed subacute portal inflammatory reaction while the kidneys showed focal sub-acute interstitial nephritis. These Alterations are more severe in liver and kidneys of rats that received lead when compared with that received cadmium. All these histopathological alterations observed were improved by the treatments especially in the groups that received fermented camel milk with spirulina extract and the group on fermented camel milk with spirulina extract and dandelion.

KEYWORDS: Pro, Pre and Syn-biotic, Lead, Cadmium, Hepatotoxicity, Nephrotoxicity, Histopathology, Rats

INTRODUCTION

Adverse health effects of heavy metals have been known for a long time; exposure to heavy metals continues and is even increasing in some countries. Lead and cadmium are known to be the most important contaminants in the environment. Both Pb and Cd can seriously affect organs and various systems of an organism and can cause severe acute and especially chronic intoxications. Lead (Pb), is an environmental pollutant and can be detected in all phases of biological systems and environments. It is an accumulative toxic element that has no known beneficial or desirable

nutritional effect on animals (Ladrón de Guevara and Moya, 1995). Lead is a neurotoxin and known to be found in drinking water. Chronic lead exposure has been associated with reduced an intelligence quotient (IQ) in children and various other mental defects (Obiri-Danso *et al.*, 2003). Patrick, (2006) found that about 75% of lead exposure to humans comes from ingestion. Adverse health effects associated with lead ingestion include neurotoxicity, nephrotoxicity, and deleterious effects on the hematological and cardiovascular systems (ATSDR, 2007).

Recent data confirmed that levels formerly considered safe may threaten health, especially among infants and children (Guidotti and Ragain, 2007). Nowadays, lead is found everywhere in the environmental due to its significant role in modern industry (Shalan *et al.*, 2005). Up to 50% of inhaled inorganic lead is absorbed in the lungs. Adults take up 10–15% of lead in food, whereas children may absorb up to 50% via the gastrointestinal tract. This amount poses a serious threat because of every day contamination of food.

On the other hand Cadmium (Cd) is one of the most common toxic heavy metals, due to its accumulation in the liver, kidney and testis. The general exposure to Cd, mainly results from smoking, air pollution and consumption of Cd contaminated foods and water (Honda *et al.*, 2010). Cd causes a wide range of adverse health effects, including renal dysfunction, cardiovascular disease, hypertension, osteoporosis, hepato-toxicity, pancreatic activity changes and cancers of many organs (Donpunha *et al.*, 2011; Kisok, 2012; Nemmiche *et al.*, 2011).

Cadmium has been classified as carcinogenic by the International Agency for Research on Cancer (IARC, 1993). It contributes indirectly to oxidative stress. Consumption of heavy metals result in generation of highly reactive oxygen species (ROS), such as superoxide radicals (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and lipid peroxides (LPO), and result in damaging various cellular components including proteins, membrane lipids and nucleic acids (Halliwell and Gutteridge, 1989)and depletes cell antioxidant defense systems.

The beneficial role of antioxidant nutrients through exogenous supplementation of antioxidant molecules may be effective in rebalancing the challenged, prooxidant/antioxidant ratio in the body following heavy metals consumption.

Camel milk constituents are very different from that of other ruminant's constituents (Yagil, 2000). The ayurvedic and other folk systems of medicine in various countries including Saudi Arabia are interested to know more information about the beneficial effect of fresh and fermented camel milk because of its protective health and therapeutic values. Oral consumption of fresh and fermented camel milk has been associated with the prevention or cure of diverse intestinal disorders such as lactose and microbial diseases (McNaught & MacFie, 2001, Agrawal *et al.*, 2002).

Probiotic bacterial in fermented dairy products are of interest for human health and diseases. Clinical studies indicated that probiotics may not be equally effective for prevention of certain diseases. Fermented dairy products containing viable microorganisms and incorporation of probiotics as starter organisms have positive effect on probiotic cultures (Reid *et al.*, 2010, Heller, 2001).

Mohamed *et al.* (1990), observed that camel milk failed to form gel like structure after 18 h incubation with lactic acid culture, this was attributed to the presence of antibacterial factors such as lysozymes, lactoferrin immunoglobulin in camel milk (El-Agmy *et al.*, 1992, Farah *et al.*, 1990). Fresh and fermented camel milk were found to provide various potential health benefits including angiotension I-converting enzyme-inhibitory activity, hypocholesterolaemic effect, hypoglycaemic effect, antimicrobial and hypoallergenicity effects (Omar and Hamad 2010).

Taraxacum officinale, known as dandelion, is a member of the Asteraceae/Compositae family. Traditionally,

dandelion has been used as a herbal medicine due to its antidiabetic, choleretic, antirhematic, and diuretic properties (Schütz *et al.*, 2006). Recent studies have proved that it may reduce the risk of diseases, including inflammation and tumors (Kim *et al.*, 2007);

The therapeutic actions of Taraxacum species have been attributed to their bitter components, some sesquiterpenes and to several phenolic compounds, e.g. chicoric acid and its isomer, monocaffeoyltartaric, caffeoylquinic, chlorogenic, caffeic, p-coumaric, ferulic, phydroxybenzoic,protocatechuic, vanillic, syringic and p-hydroxyphenylacetic acids as well as three coumarins, umbelliferone,esculetin and scopoletin that were demonstrated in dandelion roots.

Spirulina, filamentous blue green mycobacterium and is generally known as a valuable additional food and medicinal, neutraceutical as well as therapeutic agent. *Spirulina platensis* also possess potent antiviral, antimutagenic, anticancerous and cholesterol lowering activity. Spirulina is a good source of some macro and micronutrients such as amino acids, chlorophyll, gamma linolenic acid, carotenoids, vitamins B1 and B2 and trace elements such as iron, iodine, selenium and zinc (Jayaprakash and Chinnaswamy, 2005; Kumar *et al.*, 2005)

The objective of this research is to evaluate the protective role of Pro, Pre and Synbiotic agents against hepato-, nephro toxic effects of lead acetate and cadmium chloride in male rats.

MATERIALS AND METHODS

Materials

Experimental Material

Spirulina (*Spirulina platensis*) biomass was obtained from The Algae Biotechnology Unit, National research center, Giza, Egypt. Dried dandelion (Taraxacumofficinalis) leaves and roots were obtained from local market, Buraidah Qassim,Saudi Arabia). Camel milk samples were obtained from healthy lactating animals at the Agricultural Research Station .Qassim University. Starter cultures of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from Chr. Hansen`s Laboratory, Copenhagen Denemark.

Chemical

Lead acetate, cadmium chloride, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), Folin-Ciocalteus phenol reagent and Gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).Commercial kits used for determining alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, urea and creatinine were purchased from bio-Merieux Laboratory Reagents and Products, France and kits from Nubenco Interprises INC. Paramus, New Jersey, USA

Animals

One hundred and twenty male Wistar rats average weight (140 ±10g) were obtained from the experimental animal unit, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Animals were housed in control housing unit and were kept under standard conditions of temperature and humidity (temperature at 25°C, 55% humidity (40 to 70%) and in a 12-h: 12-h light: dark cycle) in experimental animal house. The rats were fed on standard pellets of concentrated diet(20.0 % Crude protein, 4.0% Crude fat, 3.5% Crude fiber, 6.0% Ash, 0.50% Salt, 1.0% Calcium, 0.60% Phosphorous, 20.0 IU/g Vitamin A. 2.20 IU/g Vitamin D, 70.0 IU/kg Vitamin E, Energy 2850.0 Kcal/kg), clean drinking water was allowed according to AIN-93 guidelines (Reeves, et al. 1993), and the changes in body weight were recorded weekly. Animal

procedures were performed in accordance with the ethics committee of Qassim University and according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

Preparation of Aqueous Extract of Dandelion and Spirulina

Prebiotic ingredients of aqueous extract of dandelion (*Taraxacum officinalis*) and Spirulina (*Spirulina platensis*) biomass were prepared according to the method described by Abdel-Salam *et al.*, (2009 & 2010). Briefly, *Taraxacum officinalis* and *Spirulina platensis* material were pulverized separately in a grinder (6 % total dry matter). The pulverized material was then dissolved and extracted with 1000 ml hot distilled water in an electric blender for 15 min. The suspension was left at room temperature for one hour, then filtered twice, first through cheese-cloth (50% cotton/50% polyester) and then through filter paper (Whatman No.2). The clear aqueous extract was preserved in sterile dark bottles (100ml) at -20°C until further used.

Preparation of Probiotic and Synbiotic Fermented Camel Milk

Camel milk containing *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was prepared according to the traditional method described by Tamime and Robinson (1999). The fresh milk was analyzed according to the AOAC method (1990). Synbiotic Camel milk containing (probiotic and prebiotic) was prepared by combining equal volume of probiotic fermented milk with an equal volume of aqueous dandelion extract or Spirulina (1v:1v).

Methods of analysis

Determination of Total Phenolic Content

The total phenolic content of the extracts was determined colorimetrically, using the Folin-Ciocalteu method, as described by Singleton et al., (1999). The total amount of phenolic compounds was calculated and expressed as GAE (mg/g).

Antioxidant activity (DPPH free radicals scavenge) assay

The ability of the extracts to scavenge DPPH free radicals was determined by the method described by Blois (1958).

Scavenging activity % = [(Abs control – Abs sample)/Abscontrol] x 100

Biological experiment procedure:

Animals were divided randomly into fifteen groups of eight animals each and received the following treatment daily for ten weeks:

1. Control group; rats received tap water as drinking water.
2. Rats received tap water containing lead acetate (pb) at a dose of 0.6 % ppm as drinking water
3. pb-acetate + dandelion extract.
4. pb-acetate + spirulina extract,
5. pb-acetate + fermented camel milk,

6. pb-acetate + fermented camel milk with dandelion extract,
7. pb-acetate + fermented camel milk with spirulina extract,
8. pb-acetate + fermented camel milk with dandelion & spirulina extract groups
9. Rats received tap water containing Cd-chloride, at a dose of 500 ppm as drinking water,
10. Cd-chloride + dandelion extract,
11. Cd-chloride + spirulina extract,
12. Cd-chloride + fermented camel milk,
13. Cd-chloride + fermented camel milk with dandelion extract,
14. Cd-chloride + fermented camel milk with spirulina extract,
15. Cd-chloride + fermented camel milk with dandelion & spirulina extract groups

BIOCHEMICAL INVESTIGATION

Blood Collection and Serum Preparation

Animals were anesthetized and blood samples were collected in dry clean tubes from ocular vascular bed using capillary tubes then centrifuged for 10 min to isolate serum and stored at -20ºC.

Evaluation of Liver Function

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to the method of Reitman and Frankel (1957). Total protein was determined colorimetrically according to the method adopted by Rodkey (1964). While, the activities of alkaline phosphatase (ALP) was carried out according to the method of Tietz *et al.*, (1983).

Evaluation of Kidney Function

Enzymatic colorimetric determination of urea was carried out according to the method of Searcy *el al.,* (1967). Kinetic determination of creatinine was carried out according to the method of Henry (1974).

Histopathological Investigation

Autopsy samples were taken from the liver and kidneys of the sacrificed rats of the different groups and fixed in 10% formal saline solution. Washing was done in tap water then serial dilutions of ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin wax tissue blocks were sectioned at 4 micron thickness by sledge microtome. The sections were collected on the glass slides, deparaffinized and stained by hematoxylin and eosin stain for routine examination by the light electric microscope (Banchroft *et al.*, 1996).

Statistical Analysis

Descriptive values of data were represented as means ± standard errors. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test with $P \leq 0.05$ being considered statistically significant (Snedecor and Cochran, 1980).Statistical analysis was conducted with SAS program (SAS, 1996).

RESULTS

Bioactive Compounds in Pro, Pre and Synbiotic

The results of total phenolics and scavenging activity of the experimental materials are presented in Table (1). The levels of total phenolics in dandelion extract, spirulina extract, fermented camel milk, fermented camel milk with dandelion extract, fermented camel milk with spirulina extract and fermented camel milk with dandelion & spirulina extracts were 2.09, 16.0, ND, 1.99, 15.52, 18.02 mg GAE/g respectively. As can be noticed from the results that camel milk contain no phenolic compounds and spirulina extract contain significantly higher level when compared with other individual ingredient. However, the highest phenolic contents were recorded when the fermented camel milk was mixed with extracts of dandelion & spirulina. The scavenging activity (%) of the experimental materials followed the same trend of the levels of the total phenolics except that analysis of fermented camel milk alone indicated a high scavenging activity (84.03%) and this scavenging activity was further improved by addition of extract of dandelion & spirulina (98.32 %)

Table 1: Bioactive Compound in Pro, Pre and Syn-Biotic

Experimental Material	Total phenolics (mg GAE/g)	Scavenging activity (%)
Dandelione E xtract	2.09 ± 0.57^{d}	20.58 ± 0.17^{e}
Spirulina extract	16.0 ± 0.7^{c}	71.09 ± 0.28^{d}
Fermented camel milk	ND	84.03 ± 0.88^{c}
Fermented camel milk with dandelion extract	1.99 ± 0.51^{d}	88.15 ± 0.27^{b}
Fermented camel milk with spirulina extract	15.52 ± 0.09^{b}	92.11 ± 0.11^{b}
Fermented camel milk with dandelion & spirulina extracts	18.02 ± 0.06^{a}	98.32 ± 0.08^{a}

Data are the mean ± SE, n = 3, Mean values in the same column bearing the same superscript do not differ significantly ($P \leq 0.05$), ND: Not Detect.

Effect of Experimental Treatments on body Weight gain and Relative Organs Weight

The effect of treatment with lead acetate on body weight of rats and the weight of liver and kidneys is shown in Table 2.There was a variable gain in body weight of all experimental groups studied however, the positive control group scored the lowest gain in body weight (92.0g).The highest gain was reported in the group that received dandelion (154.63g) but this was not significantly higher than the gain scored by the other groups. The weight of livers decreased significantly in all groups except groups that received dandelion extract, fermented camel milk with spirulina extract and Fermented camel milk with dandelion & spirulina extracts. As for the changes in weight of kidneys, all groups showed changes in weight of kidneys in the different groups. Groups that showed a significant decrease in kidney weight when compared with the negative control group were the positive control, Spirulina extract group, dandelion extract group and the group that received fermented camel with dandelion.

Table 2: Effects of Experimental Treatments on Body Weight gain and Relative Organs Weight in Rats Exposed to Pb-acetate

Pb-acetate Treatments	Initial body weight (g)	Final body weight (g)	Body weight Gain (g)	Organs weight (g)	
				Liver	Kidney
1	145.13 ± 0.02^{a}	299.38 ± 0.54^{a}	154.25 ± 0.21^{a}	9.099 ± 0.52^{a}	2.461 ± 0.06^{a}
2	135.50 ± 0.06^{a}	227.5 ± 0.11^{c}	92.0 ± 0.47^{b}	6.298 ± 0.64^{dc}	1.939 ± 0.18^{bc}
3	131.00 ± 0.12^{a}	285.63 ± 0.43^{ab}	154.63 ± 0.95^{a}	8.875 ± 0.72^{ab}	1.638± 0.2^{c}
4	128.38 ± 0.25^{a}	250.38 ± 0.48^{bc}	122 ± 0.45^{ab}	6.110 ± 0.61^{d}	1.753± 0.19^{bc}
5	134.63 ± 0.58^{a}	272.13 ± 0.56^{abc}	137.5 ± 0.63^{ab}	8.055 ± 0.88^{bc}	2.166± 0.16^{ab}
6	132.38 ± 0.18^{a}	255.0 ± 0.15^{abc}	122.63 ± 0.48^{ab}	7.098 ± 0.13^{cd}	1.895 ± 0.09^{bc}
7	129.50 ± 0.41^{a}	264.5 ± 0.52^{abc}	135.0 ±0.63^{ab}	8.768 ± 0.58^{ab}	2.443± 0.19^{a}
8	134.50 ± 0.11^{a}	262.5 ± 0.33^{abc}	128.0 ± 0.14^{ab}	8.560 ± 0.19^{ab}	2.448± 0.09^{a}

Means in the same column having different letters are significantly different ($P \leq 0.05$).

Effects of treatments with Cd-chloride on body weight of rats and the weight of liver and kidneys is shown in Table 3.The lowest body weight gain was observed in the positive control group and in the group that received dandelion extract. In the other groups, although there was reduction in body weight gain but was not significant when compared with the negative control which scored the highest gain in body weight (154.25g).The highest gain in body weight was observed in the groups that received fermented camel milk with dandelion & spirulina extract and in the group that was treated with spirulina extract. The weight of livers decreased significantly in all groups when compared with the negative control. The positive control group showed the lowest liver weight when compared with the other groups. The same trend was noticed in the weight of kidneys of the different groups.

Table 3: Effects of Experimental Treatments on Body Weight Gain and Relative Organs Weight in Rats Exposed to Cd-chloride

Cd-chloride Treatments	Initial body weight (g)	Final body weight (g)	Body weight Gain (g)	Organs weight (g)	
				Liver	Kidney
1	145.13 ± 0.14^{a}	299.38 ± 0.25^{a}	154.25 ± 0.07^{a}	9.099 ± 0.52^{a}	2.461 ± 0.06^{a}
9	129.75 ± 0.22^{a}	228.13 ± 0.54^{b}	98.38 ± 0.16^{b}	5.859 ± 0.38^{c}	1.839 ± 0.05^{bc}
10	132.00 ± 0.61^{a}	237.63 ± 0.64^{b}	105.63 ± 0.45^{b}	7.083 ± 0.19^{bc}	1.911± 0.09^{bc}
11	135.38 ± 0.41^{a}	256.13 ± 0.44^{b}	120.75 ± 0.34^{ab}	7.242 ± 0.71^{bc}	2.064 ± 0.15^{b}
12	127.25 ± 0.09^{a}	238.63 ± 0.69^{b}	111.38 ± 0.65^{ab}	6.493 ± 0.36^{bc}	1.931 ± 0.08^{bc}
13	127.13 ± 0.18^{a}	234.25 ± 0.63^{b}	107.13 ± 0.28^{ab}	6.145 ± 0.52^{bc}	1.744 ± 0.1^{c}
14	134.63 ± 0.26^{a}	250.75 ± 0.32^{b}	116.13 ± 0.87^{ab}	6.809 ± 0.51^{bc}	2.048 ± 0.11^{b}
15	133.88 ± 0.19^{a}	253.00 ± 0.37^{b}	119.13 ± 0.46^{ab}	7.452 ± 0.25^{b}	2.042 ± 0.06^{bc}

Means having different letters in the same column are significantly different ($P \leq 0.05$).

Liver Function Parameters

The concentration of total proteins and the activities of ALP, AST and ALT in serum, as indicators of the protective effect of Pro, Pre and Synbiotic preparations against lead and cadmium toxicity, are presented in table 4&5. In the Pb experiment, administration of Pb decreased significantly the concentration of total protein in the group that received Pb only (positive control and in all other groups except the group that was treated with fermented camel containing spirulina group 7). The activities of ALP, AST and ALT are presented in table 4. Activities of ALP, AST and ALT in normal rats were found to be 120.2, 54.43, 35.57 U/L respectively .These values increased significantly in rats that received Pb only to170.3, 81.37 and 81.28 U/L respectively. Following treatment there was a significant reduction in the activities of these enzymes

Table 4: Effect of Experimental Treatments on Serum Liver Function in rats Exposed to Pb-Acetate

Pb-acetate Treatments	T. Protein g/dl	ALP U/L	AST U/L	ALT U/L
1	7.43 ± 0.01^{a}	120.2 ± 0.04^{h}	54.43 ± 0.12^{f}	35.57 ± 0.1^{g}
2	5.1 ± 0.11^{f}	170.3 ± 0.08^{a}	81.37 ± 0.09^{a}	81.28 ± 0.12^{a}
3	6.12 ± 0.03^{e}	154.08 ± 0.02^{b}	70.44 ± 0.47^{b}	64.96 ± 0.02^{b}
4	6.83 ± 0.08^{d}	148.2 ± 0.12^{c}	62.05 ± 0.03^{c}	34.22 ± 0.07^{h}
5	7.06 ± 0.02^{c}	142.26 ± 0.21^{e}	59.23 ± 0.08^{d}	48.23 ± 0.15^{c}
6	6.83 ± 0.03^{d}	146.1 ± 0.33^{d}	55.21 ± 0.06^{e}	40.84 ± 0.09^{d}
7	7.39 ± 0.06^{ab}	126.3 ± 0.17^{f}	53.47 ± 0.1^{g}	39.02 ± 0.03^{e}
8	7.35 ± 0.01 ^{b}	121.56 ± 0.41^{g}	51.24 ± 0.13^{h}	36.67 ± 0.08^{f}

Means having different letters in the same column are significantly different (P ≤ 0.05).

The concentration of total proteins and the activities of ALP, AST, and ALT in serum, as indicators of the protective effect of Pro, Pre and Synbiotic preparations against cadmium toxicity, are presented in table 5.There was a significant decrease in the concentration of total protein in all groups, however experimental treatments with Pro, Pre and Synbiotic preparations increased the concentration of total serum protein. The best increase was achieved in the group that received fermented camel milk with dandelion & spirulina extract (group 15).The activities of ALP, AST, ALT in normal rats were found to be 120.2, 52.4, 35.57 U/L respectively ,however, these activities increased significantly in the group that received Cd only to 168.25, 85.47, 108.47 U/L respectively. Experimental treatment with Pro, Pre and Synbiotic preparations decreased the elevated activities of the enzymes and the best lowering effect was achieved by treatment with fermented camel milk with dandelion & spirulina extract and with the treatment with fermented camel milk and spirulina.

Table 5: Effect of Experimental Treatments on Serum Liver Function in Rats Exposed to Cd-Chloride

Cd-chloride Treatments	T. Protein g/dl	ALP U/L	AST U/L	ALT U/L
1	7.43 ± 0.01^{a}	120.2 ± 0.04^{g}	52.4 ± 0.12^{f}	35.57 ± 0.10^{h}
9	4.18 ± 0.06^{h}	168.25 ± 0.09^{a}	85.47 ± 0.10^{a}	108.47 ± 0.14^{a}
10	5.05 ± 0.04^{g}	154.08 ± 0.05^{b}	71.05 ± 0.03^{b}	88.98 ± 0.02^{b}
11	6.07 ± 0.2^{f}	144.6 ± 0.28^{c}	68.71 ± 0.22^{c}	74.13 ± 0.17^{d}
12	6.71 ± 0.14^{e}	132.23 ± 0.11^{e}	61.20 ± 0.08^{d}	78.37 ± 0.19^{c}
13	6.91 ± 0.22^{d}	136.23 ± 0.06^{d}	59.05 ± 0.06^{e}	61.23 ± 0.09^{e}
14	7.20 ± 0.31^{c}	119.37 ± 0.08^{h}	52.40 ± 0.11^{g}	59.03 ± 0.03^{f}
15	7.30 ± 0.4^{b}	121.5 ± 0.17^{f}	51.18 ± 0.09^{h}	46.60 ± 0.22^{g}

Means having different letters in the same column are significantly different (P ≤ 0.05).

Concentration of serum Urea and Creatinine in rats Exposed to Pb-acetate

Concentration of serum Urea and Creatinine in rats exposed to Pb and Cd and the protective effect of Pro, Pre and Syn-biotic preparations are shown in table 6 &7. The concentration of serum urea in the control group was 5.81 mg/dl and increased to 11.36 and 10.61 mg/dl in the groups that received Pb and Cd respectively. Treatment with Pro, Pre and Syn-biotic preparations decreased significantly, the elevated concentrations of urea and the best reduction was recorded with the treatment with fermented camel milk with dandelion & spirulina extract in both lead and cadmium treatment.

Table 6: Effect of Experimental Treatments on Serum Urea and Creatinine in rats Exposed to Pb-Acetate

Pb-acetate Treatments	Urea (mg/dl)	Creatinine (mg/dl)
1	5.81 ± 0.01^{f}	0.61 ± 0.08^{ed}
2	11.36 ± 0.04^{a}	1.073 ± 0.03^{a}
3	7.99 ± 0.08^{b}	0.83 ± 0.81^{c}
4	7.77 ± 0.06^{c}	0.96 ± 0.14^{b}
5	7.23 ± 0.1^{d}	0.60 ± 0.06^{ed}
6	7.81 ± 0.21^{c}	0.64 ± 0.16^{d}
7	6.12 ± 0.29^{e}	0.62 ± 0.31^{d}
8	6.05 ± 0.26^{e}	0.57 ± 0.25^{e}

Means having different letters in the same column are significantly different ($P \leq 0.05$).

The concentration of serum creatinine in the control group 0.61 mg/dl and increased to 1.073 and 1.20 mg/dl in the groups that received Pb and Cd respectively. Treatment with Pro, Pre and Syn-biotic preparations decreased significantly the elevated concentrations of creatinine and the best reduction in the concentration of creatinine was recorded with the treatment with fermented camel milk with dandelion & spirulina extract in both lead and cadmium treatment.

Table 7: Effect of Experimental Treatments on Serum Urea and Creatinine in Rats Exposed to Cd-Chloride

Cd-chloride Treatments	Urea (mg/dl)	Creatinine (mg/dl)
1	5.81 ± 0.01^{g}	0.61 ± 0.08^{e}
9	10.61 ± 0.12^{a}	1.20 ± 0.05^{a}
10	8.97 ± 0.02^{b}	1.12 ± 0.12^{b}
11	8.63 ± 0.15^{c}	1.02 ± 0.41^{c}
12	7.70 ± 0.08^{d}	1.11 ± 0.03^{b}
13	6.94 ± 0.11^{e}	0.97 ± 0.39^{c}
14	6.23 ± 0.04^{f}	0.72 ± 0.25^{d}
15	6.13 ± 0.09^{f}	0.56 ± 0.02^{e}

Means having different letters in the same column are significantly different ($P \leq 0.05$).

Histopathological Finding

Data concerning the histopathological examinations in the liver and kidney of treated rats when orally administered with lead acetate and cadmium chloride are presented in figures from 1 to 4 and table 8. Examination of the obtained results, revealed the influence of lead and cadmium on the tested animal organs. To facilitate the presentation of data, each organ is discussed separately as follows:

Lead Acetate Treated Groups

- **Liver**

The histology of liver of the control group is presented in (Figure 1), Micrograph1. The portal area in Pb-acetate treated rat showed inflammatory cells infiltration and fibroblastic cells proliferation with congestion in the portal vein (Micrograph 2).

The liver sections obtained from rat treated with Pb-acetate plus dandelion extract showed inflammatory cells infiltration with few fibrosis (Micrograph 3). Karyomegalic nuclei as well as double nuclei were detected in some of the hepatocytes (Micrograph 4). On the other hand, the liver section from rat treated with Pb-acetate plus spirulina extract

appeared diffuse kupffer cells proliferation in between the hepatocytes while the portal area showed fibrosis with inflammatory cells infiltration (Micrograph 5). The liver sections obtained from animals treated with Pb-acetate plus fermented camel milk showed few inflammatory cells infiltration in between the hepatocytes as well as in the portal area in association with diffuse kupffer cells proliferation in between the hepatocytes (Micrograph 6). While, the histopathological changes in the liver sections treated with Pb-acetate plus Fermented camel milk incorporated with dandelion extract revealed diffuse kupffer cells proliferation in between the hepatocytes (Micrograph 7). On the other hand, the liver sections treated group with Pb-acetate plus Fermented camel milk incorporated with spirulina extract and the combination group (Fermented camel milk plus dandelion extract plus spirulina extract) they revealed no histopathological alteration as recorded in (Micrograph 8).

- **Kidney**

The photomicrograph in (Figure 2) showed that the control group showed no histopathological alteration and the normal histological structure of the glomeruli and tubules at the cortex were recorded in (Micrograph 1). The Pb-acetate treated rat showed vacuolization in the lining endothelium of the glomerular tufts associated with coagulative necrosis in the tubular lining epithelium as well as focal inflammatory cells infiltration in the perivascular tissue surrounding the congested blood vessels at the cortex. The corticomedullary portion showed focal inflammatory cells infiltration in between the tubules (Micrograph 2). The kidney sections obtained from rat treated with Pb-acetate plus dandelion extract noticed Focal inflammatory cells infiltration was noticed in between the tubules at the corticomedullary portion (Micrograph 3). On the other hand, the kidney section from rat treated with Pb-acetate plus spirulina extract appeared focal fibrosis with inflammatory cells infiltration in between the tubules with congestion in the blood vessels at the cortex. The corticomedullary portion showed congestion in the blood vessels (Micrograph 4). The kidney sections obtained from animals treated with Pb-acetate plus fermented camel milk was detected focal fibrosis in between the cystic dilated tubules (Micrograph 5). While, the kidney sections treated with Pb-acetate plus Fermented camel milk incorporated with dandelion extract revealed focal few inflammatory cells infiltration in between the tubules at the cortex (Micrograph 6). On the other hand, the liver sections treated group with Pb-acetate plus Fermented camel milk incorporated with spirulina extract was noticed focal fibrosis with inflammatory cells infiltration in between the tubules (Micrograph 7)and the combination group (Fermented camel milk plus dandelion extract plus spirulina extract) revealed no histopathological alteration as recorded in (Micrograph 8).

Cadmium Chloride Treated Groups

- **Liver**

The photomicrograph in (Figure 3) showed that the control group showed no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma were recorded in (Micrograph 1). The portal area from Cd-chloride treated rat showed inflammatory cells infiltration with multiple numbers of newly formed bile ducts; in association with sever dilatation in the portal vein (Micrograph 9). The liver sections obtained from rat treated with Cd-chloride plus dandelion extract noticed fibrosis with inflammatory cells infiltration in the portal area (Micrograph 10). On the other hand, the liver section from rat treated with Cd-chloride plus other treatments group revealed no histopathological alteration as recorded in (Micrograph 11-15)

- **Kidney**

The photomicrograph in (Figure 4) showed that the control group showed no histopathological alteration and the normal histological structure of the glomeruli and tubules at the cortex were recorded in (Micrograph 1). The Cd-chloride treated rat showed congestion in the blood vessels and glomeruli (Micrograph 9). The kidney sections obtained from rat treated with Cd-chloride plus dandelion extract noticed that the glomeruli were congested associated with degeneration in the lining tubular epithelium (Micrograph 10). On the other hand, the kidney section from rat treated with Cd-chloride plus spirulina extract appeared degeneration in the lining epithelium (Micrograph 11). Boss the kidney sections obtained from animals treated with Cd-chloride plus fermented camel milk and Cd-chloride plus fermented camel milk with dandelion extract groups were noticed no histopathological alteration as recorded in (Micrograph 12-13). While, the kidney sections obtained from animals treated with Cd-chloride plus fermented camel milk with spirulina extract showed Congestion was observed in the cortical blood vessels (Micrograph 14) and finally the combination group (Cd-chloride plus Fermented camel milk with dandelion and spirulina extract) revealed Congestion was noticed in the glomerular tufts as recorded in (Micrograph 15).

Table 8: The Severity of the Reaction in Hepatic and Renal Tissue According to Histopathological Alterations of Different Experimental Groups

Toxicant	Organs	Groups No. Alterations	1	2	3	4	5	6	7	8
Pb-acetate	Liver	Subacute portal inflammatory reaction	-	+++	+	++	+	+	-	-
	Kidney	Focal subacute interstitial nephritis	-	+++	+++	+++	+	+	+	-
			1	9	10	11	12	13	14	15
Cd-chloride	Liver	Subacute portal inflammatory reaction	-	++	+	-	-	-	-	-
	Kidney	Focal subacute interstitial nephritis	-	+	-	-	-	-	-	-

: Nil; +: Mild; ++: Moderate; +++: Severe effect.

DISCUSSIONS

For various reasons in recent years the popularity of complementary medicine has increased. Plant therapies are widely practiced in many countries (WHO, 1999). It was reported that 34% of the American population used unconventional therapy including herbal medicine (Eisenberg *et al.*, 1993). In the present investigation potential protective effect of fermented camel milk containing probiotic, prebiotic against lead and cadmium intoxicated male rats was studied. Cd and Pb toxicity have been comprehensively studies and various cellular and molecular mechanisms have been suggested (Matović *et al.*, 2015; Wang *et al.*, 2014). One of these mechanisms for both Pb and Cd toxicity is through their binding to sulfhydryl (-SH) groups and by so doing affecting many enzymes and molecules. Various physiological and biochemical processes will be affected. The level of glutathione (GSH), a tripeptide and important non-enzyme antioxidant, will be reduced following lead and cadmium exposure.

In the present study liver and kidney functions were altered following exposure to these heavy metals. This can be authenticated by the increase in the activities of liver enzymes (ALP, AST, and ALT) and a decrease in the concentration total serum proteins. There were also increases in the concentrations of serum urea and creatinine and histopathological alterations in the liver and kidneys following exposure.

Many studies suggested that exposure to Pb or Cd can cause oxidative damage to various biological macromolecules and can cause disturbance in the oxidative status (Gurer and Ercal, 2000; Soltaninejad *et al.*, 2003; Olaleye *et al.*, 2007).Chronic exposure to cadmium (Cd) or Pb causes hepatotoxicity and nephrotoxicity (Webb and Cain, 1982; Friberg, 1984; Dudley *et al.*, 1985). Cd accumulates mainly in the liver and to a lesser extent in the kidney and other tissues.

In agreement with other authors (Halliwell and Gutteridge, 1989; McNaught& MacFie, 2001, Agrawal *et al.*, 2002) we found that most of these changes reported in the present study were due to lipid peroxidation and liberation of free radicals by lead and cadmium. However, most of these changes were improved to normal by the different treatments. Bioactive compounds in Pro, Pre and Synbiotic materials tested in this study indicated high level of total polyphenols in spirulina extracts, fermented camel milk with spirulina extract, Fermented camel milk with dandelion & spirulina (16.0; 15.52; 18.02 mg GAE/g respectively). Hepatoprotective effects of the aqueous extract from dandelion root against alcohol-induced oxidative stress was reported by Yanghee et *al.*, (2010).

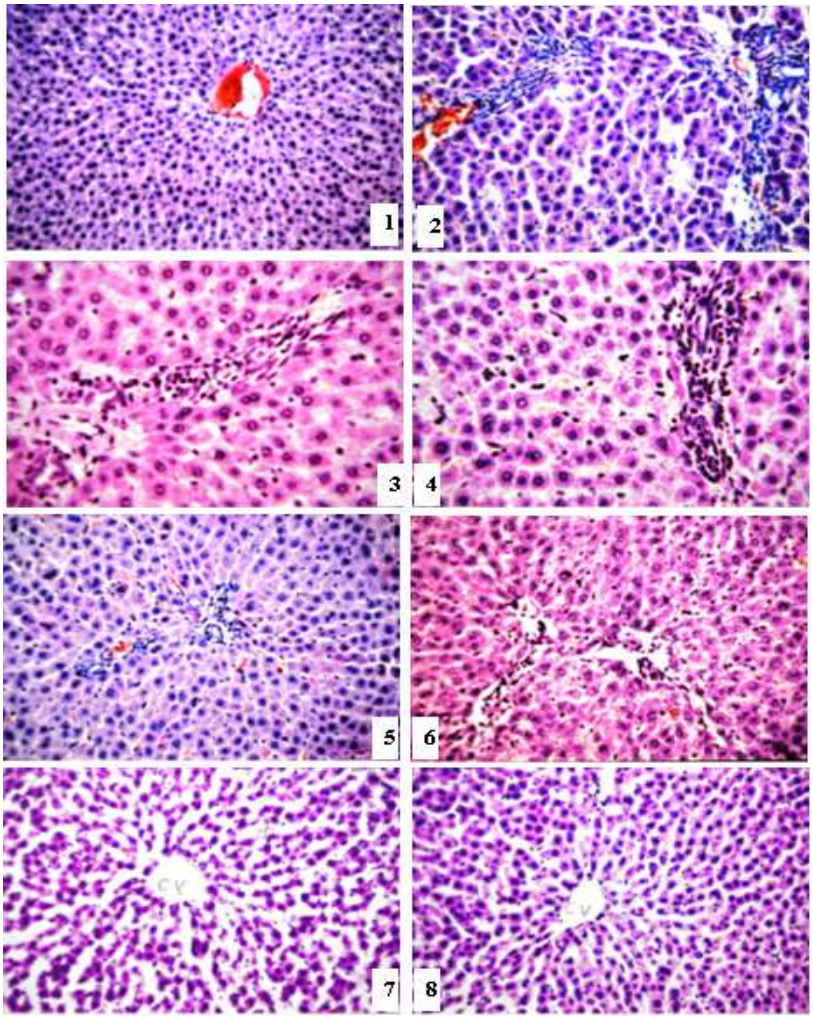

Figure 1: Photomicrography sections in the liver of rat exposed to Pb-acetate as shown in micrograph (1) control, (2) pb-acetate, (3) pb-acetate + dandelion extract, (4) pb-acetate + spirulina extract, (5) pb-acetate + fermented Camel Milk, (6) pb-acetate + fermented Camel Milk with Dandelion extract, (7) pb-acetate + fermented Camel Milk with Spirulina extract, (8) pb-acetate + fermented Camel Milk with Dandelion & Spirulina extract groups (H & E, 40X)

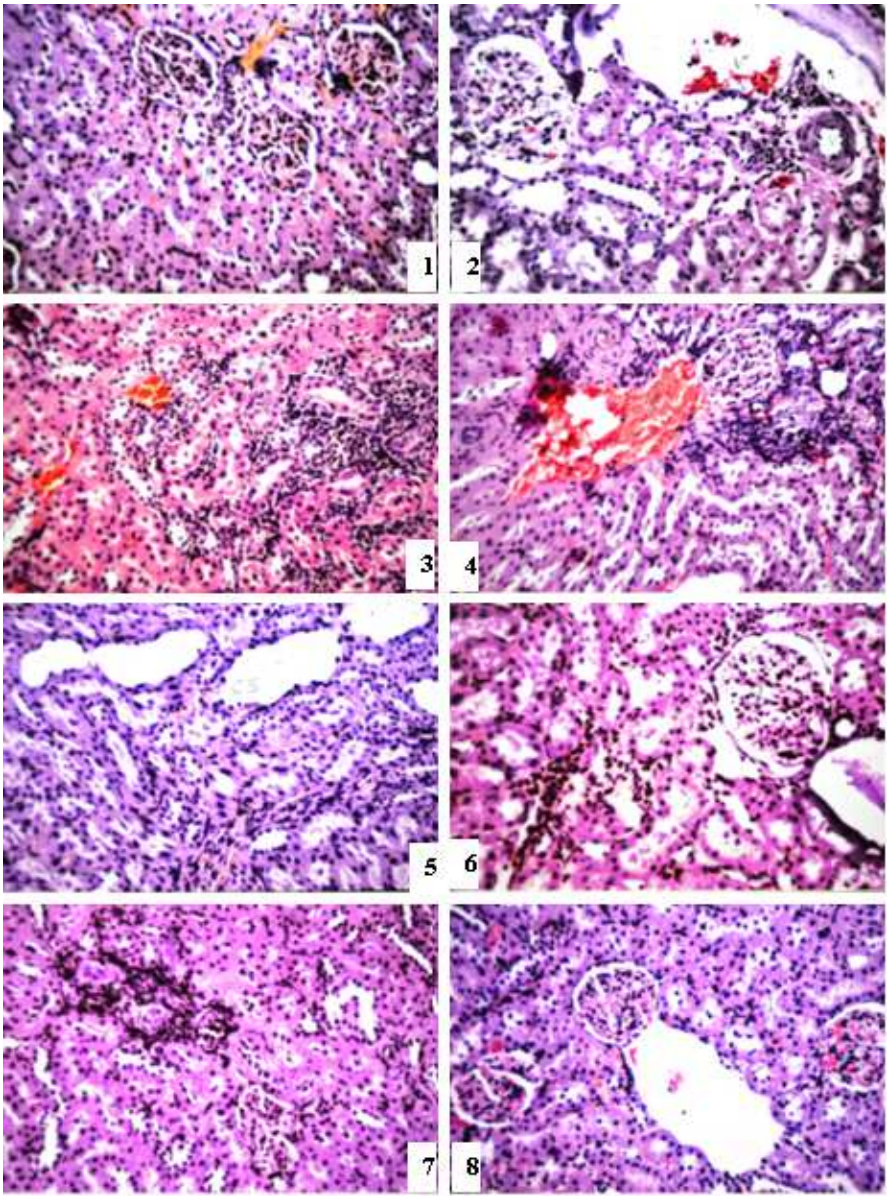

Figure 2: Photomicrography Sections in the Kidney of rat exposed to Pb-acetate as shown in micrograph (1) control, (2) pb-acetate, (3) pb-acetate + dandelion extract, (4) pb-acetate + spirulina Extract, (5) pb-acetate + fermented Camel Milk, (6) pb-acetate + fermented Camel Milk with dandelion extract, (7) pb-acetate + fermented Camel Milk with spirulina extract, (8) pb-acetate + fermented Camel Milk with Dandelion & Spirulina Extract Groups (H & E, 40X)

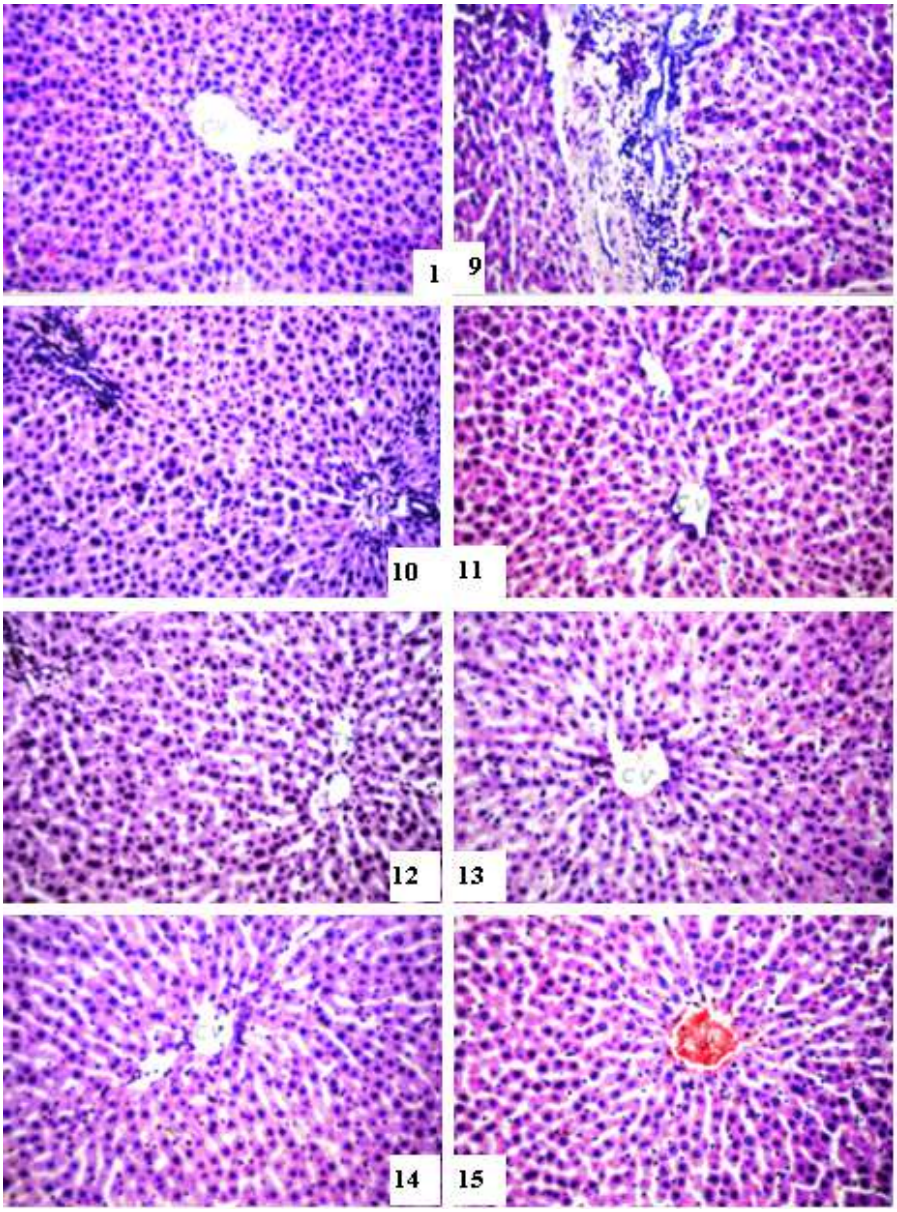

Figure 3: Photomicrography Sections in the Liver of Rat Exposed to Cd-chloride as shown in Micrograph (1) control, (9) Cd-chloride, (10) Cd-chloride + dandelion extract, (11) Cd-chloride + spirulina extract, (12) Cd-chloride + Fermented Camel Milk, (13) Cd-chloride + fermented Camel Milk with Dandelion Extract, (14) Cd-chloride + Fermented Camel Milk with spirulina extract, (15) Cd-chloride + fermented Camel Milk With Dandelion & Spirulina Extract Groups (H & E, 40X)

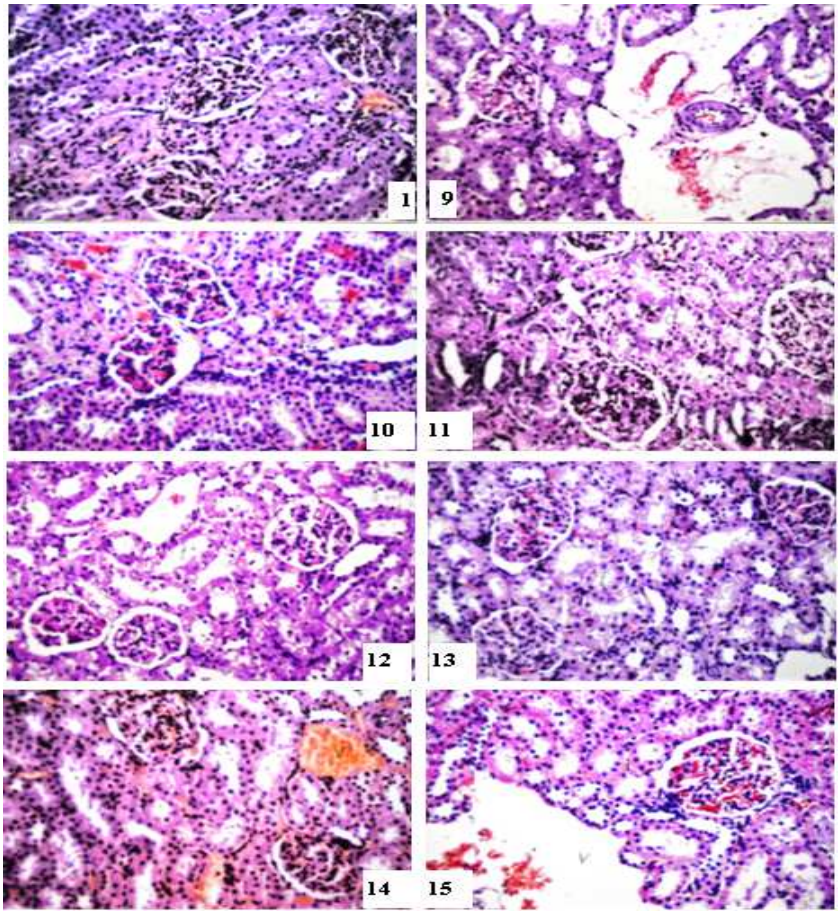

Figure 4: Photomicrography sections in the kidney of rat exposed to Cd-chloride as shown in micrograph (1) control, (9) Cd-chloride, (10) Cd-chloride + dandelion extract, (11) Cd-chloride + spirulina extract, (12) Cd-chloride + fermented camel milk, (13) Cd-chloride + fermented camel milk with dandelion extract, (14) Cd-chloride + fermented camel milk with spirulina extract, (15) Cd-chloride + fermented camel milk with dandelion & spirulina extract groups (H & E, 40X)

CONCLUSIONS

Based on the above results, it could be concluded that the Pro, Pre and Syn-biotic have the potential to protect from lead and cadmium toxicity, mitigates changes in liver and kidney function biochemical of pb-acetate and Cd-chloride exposure in drinking water, and especially fermented camel milk containing probiotic, prebiotic have a potent protective. This could be due to its natural antioxidant contents, which combines free radical scavenging with metal chelating properties. Therefore, Pro, Pre and Syn-biotic can be given as dietary supplements to human populations exposed to environmental toxicants and can provide protection against toxic effects.

ACKNOWLEDGEMENTS

This study was supported by Deanship of the Scientific Research, Qassim University. Project No. SR-S-13-14; Title: Potential protective effect of fermented camel milk containing probiotic, prebiotic against lead and cadmium -intoxicated male rats.

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