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CAMEL MILK EFFICIENCY IN PROTECTING RAT TESTES AGAINST LEAD ACETATE TOXICITY

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ABSTRACT

Two experiments were conduted to evaluate the role of camel milk in preventing the detrimental effect of lead on rat. In the first, 6 groups of adult male rats were administered daily for 60 days the following : group 1 saline; group 2 camel milk; group 3 cow milk, group 4 lead acetate; group 5 camel milk plus lead and group 6 cow milk plus lead. In the second, pregnant female rats were divided and treated following the first experiment. The female were allowed to deliver pups, the treatment continued until weaning of the pups then, the male pups were left without treatment until puberty. Lead caused significant reduction in the body and reproductive organ weights; plasma and testicular testosterone, testicular zinc; antioxidant enzymes, luteinising and follicle stimulating hormones and semen characteristics, while it caused significant increase in malondialdehyde; testicular cholesterol and testicular and plasma lead. Camel milk treatment improved the estimated parameters in adult male rat. However, it could not alleviate these parameters in male rats born for mothers exposed to lead during pregnancy and lactation periods. Camel milk treatment improved the evaluated parameters in adult male rats exposed to lead intoxication albeit not all were identical to the control levels, however, it could not improve these parameters in adult male rats born for mothers exposed to lead during pregnancy and lactation.

Key words: Camel milk, lead acetate, oxidative stress, rat, testes, toxicity

Many metal ions drastically affect the reproductive process in both male and female. Lead can cross blood-testis barrier, produces its toxic effect on the primary and the secondary reproductive organs (Elgawish and Abdelrazek, 2014). Lead passes through placenta and mammary gland of mice and produces physiological and pathological adverse effects in the testes of both neonate and adult males (Sharma and Garu, 2011). Lead induced its toxicity in most biological systems via oxidative stress (Flora *et al,* 2012). Many researchers attempted to use nutrients and medicinal plants with antioxidant activity to protect against this toxicity. In addition, some chelating agents and certain antioxidants such as vitamin C, E, methionine, N-acetylcysteine, homocysteine and α-lipoic acid were used to reduce lead toxicity (Jackie *et al,* 2011). However, most of these chelating agents elicit many side effects and are ineffective to reduce lead exposure (Flora and Pachauri, 2010).

Camel milk contains high minerals, vitamins and insulin contents but have low protein, cholesterol and sugar (Alavi *et al,* 2017). Thus imparts medicinal properties, which are exceedingly exploited for human health, as in many developing countries and ex-Soviet Union (Konuspsyeva *et al,* 2009). Many potential therapeutic advantages for camel milk were reported for few diseases e.g. diabetes and wound healing in diabetic patient (Badr *et al,* 2012), hepatitis C infection (El-Fakharany *et al,* 2017), autism (Adams, 2013) and hypertension (Ayyash *et al,* 2018). Camel milk diminished oxidative stress status and free radicals production in aluminum chloride (Al-Hashem, 2009) and cadmium (Al-Hashem *et al,* 2009) treated rats.

Effect of camel milk against lead-induced testicular toxicity are not traceable in available literature. Hence, present study was done to assess the conservative efficiency of camel milk in the induced lead toxicity in the testes of rats.

Materials and Methods

Camel and cow milk samples were collected daily early in the morning, by hand milking from a healthy 5 and 7 years old she camel and Holstein cow, respectively kept at Experimental Veterinary and

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Agriculture Station, King Faisal University, Al-Ahsa, Saudi Arabia. The milk was collected in sterile screw capped bottles and kept in cool boxes until transported to the laboratory. The unpasteurised camel milk (pH 6.3 and specific gravity 1.029) and cow milk (pH 6.5 and specific gravity 1.034) were intubated daily to rats at dose of 2ml/rat at a fixed time.

Experimental animals and protocol

Experiment 1

Thirty six 36 adult male Wister rats, weighed 215-240 g obtained from the Experimental Veterinary and Agriculture Station, King Faisal University, at Al-Ahsa, Saudi Arabia, were housed in plastic cages at Physiology Laboratory. The rats were acclimatised in controlled environment (20-22°C and 12hours light/12 hours dark schedule). The rats were fed with standard food pellets (15% crude protein, 3.8 crude fat, 6% crude fiber, 1.1% calcium, 0.8% phosphorus) and water *ad libitum*. The maintenance and handling of the animals was done according to King Faisal University guidance from the Ethical Committee for Research on Laboratory Animals (KFU-REC/2017-04-04). Following one-week acclimatisation, the animals were assigned into 6 groups with 6 rats each. Group 1 was intubated with 2ml saline (S), group 2 was intubated with 2ml camel milk (CM); (Al-Hashem, 2009) group 3 was intubated with 2ml cow milk (W), group 4 was intubated with saline containing 20 mg lead acetate (Pb) (Sigma Chemical Co. St Louis, MO, USA)/ kg body weight (Abdel Moneim, 2016). Group 5 was intubated with camel milk 2 hours before intubation of lead acetate (CM + Pb). Group 6 was intubated with cow milk 2 hours before intubation of lead acetate (W+Pb). The treatment was performed daily for 60 days which represent complete spermatogenic cycle. Rats were weighed by the end of treatment.

Experiment 2

Female and male Wister rats (4-5 months) in the ratio of 2:1 were kept in plastic cages and the day at which sperm was detected in the vaginal smear was designed as day 1 of pregnancy. Pregnant rats were allocated into 6 groups (6 rats each) and were treated as in the experiment 1. All the animals allowed deliver pups. The treatments further continued throughout the lactation period (up to 22 days). At the time of weaning, the mothers were isolated and the pups grew alone without treatment until sexual maturity (60 days) in this experiment. Six male rat pups from each group were randomly selected and their body weight were recorded.

Reproductive organ weights

Twenty four hours after the end of the experimental period, all rats in both experiments, were anaesthetised intramuscularly with (85 mg and 15 mg of ketamine and xylazine/kg body weight respectively). Individual blood samples were collected by heart puncture. Then, all male rats were sacrificed by decapitation at the end of the 2 experiments. Blood samples were collected into heparinised test tubes. The samples were divided into 2 sets, one for lead determination and the other set was used for testosterone, Luteinising hormone (LH) and Follicle stimulating hormone (FSH) assay. Immediately after blood collection, the testes, prostate, seminal vesicles and epididymis were removed, blot dry; grossly examined and weighed. One testis was used for determination of antioxidant and oxidative stress biomarkers; the other one cut into 2 halves, one-half for lead determination and the other half for histopathological examination.

Semen analysis

Epididymal spermatozoa were counted by a modified method of Yokoi *et al* (2003). The epididymis was cut into head; body and tail, then they minced in 5 ml phosphate buffer (pH 7.4), after that it was shaken vigorously for homogeneity and dispersal of sperm cells. An aliquot (10µl) of epididymal sperm suspension was placed in the counting chamber of the haemocytometer and allowed to stand for 5 min for count under a microscope (×200). The heads of sperm were counted and expressed as million/ml. A drop of epididymal content of each rat was mixed with an equal drop of eosin–nigrosine stain and a thin film was made on a clean slide. The average viability % was determined from 200 sperms examined per slide. The progressive motility was evaluated. The content of cauda epididymis was obtained with a pipette and diluted to 2ml with tris buffer solution. The motility was evaluated at × 400 magnification and the average final motility score was estimated from the 3 different fields in each sample. The morphologically abnormal spermatozoa percentages were recorded, i.e. 40 µl of sperm suspension mixed with 10 µl of 1% eosin and nigrosine, 200 sperm examined on each slide using a microscope (×400) and the average taken.

Hormonal assay

Testicular and plasma testosterone levels were estimated using radioimmunoassay (RIA) kits from Diagnostic Products Cooperation (Los Angeles, California). The sensitivity was 0.2 ng/ml and intra-assay coefficient of variation was 12.8%.

LH and FSH levels were determined by generated electrochemiluminscence using kits supplied by Roche Diagnostics and using automat (Elecsys 2010, Roche Diagnostics, Mannheim). The hormones were assayed according to manufacture guide of the kits.

Antioxidant enzyme activities and oxidative stress assays

One of the 2 testes was kept at −70°C, then in cold potassium phosphate buffer (pH 7.4). This testis was homogenised, then centrifuged at 4°C for 10 minutes at 5000 rpm. The supernatant used for determination of glutathione (GSH), (SOD) superoxide dismutase, Catalase (CAT), malondialdehyde (MDA) using commercial available colorimetric assay kits (Cayman Chemical, Ann Arbor, Michigan, USA) according to the manufacturer guides.

Blood and testicular lead determination

In polystyrene test tube 100 µl, blood was placed then 150 µl deionised water and 150 µl of 2 M nitric acid was added, vigorously mixed for 30 seconds then centrifuged at 4000x g for 15 minutes. Ten µl of the supernatant was combusted in graphite furnace at 500°C for 24 hrs. The obtained ash was diluted with 5ml of 0.1 M nitric acid (Parsons and Slavin, 1993). The testicular tissue was thoroughly washed in distilled water and then dried for 48 hours at 60°C and finally combusted. The combusted tissue was digested with 10 ml nitric acid, after that few drops of perchloric acid were added then distilled water added to be 50ml (US EPA, 1986). Lead concentration was determined by AAS (Perkin Elmer Analyst, model 2180).

Histopathological examination

After the fixation of half of the testicle in 10% formalin solution, it was processed and sections were stained with hematoxylin and eosin (H & E) and examined under light microscopy (Lillie, 1965).

Statistical analysis

The obtained data expressed as means ± standard errors. The significance of differences calculated by one-way analysis of variance (SAS, 2001) followed by Duncan's multiple range test (Duncan, 1955). The difference between means was considered significant when $P<0.05$.

Results and Discussion

The final body weight and the index weight of the testis, accessory sex glands and the epididymis decreased significantly ($P<0.05$) in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control group. However, this reduction was less detected in the group treated with lead acetate plus camel milk (Table 1).

Epididymal sperm count, alive sperm and motility per cent decreased significantly ($p<0.05$) in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control group, this reduction was less conspicuous in the group treated with lead acetate plus camel milk (Table 2). Furthermore, there was a significant increase ($p<0.05$) in sperm abnormalities percentage in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control one. This increase was less marked in lead acetate plus camel milk treated group (Table 2).

Table 3 showed that MDA increased significantly ($p<0.05$) in lead acetate and lead acetate plus camel or cow milk treated groups compared to control one. The greatest increase was in lead acetate and lead acetate plus cow milk treated groups. Moreover, there was a significant decrease in GSH, SOD and CAT in lead acetate and lead acetate plus

Table 1. Effect of lead acetate, camel milk and cow milk on the body and reproductive organs index weight of adult male rats.

Groups	Parameters*					
	Initial body weight (gm)	Final bod weight (gm)	Feed intake	Testes (I.W)	Epididymis (I.W)	Accessory glands (I.W)
Group 1 (S)	226.17±3.22^{a}	285.33±3.70^{a}	20.92±0.48^{a}	1.64±0.02^{a}	0.70±0.02^{a}	0.82±0.02^{a}
Group 2 (CM)	226.67±3.11^{a}	285.83±5.07^{a}	15.87±0.26^{b}	1.69±0.03^{a}	0.73±0.02^{a}	0.83±0.02^{a}
Group 3 (W)	228.00±3.24^{a}	287.17±2.09^{a}	15.55±0.54^{b}	1.64±0.02^{a}	0.70±0.02^{a}	0.82±0.02^{a}
Group 4 (Pb)	225.83±3.75^{a}	235.00±3.42^{b}	12.87±0.21^{c}	1.04±0.02^{b}	0.53±0.01^{b}	0.63±0.01^{b}
Group 5 (CM+Pb)	226.50±0.43^{a}	251.67±4.01^{c}	13.22±0.15^{c}	1.37±0.03^{c}	0.62±0.01^{c}	0.72±0.01^{c}
Group 6 (W+Pb)	227.00±1.00^{a}	238.17±1.56^{b}	12.86±0.32^{c}	1.05±0.02^{b}	0.54±0.01^{b}	0.64±0.01^{b}

N= 6 * Means ± SE I.W = index weight
Means having different superscript letters in the same column are significantly different ($P<0.05$).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

camel or cow milk treated groups compared to control one. The greatest reduction was in lead acetate and lead plus cow milk treated groups.

Plasma testosterone, FSH and LH levels decreased significantly ($p<0.05$) in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control group, this reduction was less conspicuous in the groups treated with lead acetate plus Camel's milk (table 4). Furthermore, there was a significant increase ($p<0.05$) in blood lead concentration in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control one. This increase was less marked in lead acetate plus camel milk treated group (Table 4).

Table (5) showed that both testicular lead and cholesterol content increased significantly ($p<0.05$) in lead acetate and lead acetate plus camel or cow milk treated groups compared to control one. The greatest

Table 2. Effect of lead acetate, camel milk and cow milk on the epididymal sperm characteristics in adult male rats.

Groups	Parameters*			
	Sperm count ($x10^6$)	Sperm motility (%)	Alive sperm (%)	Sperm abnormalities (%)
Group 1 (S)	315.50 ± 3.27^a	91.00 ± 0.58^a	89.50 ± 0.76^a	7.33 ± 0.71^a
Group 2 (CM)	317.67 ± 0.49^a	91.67 ± 0.56^a	90.67 ± 0.33^a	6.83 ± 0.48^a
Group 3 (W)	318.00 ± 1.39^a	91.00 ± 1.26^a	90.50 ± 1.43^a	7.50 ± 0.76^a
Group 4 (Pb)	218.17 ± 3.24^b	69.00 ± 0.97^b	68.17 ± 1.72^b	17.33 ± 0.67^b
Group 5 (CM+Pb)	282.50 ± 4.01^c	80.00 ± 1.71^c	79.50 ± 1.23^c	12.00 ± 0.73^c
Group 6 (W+Pb)	221.00 ± 3.51^b	67.83 ± 1.17^b	69.17 ± 1.17^b	17.67 ± 0.76^b

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different ($P<0.05$).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 3. Effect of lead acetate, camel milk and cow milk on the oxidative stress markers and antioxidant in adult male rats.

Group	Parameters*			
	MDA (μmol/g)	GSH (mg/g)	SOD (μ/g)	CAT (μ/g)
Group 1 (S)	6.19 ± 0.25^a	16.47 ± 0.45^a	1.58 ± 0.13^a	18.36 ± 0.35^a
Group 2 (CM)	6.14 ± 0.25^a	17.33 ± 0.72^a	1.64 ± 0.05^a	16.76 ± 1.61^a
Group 3 (W)	6.22 ± 0.30^a	$16..61 \pm 0.23^a$	1.67 ± 0.03^a	17.97 ± 0.25^a
Group 4 (Pb)	22.01 ± 0.69^b	6.87 ± 0.37^b	0.70 ± 0.03^b	8.61 ± 0.24^b
Group 5 (CM+Pb)	10.63 ± 0.57^c	10.96 ± 0.48^c	0.98 ± 0.02^c	11.16 ± 0.20^c
Group 6 (W+Pb)	22.52 ± 0.72^b	7.01 ± 0.23^b	0.71 ± 0.04^b	8.55 ± 0.17^b

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different ($P<0.05$).
MDA= malondialdehyde GSH= glutathione SOD= superoxidedimutase CAT= Catalase.
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 4. Effect of lead acetate, camel milk and cow milk on plasma testosterone; FSH & LH and blood lead content in adult male rats.

Groups	Parameters*			
	Plasma testosterone (ng/ml)	Plasma FSH (ng/ml)	Plasma LH (ng/ml)	Blood lead (µg/ml)
Group 1 (S)	5.53 ± 0.22^a	2.83 ± 0.02^a	6.33 ± 0.35^a	1.90 ± 0.10^a
Group 2 (CM)	5.57 ± 0.37^a	2.87 ± 0.03^a	6.45 ± 0.39^a	1.83 ± 0.19^a
Group 3 (W)	$5 .55 \pm 0.11^a$	2.84 ± 0.02^a	6.60 ± 0.08^a	1.89 ± 0.03^a
Group 4 (Pb)	3.46 ± 0.10^b	1.52 ± 0.10^b	3.31 ± 0.06^b	17.34 ± 1.69^b
Group 5 (CM+Pb)	3.74 ± 0.60^b	1.63 ± 0.08^b	4.51 ± 0.11^c	14.72 ± 0.48^c
Group 6 (W+Pb)	3.56 ± 0.71^b	1.59 ± 0.02^b	3.23 ± 0.19^b	17.95 ± 0.60^b

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different ($P<0.05$).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

increase was in lead acetate and lead acetate plus cow milk treated groups. Moreover, there was a significant ($p<0.05$) decrease in both of testicular testosterone and zinc content in lead acetate and lead acetate plus camel or cow milk treated groups compared to control one. The greatest reduction was in lead acetate and lead plus cow milk treated group.

The body weight and the index weight of the testes, accessory sex glands and the epididymis decreased significantly ($P<0.05$) in male rats born for mothers treated with lead acetate and lead acetate plus camel or cow milk during pregnancy and lactation periods compared to control group. There was significant ($p<0.05$) increase in body and index weight in the epididymis of lead acetate plus camel milk group compared to lead acetate and lead plus cow milk groups (Table 6).

Epididymal sperm count, alive sperm and motility percentage decreased significantly ($p<0.05$) in male rats born for mothers treated with lead acetate and lead acetate plus camel or cow milk during pregnancy and lactation periods compared to control group (Table 7). Furthermore, there was a significant increase ($p<0.05$) in sperm abnormalities percentage in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control one. There was no significant ($p<0.05$) difference between lead acetate and acetate plus camel or cow milk treated groups in all parameters estimated (Table 7).

Plasma testosterone, FSH and LH levels decreased significantly ($p<0.05$) in male rat born for mothers treated with lead acetate and lead acetate plus camel or cow milk during pregnancy and lactation periods compared to control. Furthermore, there was a significant increase ($p<0.05$) in blood lead concentration in male rat born for mothers treated with lead acetate and lead acetate plus camel or cow milk during the pregnancy and lactation period compared to control one (Table 8). There was no significant ($p<0.05$) difference between lead acetate and lead plus camel or cow milk treated groups in plasma testosterone, FSH and LH levels and blood lead concentration (Table 8).

Table 5. Effect of lead acetate, camel milk and cow milk on testicular lead; testosterone cholesterol and zinc content in adult male rats.

Groups	Parameters*			
	Testicular lead (ng/g)	Testicular testosterone (ng/g)	Testicular cholesterol (mg/g)	Testicular zinc (µg/g)
Group 1 (S)	63.67 ± 1.45^a	8985.08±85.64^a	15.98±0.24^a	17.82±0.28^a
Group 2 (CM)	63.50 ± 0.50^a	8962.85±100.43^a	15.65±0.20^a	17.98±0.25^a
Group 3 (W)	63.44±0.35^a	8924.38±23.25^a	15.54±0.60^a	18.11±0.14^a
Group 4 (Pb)	128.91±0.51^b	5406.98±113.73^b	35.69±0.34^b	8.06±0.18^b
Group 5 (CM+Pb)	102.17 ±3.61^c	6400.53±79.25^c	20.95±0.28^c	12.47±0.17^c
Group 6 (W+Pb)	127.50±1.34^b	5391.96±41.61^b	35.49±0.0.53^b	8.16±0.22^b

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different ($P<0.05$).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 6. Effect of lead acetate, camel milk and cow milk on the body and reproductive organs weight of male rats, which their mother exposed to lead acetate at pregnancy and lactation periods.

Groups	Parameters*				
	Body weight (g)	Testes (I.W)	Epididymis (I.W)	Prostate (I.W)	Seminal vesical (I.W)
Group 1 (S)	193.00±2.08^a	1.17±0.03^a	1.85±0.02^a	0.13±0.01^a	0.47±0.01^a
Group 2 (CM)	208.00±2.08^b	1.25±0.02^b	1.92±0.02^b	0.14±0.01^a	0.52±0.02^a
Group 3 (W)	208.17±0.79^b	1.14±0.03^a	1.83±0.03^a	0.15±0.01^a	0.54±0.02
Group 4 (Pb)	149.00±1.53^c	0.88±0.03^c	1.24±0.04^c	0.10±0.00^b	0.28±0.02^b
Group 5 (CM+Pb)	165.00±2.89^d	0.90±0.03^c	1.47±0.03^d	0.11±0.00^b	0.32±0.02^b
Group 6 (W+Pb)	157.17±2.76^e	0.88±0.01^c	1.25±0.0.1^c	0.11±0.01^b	0.28±0.1^b

N= 6 * Means ± SE I.W = index weight
Means having different superscript letters in the same column are significantly different ($P <0.05$).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

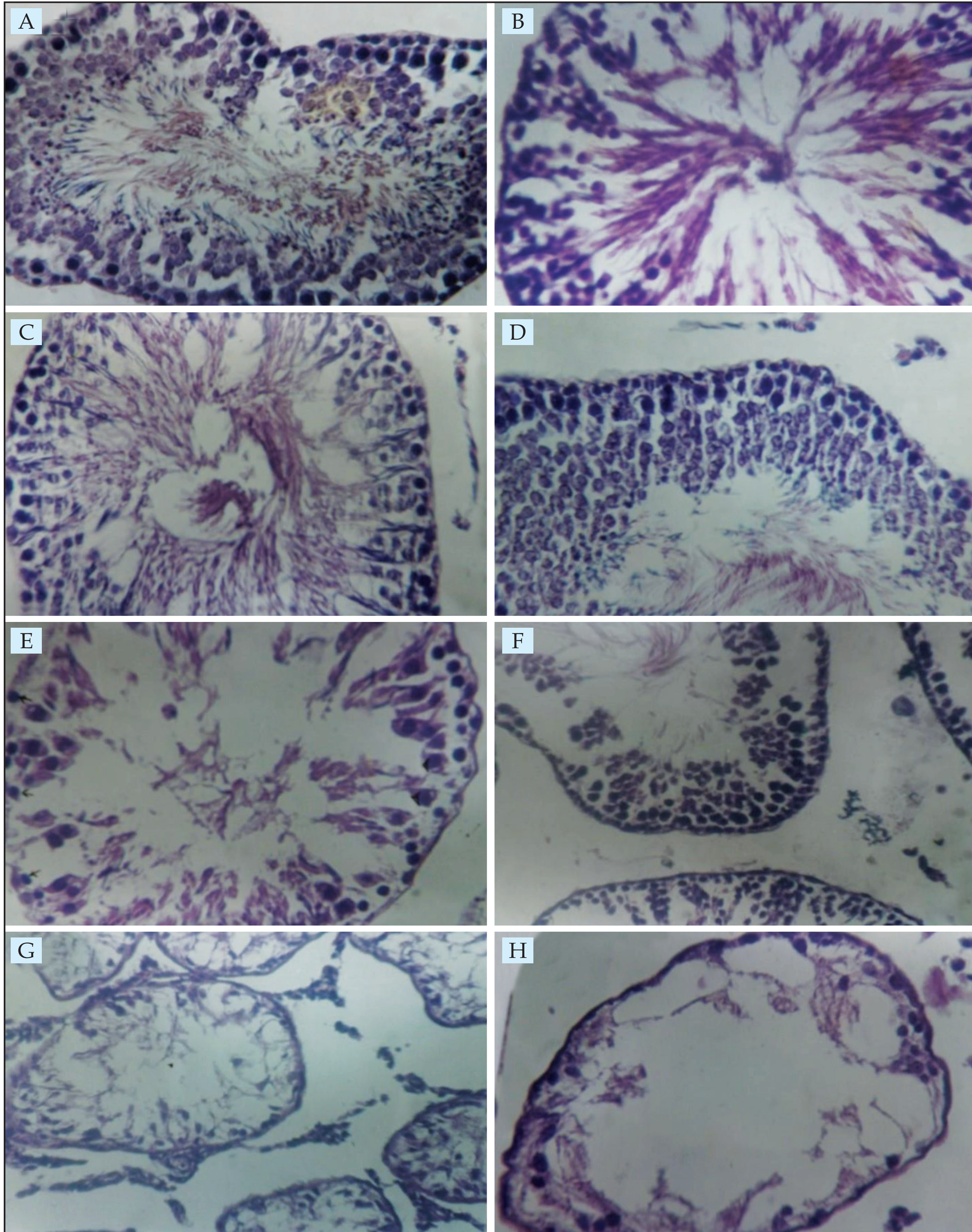

Fig 1. (A-H): Photomicrographs of rats testes (H&E): **A.** mature male (control) showing active seminiferous tubules containing all layers of spermatic cells (X100). **B.** mature rats treated with camel milk showing active seminiferous tubules containing all layers of spermatogenic cells (X400). **C.** male (control) born to mothers treated with saline during pregnancy and lactation periods (X400). **D.** male born to mothers treated with camel's milk during pregnancy and lactation period showing active spermatogenesis (X400). **E.** adult male treated with lead acetate showing degenerated spermatogonia cells and spermatocytes (X400). **F.** adult male treated with lead acetate plus camel milk showing poor spermatogenesis and few spermatozoa inside seminiferous tubules (X400). **G.** adult male born for mother exposed to lead acetate during pregnancy and lactation periods showing absence of spermatids and spermatocytes (X400). **H.** male treated with lead acetate plus cow milk showing degenerated seminiferous tubules and absence of 1ry & 2ndry spermatocytes and spermatids(X400).

Table 9 showed that both testicular lead and cholesterol content increased significantly ($p<0.05$) in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one and there were significant ($p<0.05$) decrease in testicular testosterone and zinc level in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one. There was no significant ($p<0.05$) difference between lead acetate and lead acetate plus camel or cow milk treated groups in all parameters estimated (Table 9).

Table 10 showed that MDA increased significantly ($p<0.05$) in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one. Moreover, there was a significant decrease in GSH, SOD and CAT in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one. There was no significant ($p<0.05$) difference between lead acetate and camel or milk plus lead acetate treated groups in all parameters estimated (Table 10).

Histopathological examination

Testes of mature rats control (treated saline); treated with camel milk, male born to mother treated with saline during pregnancy and lactation and male born to mother treated with camel milk during pregnancy and lactation) showed normal structure of seminiferous tubules containing all layers of spermatogenic cells with huge amount of sperms (Figs 1A, B, C and D). Photomicrograph of the testes of male rats treated with lead acetate showed degenerative spermatogenic cells without sperm inside the seminiferous tubules (Fig 1E). Testes of mature rats treated with camel milk plus lead acetate show some activity of seminiferous tubules with few sperm inside seminiferous tubules (Fig 1F). Testes of mature rats born for mothers treated during pregnancy and lactation periods with camel or cow milk plus lead acetate showed degeneration of seminiferous tubules characterised by degeneration and necrosis of germ cells with the reduction of sperm in center of the seminiferous tubules with reduction of the interstitial cells (Figs 1 G and H).

The present study indicated that exposure to lead produced decrease in the final body weight. Aprioku and Siminialayi (2013) reported that the growth rate decreased in rat intoxicated with lead. The decrease in the body weight might be due to imbalance metabolism as result of disturbing zinc status in zinc dependent enzymes, which are essential for several metabolic processes. Testes, seminal vesicles, epididymis and prostate weights decreased in adult rats administered lead acetate for 60 days or in adult rat born for dam exposed to lead during pregnancy and lactation periods. Similar results were obtained in lead acetate exposed male rats (El Sayed and El-Neweshy, 2010). The decrease in the weights of the reproductive organs referred to the decrease in testosterone level or to the loss of the body weight. The decrease in the testosterone production and accessory sex gland weights may be a result of the decrease in the body weight (Rehm *et al,* 2008). Lead treated group (adult or born for mother exposed to lead at pregnancy or lactation period) in our study showed significant decrease in serum testosterone levels. El Sayed and El-Neweshy (2010) found that serum testosterone level fall in animals treated with lead. The decrease in plasma testosterone level might be due to reduction of testosterone production by Leydig cells which confirmed by its reduction in testicular tissues and increase in cholesterol testicular content. The reduction in testosterone level in the lead acetate treated rats may be due to reduction of utilisation of cholesterol by the Leydig cells. The stimulated Leydig cells function are impaired by the high cholesterol level (Tong *et al,* 2004). In the present study, lead acetate reduced LH and FSH levels in the lead treated group. Similar results were obtained by El Sayed and El-Neweshy (2010). FSH stimulates synthesis of LH receptors in Leydig cells the latter produce testosterone in response to LH stimulation (Ramaswamy and Weinbauer, 2014). Therefore, the reduction of testosterone production attributed to alteration in testicular steroidogenic enzyme activities because of decrease of FSH and LH secretion from pituitary. In the present study, sperm concentration, motility and viability reduced in lead acetate exposed rats (adult or born for mothers exposed to lead during pregnancy and lactation). These results are in accordance with the results of El-Sayed and El-Neweshy (2010). The decrease in the semen characteristics may be due to decrease of testosterone, FSH and LH or might be due to oxidative stress. The suppression of sperm motility is due to alteration of antioxidant system of spermatozoa (Ramah *et al,* 2015). In the present study, exposure to lead acetate for 60 days or at pregnancy and lactation periods resulted in significant decrease in SOD, GSH and CAT and increase in MDA. Exposure to lead in the present study produced testicular damage, which led to spermatogenic arrest. Similar results were noted by El-Sayed and El-Neweshy (2010). The

Table 7. Effect of lead acetate, camel's milk on the epididymal sperm characteristics in male rats, which their mother exposed to lead acetate at pregnancy and lactation periods.

Groups	Parameters*			
	Sperm count ($x10^6$)	Motility %	Live sperm %	Abnormal sperm %
Group 1 (S)	$62.14±0.53^a$	$88.50±0.43^a$	$89.67±0.61^a$	$12.00±0.97^a$
Group 2 (CM)	$64.25±0.54^b$	$90.00±0.58^a$	$91.83±0.48^a$	$12.83±0.83^a$
Group 3 (W)	$62.39±0.49^a$	$89.16±0.23^a$	$90.33±1.09^a$	$12.50±0.76^a$
Group 4 (Pb)	$0.57±0.17^c$	$20.14±1.03^c$	$49.5±1.82^c$	$29.50±0.99^b$
Group 5 (CM+Pb)	$0.60±0.13^c$	$20.20±2.01^c$	$51.17±1.66^c$	$28..17±2.15^b$
Group 6 (W+Pb)	$0.58±0.01^c$	$20.13±1.14^c$	$50.50±1.61^c$	$28.83±0.79^b$

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different (P<0.05).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 8. Effect of lead acetate, camel milk and cow milk on plasma testosterone; FSH & LH and blood lead content in adult male rats, which their mother exposed to lead acetate at pregnancy and lactation periods.

Groups	Parameters*			
	Plasma testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)	Lead (µg/dl)
Group 1 (S)	$2.03±0.05^a$	$6.05±0.09^a$	$2.71±0.04^a$	$0.93±0.04^a$
Group 2 (CM)	$2.04±0.09^a$	$6.34±0.18^a$	$2.75±0.04^a$	$0.97±0.09^a$
Group 3 (W)	$2.07±0.05^a$	$6.13±0.09^a$	$2.72±0.03^a$	$0.95±0.03^a$
Group 4 (Pb)	$0.86±0.01^c$	$3.41±0.09^b$	$1.53±0.02^b$	$7.62±0.40^b$
Group 5 (CM+Pb)	$0.87±0.01^c$	$3.50±0.08^b$	$1.58±0.01^b$	$7.36±0.25^b$
Group 6 (W+Pb)	$0.89±0.03^c$	$3.45±011^b$	$1.55±0.02^b$	$7.37±0.24^b$

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different (P<0.05).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk
LH =luteinizing hormone FSH =Follicle stimulating hormone

Table 9. Effect of lead acetate, camel milk and cow milk on testicular lead; testosterone cholesterol and zinc and testosterone content in adult male rats, which their mother exposed to lead acetate at pregnancy at lactation period.

Groups	Parameters*			
	Lead (ng/g)	Testosterone (ng/gm)	Cholesterol (mg/g)	Zinc (µg/g)
Group 1 (S)	$36.78±1.01^a$	$7105.24±176.84^a$	$14.67±0.44^a$	$15.24±0.61^a$
Group 2 (CM)	$36.08±1.08^a$	$7209.01±258.86^a$	$14.71±0.35^a$	$15.49±0.36^a$
Group 3 (W)	$36.53±0.40^a$	$7185.55±80.13^a$	$14.80±0.23^a$	$15.42±0.29^a$
Group 4 (Pb)	$100.80±4.59^b$	$4973.08±150.35^b$	$36.78±0.035^b$	$5.71±0.57^b$
Group 5 (CM+Pb)	$98.86±2.31^b$	$4990.92±133.92^b$	$36.78±0.47^b$	$5.64±0.45^b$
Group 6 (W+Pb)	$100.01±3.49^b$	$4979.14±3..26^b$	$36.64±0.18^b$	$5.53±0.32^b$

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different (P <0.05).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

potential toxicity of lead caused decrease in sperm count, motility and viability as well as biochemical (decrease both SOD, GSH, CAT and zinc, while increase in MDA, lead and cholesterol) and hormones diruptions (FSH; LH and testosterone). The damage to the testicular cells induced by lead attributed to oxidative stress because it contains high quantity of unsaturated fatty acid. Lead exposure increased the level of ROS in male rat reproductive organs (Ramah *et al,* 2015). The lipid peroxidation oxidises the cell constituents resulting in their inactivation and finally damage of its membrane impartiality (Ramah *et al,* 2015). From our results, it is clear that administration of camel but not cow milk before exposure to lead significantly amended the estimated parameters although, not all were similar to control

Table 10. Effect of lead acetate, camel milk and cow milk on plasma oxidative stress marker and antioxidant content in adult male rats, which their mother exposed to lead acetate at pregnancy and lactation period.

Groups	Parameters*			
	MDA (µmol/g)	GSH (mg/g)	SOD (µ/g)	CAT (µ/g)
Group 1 (S)	5.69±0.21[a]	14.66±0.54[a]	1.51±0.07[a]	15.98±0.36[a]
Group 2 (CM)	5.61±0.32[a]	14.72±0.60[a]	1.52±0.95[a]	16.03±0.39[a]
Group 3 (W)	5.72±0.11[a]	14.42±0.37[a]	1.49±0.08[a]	16.21±0.31[a]
Group 4 (Pb)	20.47±0.36[b]	5.87±0.13[b]	0.62±0.04[b]	7.24±0.24[b]
Group 5 (CM+Pb)	20.17±0.62[b]	5.83±0.09[b]	0.61±0.04[b]	7.32±0.30[b]
Group 6 (W+Pb)	20.29±0.45[b]	5.79±0.19[b]	0.61±0.10[b]	7.33±0.11[b]

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different (P <0.05).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk
MDA= malondialdehyde GSH= glutathione SOD= superoxidedimutase CAT= Catalase

levels. Vitamin C content in camel milk is higher than cow milk (EL-Hatmi *et al,* 2015). Moreover, the whey protein fraction and zinc in camel milk are higher than those of cow. The protective effect of camel milk against oxidative stress might be due to its high content of Ca++ and casein (Al-Ayadhi and Elamin, 2013), zinc and high content of vitamin C which act as antioxidants (Alavi *et al,* 2017). Interactions between lead and zinc inspected at absorptive and enzymatic sites, zinc occupy the same binding sites of lead on gastrointestinal transporting protein, metallothionin hence; it reduces the availability and absorption of lead and hence reducing its toxicity. Al-Hashem (2009) reported that camel milk could attenuate changes in oxidative stress parameters in testes intoxicated with aluminum chloride. Ebaid *et al* (2015) suggested that camel whey protein modulates oxidative stress and antioxidant defense system. El Bahr (2014) reported that camel milk improves liver damage induced by CCl_4 *via* activation of genes expression; upregulation of antioxidant enzyme gene expression and increasing GSH availability. Moreover, Ebaid *et al* (2015) found that camel milk peptides, digested by trypsin decreases nitric oxide, ROS and MDA. There were non-significant changes in parameters of the rat exposed to lead acetate during intrauterine life and lactation period when compared to the rats treated with lead acetate and camel's milk. It suggested that the amount of antioxidant present in the camel milk may pass through the placenta or mammary gland with low concentration that could not able to alleviate the deleterious effect of lead on these rats during pregnancy and lactation periods.

In conclusions, camel milk can alleviate the effect of lead intoxication in adult rats through its antioxidant properties, however, it could not able to do the same impact in rats born to mothers exposed to lead during pregnancy and lactation periods.

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Conflict of interests

The authors declare that there is no conflict of interest

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