Original article

Lead acetate versus cadmium sulfate in the modulation of main physiological pathways controlling detrusor muscle contractility in rat

Safaa S. Taha [a,*], Tahia T. Daabees [a], Rania G. Aly [b], Amira M. Senbel [a,c]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Alexandria University, Egypt
[b] Department of Pathology, Faculty of Medicine, Alexandria University, Egypt
[c] Biological and Clinical Sciences Division, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT), Egypt

ARTICLE INFO

ABSTRACT

Heavy metals have a deleterious effect on lower urinary tract functions. Scant data has been reported about metals' effect on altering detrusor muscle contractility. Rats were given lead acetate (3, 30 mg/kg), cadmium sulfate (0.1, 1 mg/kg) or ferrous sulfate-iron overload-(3, 30 mg/kg), in a subacute toxicity study (21 days, ip). *In-vitro* tension experiments were conducted using isolated rat detrusor muscle. Measurement of heavy metal concentrations in blood and tissue homogenates was performed, as well as histopathological examinations. Subacute toxicity induced by treatment with lead and cadmium was manifested as a decrease in EFS, ACh, and ATP-mediated contraction of isolated detrusor muscle. Iron overload only decreased E_{MAX} of EFS and ACh-mediated contraction. Lead (30 mg/kg) caused an upward shift in the dose response curve of isoprenaline-induced relaxation, with a significant decrease in E_{MAX}. Lead (30 mg/kg) or cadmium (1 mg/kg) inhibited adenosine (10^{-5} M)-induced relaxation. Comparisons to control tissues showed a selective accumulation of metals in the detrusor muscle. Histopathological examinations revealed edema and inflammation in the urinary bladder. Directly added lead (10 mM) inhibited detrusor muscle contraction *in-vitro*, and its effect was decreased in presence of atropine, and potentiated in presence of TEA, L-NAME, or MB. Cadmium's (0.1 mM) inhibitory effect was reduced in presence of nifedipine or trifluoperazine. In conclusion, lead, cadmium, or iron induce detrusor hypoactivity: The inhibitory effect of lead may be mediated by modulating muscarinic receptors but not the K^+/NO/cGMP pathway, whereas cadmium inhibitory effect may be mediated by inhibiting the Ca^{2+}/calmodulin pathway.

1. Introduction

Heavy metal toxicity is one of the most dangerous threats to human health in both occupational and environmental settings (Atieh et al., 2017). Heavy metals have been extensively used in a variety of daily activities, which results in excessive human exposure. They have been shown to be deposited in the aquatic ecosystem, soil, and plants, and have subsequently become a major source of human toxicity (Hamid et al., 2016; Sulaiman and Hamzah, 2018).

Several studies have reported the negative effect of lead on the viability of human lung epithelial cells (Ahamed et al., 2019; Ahamed et al., 2020b). Moreover, other studies have documented cadmium's cytotoxicity and apoptotic effect on different human cells (Ahamed et al., 2020a; Ahamed et al., 2020c). Additionally, lead and cadmium toxicity play a role in the alteration of the lower urogenital tract functions; lead and cadmium have been shown to accumulate in corpus cavernosum tissues and to have anti-erectile effects in rats following acute and subacute toxicity studies where lead acetate has been found to modulate NO/cGMP pathway (Senbel et al., 2016). Previous results have also suggested a possible role of heavy metals in the pathogenesis of bladder dysfunction (Romaniuk et al., 2017). Moreover, the onset of bladder cancer has been linked to exposure to lead, or cadmium (Feki-Tounsi and Hamza-Chaffai, 2014; Golabek et al., 2009). It is also assumed that

the excretion of more iron in the urine may exacerbate inflammation of the cells (Bauckman et al., 2019).

The main function of the urinary bladder is the storage of urine for a period of time and then voiding it upon demand at the proper time (Hill, 2015). This requires quite good coordination between the structures of the lower urinary tract tissues and the central nervous system (Andersson and Arner, 2004). The interaction between the autonomic and somatic nervous systems is the main regulator of bladder function, and any imbalance between those systems results in disorders of urinary bladder reflexes, such as urgency or urinary retention (Fowler et al., 2008). There is a variety of neurotransmitters that are responsible for initiating contraction and relaxation (Andersson and Arner, 2004). Acetylcholine (ACh) and adenosine triphosphate (ATP) are the main neurotransmitters involved in bladder smooth muscle contraction. Upon activation of muscarinic (M_2 and M_3) and purinergic (P_2X_1) receptors, it results in an elevation in calcium (Ca^{2+}) level and then activation of voltage-dependent L-type Ca^{2+} channels, which causes detrusor smooth muscle contraction (Ding et al., 2009). In addition, parasympathetic stimulation induces relaxation of the urethra during voiding (Andersson and Arner, 2004). ACh mediates contraction in the bladder, while the use of nitric oxide synthase (NOS) inhibitors blocks the relaxation of the urethra in *in-vivo* and *in-vitro* experiments. Therefore, NO is considered to be a vital inhibitory transmitter involved in urethral relaxation (de Groat and Yoshimura, 2015).

Adrenergic receptors also play an important role in urinary bladder relaxation. β receptors, mainly $β_3$, are more expressed in the detrusor muscles, while $α_1$-receptors are mainly expressed near the bladder neck and urethra (Andersson and Arner, 2004). Activation of $β_3$-receptors results in an elevation of the cyclic adenosine monophosphate (cAMP) level, which in turn causes smooth muscle relaxation. It also involves other signaling pathways, such as potassium (K^+) channels (Schena and Caplan, 2019). The large-conductance voltage-and Ca^{2+}-activated K^+ (BK) channels are the most responsible K^+ channels for urinary bladder function, and their dysfunction results in overactive bladder (Petkov, 2014). On the other hand, activation of $α_1$-receptors in the bladder neck causes its contraction and thus improves the ability of the bladder to accommodate a large volume of urine (Michel and Barendrecht, 2008).

Despite the extensive literature on heavy metals, there is little information about the deleterious effects of heavy metals on the myogenic activity of the urinary bladder wall. This study aims to investigate the potential mechanisms governing the interaction of lead, cadmium, or iron with rat detrusor muscle contractility. A better understanding of the deleterious effects of lead, cadmium, and iron on the pathophysiology of the urinary bladder ameliorates appropriate ways of protection or treatment.

2. Materials and methods

2.1. Chemicals

The chemicals used in this study and their respective sources were as follows: acetylcholine chloride (Sigma), adenosine (Sigma), atropine sulfate (Merck), cadmium sulfate (LOBA Chemie), ferrous sulfate (Oxford Lab Chem), isoprenaline hydrochloride (ISO) (Fluka), lead acetate (Winlab), N^G-Nitro-L-arginine methyl ester (L-NAME) (Sigma), nifedipine (Sigma), tetraethylammonium chloride (TEA) (Sigma), trifluoperazine hydrochloride (TFP) Stellasil® (Kahira Pharmaceuticals). Acetylcholine was prepared daily in distilled water. Nifedipine was prepared in dimethyl sulfoxide (DMSO). The latter caused no change in tissue contraction when added to the organ bath.

A stock solution (40% v/v formaldehyde) was diluted 1:9 with distilled water containing phosphate buffer (4 g/L NaH_2PO_4, 6.5 g/L Na_2HPO_4) to maintain neutral pH, for the preparation of 10% (v/v) formalin solution.

2.2. Animals

Adult male Wistar rats (250–300 g) were housed at ambient temperature with free access to chaw (16% protein, Tanta Oil and Soap Co., Egypt) and water at the animal facility, Faculty of Pharmacy, Alexandria University, Egypt. Experimental protocols and procedures were conducted in compliance with international guidelines for animal care and were approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmacy, Alexandria University (Project Number: ACUC16/11).

2.3. Tension studies using isolated rat urinary bladder smooth muscle

The detrusor muscle was isolated and prepared as described by Luheshi and Zar (1991). Rats were anaesthetized with thiopentone (50 mg/kg) intraperitoneally (ip) and then sacrificed by exsanguination. The urinary bladder was exposed by opening the lower abdomen, and then isolated away from the neck. The tissue was washed multiple times with Krebs solution (in Mm: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4·7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11, "pH = 7.4"). In a petri dish, the bladder was then unfolded by making two lateral incisions, then the muscle was cut into two longitudinal strips of 2x15 mm of detrusor muscle for *in-vitro* tension measurements. The strip of detrusor muscle was fixed at one end between two parallel platinum electrodes (4–5 mm) apart. The electrode is then mounted in an organ bath filled with Krebs solution, adequately oxygenated with 95% (v/v) O_2 and 5% (v/v) CO_2, and maintained at a temperature of 37 °C. The other end of the tissue was connected to a force displacement transducer (Grass FT-03), which was then connected to a computerized data acquisition system via an MLAC11 Grass adapter cable. Tension measurement recording was made using the Lab Chart-7 pro software (Power Lab 4/35, model ML 866/P; AD Instrument Pty Ltd, Castle Hill, Australia). A resting tension of 0.5 g was applied to the suspended tissues for 60 min, and the Krebs solution was replaced every 15 min prior to the experiment.

2.4. Protocols and experimental groups

2.4.1. Subacute toxicity study

Rats were divided into seven groups; the first group was injected with saline and served as the control group, while the second and third groups received lead acetate (3, 30 mg/kg). The fourth and fifth groups received cadmium sulfate (0.1, 1 mg/kg), and the last two groups received ferrous sulfate (3, 30 mg/kg). Rats were injected intraperitoneally daily for 21 days.

For organ bath experiments, a set of rats containing 8 rats from each group was used. Rats were sacrificed 24 h after the last injection. The urinary bladder was isolated, and half of the bladder was fixed in formalin and prepared for histopathological examination, while the other half was used for tension measurement experiments where detrusor muscle contractility was recorded in response to electrical field stimulation (EFS), ACh, and ATP-induced contraction. For EFS experiments, each strip was subjected to increasing frequencies (1, 4, and 16 Hz: voltage 80 V, pulse duration 1 ms, 3 min interval). The contractile responses were measured and compared to the control. In addition, isoprenaline and adenosine-induced relaxation of precontracted detrusor muscle was studied and compared to control.

Venous blood samples were collected from retro-orbital plexus of anaesthetized rats into a non-heparinized tube. Centrifugation

of blood samples was performed using centrifugation (Hettich zentrifugen D 78532 Tuttlingen, Germany) at 4000 r.p.m. for 10 min and serum was recovered and stored at −80 °C for later determination of metal concentration in blood. For determination of metal concentration in tissues: the bladder was isolated, immediately removed, and rinsed with ice-cold saline, dried, weighed, and stored at −80C.

2.4.2. In-vitro toxicity study: Effect of lead acetate, cadmium sulfate, or ferrous sulfate, after 10 min of incubation, and investigation of the mechanism of toxic action in isolated tissues

In this series of experiments, the effect of *in-vitro* exposure of the isolated tissue preparation to directly added metal salts has been investigated. The metal salts were added directly to the organ bath in cumulatively increasing concentrations, and each concentration was incubated for 10 min. The contractility of detrusor muscle in the absence or presence of the tested metal salt was measured, and the percentage change from its control value was calculated and compared to the time-matched control experiment.

In another series of experiments, the effect of some pharmacological modulators on the adverse effect of the directly added metal salt on detrusor muscle contractility was investigated. A submaximal concentration of lead acetate (10 mM) or cadmium sulfate (0.1 mM) was selected to produce a 40–60% change in tissue response to EFS (4 Hz) or ACh (10^{-4} M). The effect of metal salts in the presence of certain blockers (TEA, nifedipine, trifluoperazine, atropine, MB, or L-NAME) was tested. In this series of experiments, two strips of detrusor muscle were separated from the same rat, one for studying the effect of metal salt in presence and absence of each blocker, and the other serving as a time-matched control over the duration of the experiment (Fig. 1).

2.5. Histopathological examination

Histopathological examination of the urinary bladder was performed to examine potential pathological alterations in the bladder. Following the sacrifice of the rats, the bladder specimens from all groups were fixed in 10% formalin, dehydrated with an ascending ethanol gradient, cleared using xylol and embedded in paraffin to form formalin-fixed, paraffin-embedded blocks (FFPE). Blocks were sliced into (5 μ thick) sections, then stained with hematoxylin and eosin (H & E) as well as Prussian blue to detect the iron pigments. The sections were examined using (100 & 200) magnifications of the light microscope (Leica, Germany) and photographed.

2.6. Determination of lead, cadmium, and iron levels in blood and rat urinary bladder

According to Parker et al. (1967), graphite atomic absorption spectroscopy (Shimadzu model AA-6800) was used to determine lead and cadmium concentrations in blood and tissue homogenate.

For determination of iron levels in blood and tissues, MyBiosource® iron colorimetric assay kits were used.

2.7. Statistical analysis

Values are presented as mean ± SEM. The Student's *t* test was used for unpaired data analysis, while analysis of variance (ANOVA) was employed for multiple comparisons, followed by Dunnett's post-test. The criterion for statistical significance was set at the 0.05 level. GraphPad Instat 3.06 and GraphPad Prism 6.01 computer software programs were used for analysis.

3. Results

3.1. Subacute toxicity study

3.1.1. Effect of lead acetate, cadmium sulfate or ferrous sulfate on EFS, ACh and ATP-induced contraction

EFS (1–16 Hz) induced a contraction of the rat detrusor muscle, with a maximum contraction of 3.60 ± 0.23 g at the highest frequency (16 Hz). ACh (10^{-9}-10^{-3} M) also caused detrusor muscle contraction in a concentration-dependent manner, with a maximum contraction of 3.35 ± 0.23 g at (10^{-3} M). In addition, ATP (10^{-7}-10^{-4} M) induced a concentration-dependent contraction with a maximum of 0.43 ± 0.02 g at (10^{-4} M). Subacute toxicity of lead acetate (low dose and high doses, 3, 30 mg/kg, respectively) inhibited EFS-induced contractions significantly, with maximal inhibitions of 44.44 ± 2.57% and 52.77 ± 4.21%, respectively, at 16 Hz. The high dose of lead acetate also triggered a substantial reduction in ACh and ATP-induced contraction, with maximum inhibitions of 42.36 ± 4.57% and 57.45 ± 0.2.23%, respectively. On the other hand, only the high dose of cadmium sulfate (1 mg/kg) induced a significant inhibition of EFS at 16 Hz, with a maximum attained inhibition amounting to 47.77 ± 4.91%. Both low and high doses of cadmium sulfate caused a substantial inhibition of ACh-induced contraction, with maximal inhibitions of 33.14 ± 2.34% and 39.11 ± 3.32%, respectively. Similarly, a significant inhibition of ATP-induced contraction was observed at the high dose of cadmium sulfate with a maximum inhibition of 69.77 ± 11.9%.

Iron overload using ferrous sulfate (30 mg/kg, ip) was confirmed with biochemical testing of serum iron, ferritin, and transferrin saturation% (unpublished data). It was found to inhibit high frequency (16 Hz) and ACh (10^{-3} M)-induced contraction only by 42.5 ± 5.72% and 26.86 ± 3.54%, respectively. However, it had no effect on ATP-induced contraction (Fig. 2).

3.1.2. Effect of lead acetate, cadmium sulfate or ferrous sulfate on isoprenaline and adenosine-induced relaxation of isolated rat detrusor muscle

In *in-vitro* relaxation experiments, isoprenaline (a non-selective β adrenoceptor agonist, 10^{-6}-10^{-4} M) induced a concentration-dependent relaxation of rat detrusor muscle precontracted with
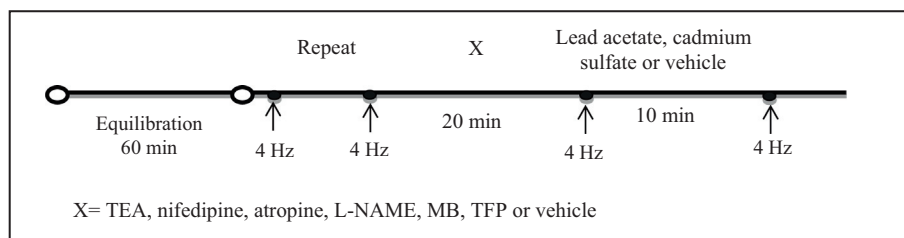

Fig. 1. Experimental protocol used to test the effect of tetraethylammonium (TEA), nifedipine, atropine, L-NAME, methylene blue (MB) or trifluoperazine (TFP) on the modulatory effect of lead acetate or cadmium sulfate on electric field stimulation (EFS)-induced contraction of isolated rat detrusor muscles.
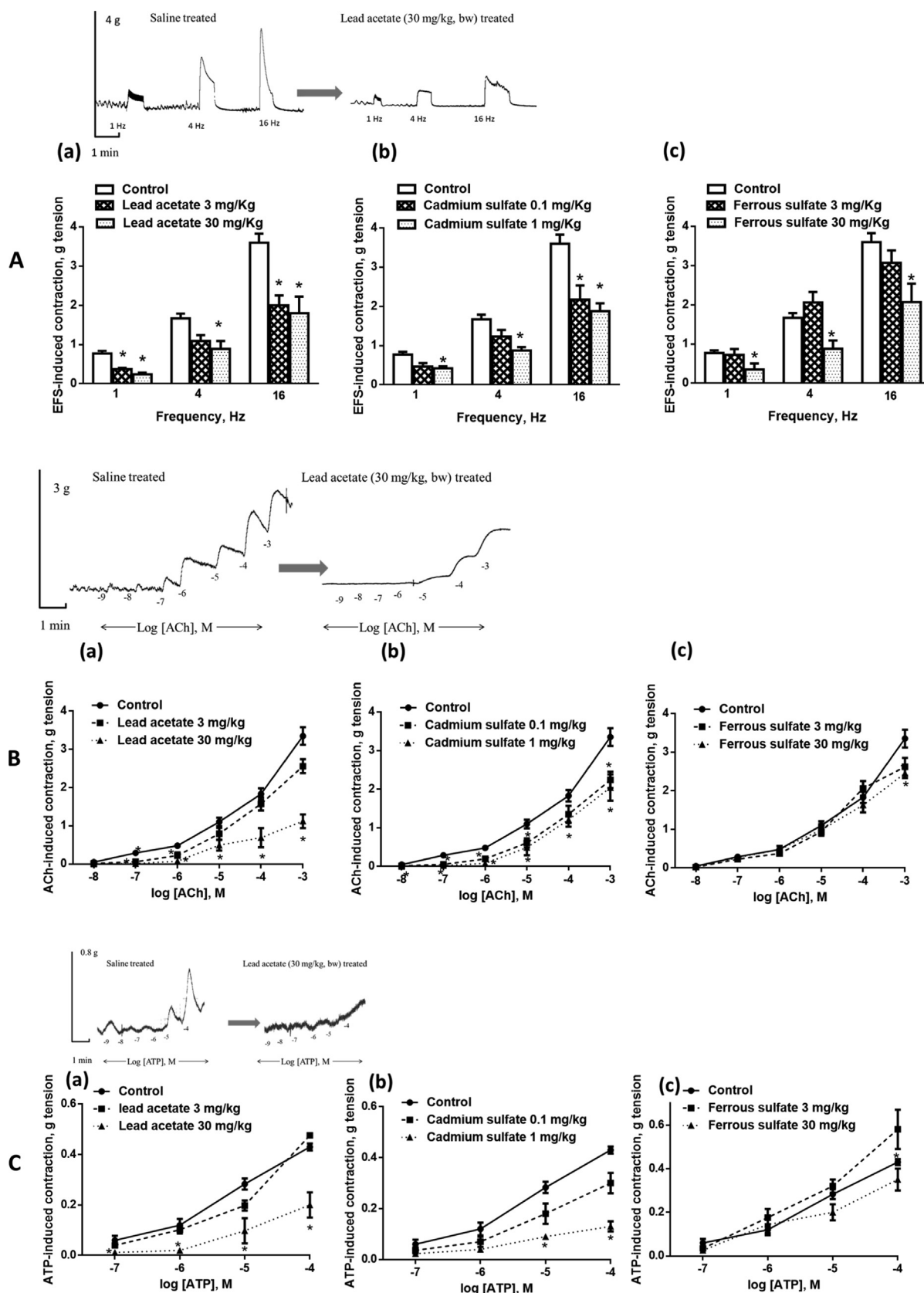
Fig. 2. Effect of lead acetate, Panel a, cadmium sulfate, Panel b, or ferrous sulfate, Panel c, on electrical field stimulation (EFS, 1–16 Hz, **A**), ACh (10^{-8}-10^{-3} M, **B**), or ATP (10^{-7}-10^{-4} M, **C**)-induced contraction (g tension) of isolated rat detrusor muscles, in subacute toxicity study (21 days, ip). Data are expressed as mean ± SEM, (n = 6–7). * denotes significant difference from control (P < 0.05).

ACh (10^{-4} M). The subacute toxicity of lead acetate, at high dose, significantly inhibited isoprenaline-induced relaxation of precontracted strips of isolated rat detrusor muscles, causing an upward shift in the dose response curve. It decreased the E_{MAX} of relaxation to 31.67 ± 2.26% vs. 64.98 ± 4.33% for control. The small dose of lead acetate had no effect, nor did cadmium sulfate or ferrous sulfate (Fig. 3). Furthermore, only high doses of lead acetate and cadmium sulfate inhibited adenosine (10^{-5} M)-induced relaxation of rat detrusor muscles precontracted strips by 10.67 ± 1.79% and 7.03 ± 0.97%, respectively, compared to a control value of 20.79 ± 1.78% (Fig. 4).

3.1.3. Histological examination

Histological examinations of the urinary bladders of treated rats revealed edema and an inflammatory infiltrate. Additionally, the urinary bladder of iron-overloaded rats showed brown pigment deposition predominantly in the surface epithelium, which was stained blue by Prussian blue special stain, as shown in Fig. 5.

3.1.4. Effect of lead acetate, cadmium sulfate or ferrous sulfate on relative urinary bladder weight, and on metal concentrations in blood and urinary bladder tissues

In the subacute toxicity study, the effects of lead acetate, cadmium sulfate, or ferrous sulfate on relative urinary bladder weight, as well as metal concentrations in blood and urinary bladder tissues, was investigated. Table 1 shows that there was no significant difference in relative urinary bladder weights between the treated groups compared to control. There was an increase in lead blood levels in groups treated with low and high doses of lead acetate, reaching 9.13 ± 2.14 μg/dl and 37.64 ± 7.91 μg/dl respectively, compared to 2.19 ± 0.17 μg/dl as control value. Cadmium blood levels rose to 0.34 ± 0.14 μg/l and 6.12 ± 1.33 μg/l respectively, after treatment with low and high doses of cadmium sulfate, respectively, compared to 0.12 ± 0.05 μg/l as the control value. A high dose of ferrous sulfate resulted in a significant elevation of iron from 146.20 ± 9.23 μg/dl in control to 320.82 ± 23.81 μg/dl as shown in Table 2. Furthermore, the three metals, lead, cadmium, and iron, were also found in higher concentrations in the urinary bladder tissues in groups given high doses of metal salts than in the control group. The concentration of lead was 0.40 ± 0.09 μg/g and 2.03 ± 0.31 μg/g after treatment with low and high doses, respectively, in

comparison to the control of 0.09 ± 0.02 μg/g. Cadmium concentrations increased in bladder tissues to 0.35 ± 0.03 μg/g and 1.75 ± 0.16 μg/g after treatment with low and high doses of cadmium sulfate, respectively, compared to a control value of 0.15 ± 0.02 μg/g. After treatment with a high dose of ferrous sulfate, iron levels also increased to 200.51 ± 14.52 μg/g, up from 126.50 ± 6.57 μg/g in the control group.

3.2. In-vitro effect of lead acetate, cadmium sulfate, or ferrous sulfate, after 10 min of incubation, on EFS and ACh-induced contraction

We tested the effects of lead acetate (1,3,10, 30 mM) or cadmium sulfate (0.01, 0.03, 0.1, 0.3 mM) on the contractile effect of a submaximal dose of ACh (10^{-4} M) and a submaximal frequency of 4 Hz in rat detrusor muscle, and then compared these to time-matched controls that were conducted over the course of the experiment. Lead acetate (10 and 30 mM) successfully inhibited EFS-induced contraction by 45.81 ± 6.52% and 96.83 ± 2.58% respectively. With the same doses, it also caused a significant decrease in ACh-induced contraction by 48.37 ± 5.10% and 91.5 ± 6.70%, respectively. Cadmium sulfate significantly decreased EFS-induced contraction at 0.03 mM, resulting in 39.13 ± 2.30% inhibition with a maximum attained inhibition of 83.2 ± 1.85% at a high concentration of 0.3 mM. Furthermore, at a concentration of 0.3 mM, cadmium sulfate inhibited ACh-induced contraction with a maximum attained inhibition of 93.26 ± 3.13% (Fig. 6). On the other hand, ferrous sulfate directly added to the organ bath had no significant effect on either EFS or ACh-induced contraction of isolated detrusor muscle.

3.3. Effect of lead acetate or cadmium sulfate on EFS and ACh-induced contraction in presence of atropine, TEA, nifedipine, trifluoperazine, L-NAME or MB

Those different sets of experiments were used to investigate the effects of K^+ channel blocker, Ca^{2+} channel blocker, a calmodulin-dependent stimulation inhibitor, and NO/cGMP modulators on the inhibitory effect of lead acetate (10 mM) and cadmium sulfate (0.1 mM) on rat detrusor muscles.

Atropine (Non-selective muscarinic blocker, 1 μM) reduced EFS-induced contraction by 43.23 ± 4.85%, as well as ACh (10^{-4} M)-
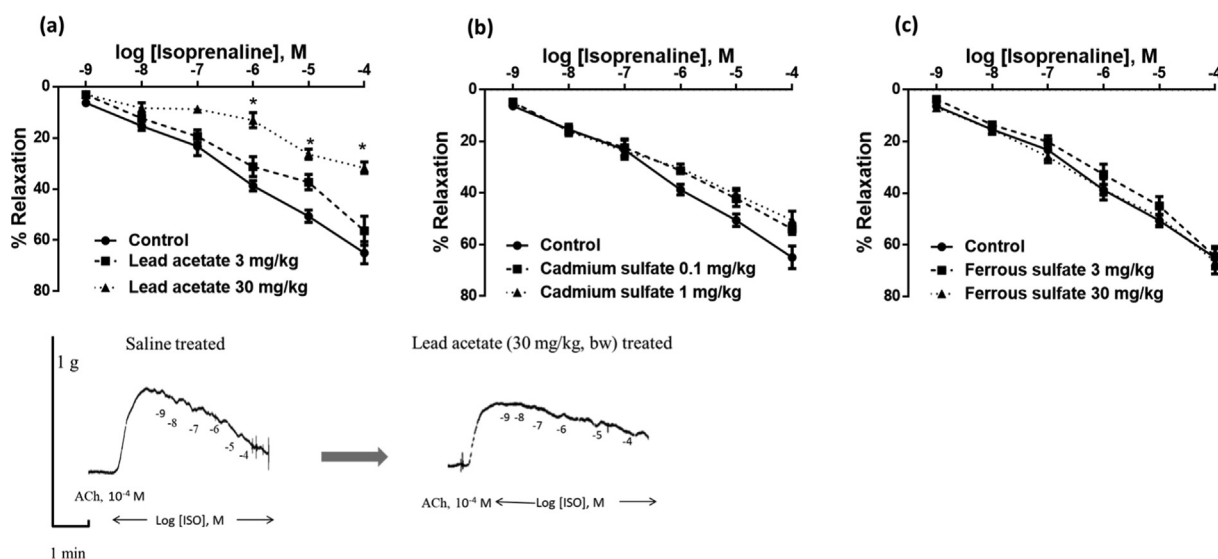

Fig. 3. Effect of lead acetate, Panel a, cadmium sulfate, Panel b, or ferrous sulfate, Panel c, on % relaxation induced by isoprenaline (10^{-9}-10^{-4} M) on isolated rat detrusor muscles in subacute toxicity study (21 days, ip). Detrusor muscle strips were precontracted with ACh (10^{-4} M). Data are expressed as mean ± SEM, (n = 6–7). * denotes significant difference from control (P < 0.05).
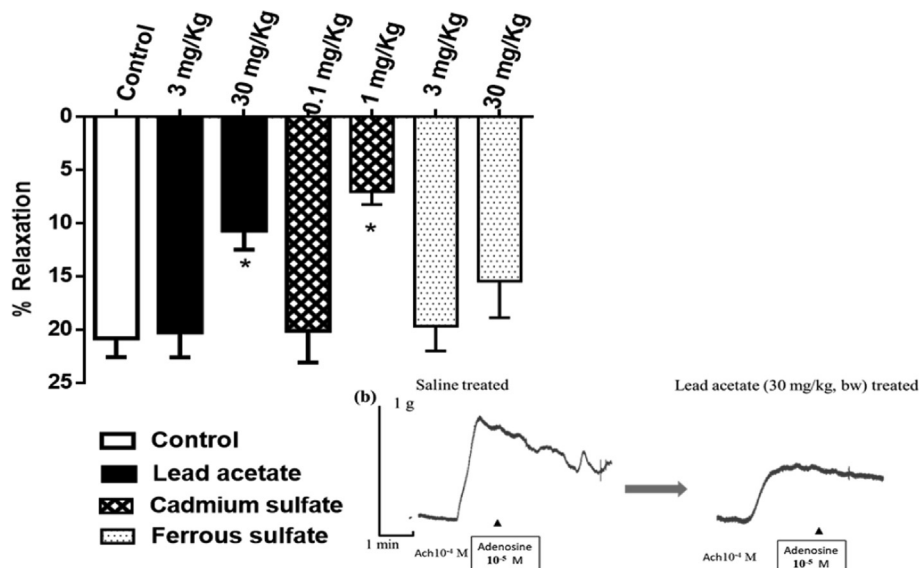
Fig. 4. Effect of lead acetate, cadmium sulfate, or ferrous sulfate on % relaxation induced by adenosine (10^{-5} M) on isolated rat detrusor muscles in subacute toxicity study (21 days, ip). Detrusor muscle strips were precontracted with ACh (10^{-4} M). Data are expressed as mean ± SEM, (n = 6–7). *denotes significant difference from control (P < 0.05).

induced contraction by 56.04 ± 7.50%, in rat detrusor muscle. Atropine reversed the inhibitory effect of lead acetate on EFS but significantly decreased lead acetate inhibitory effect on ACh-induced contraction to 25.80 ± 4.78% in presence of atropine compared to 38.33 ± 3.07% in its absence. While atropine had no effect on the inhibitory effect of cadmium sulfate (Figs. 7 & 8).

TEA (tetraethylammonium, K^+ channel blocker, 10^{-3} M) increased EFS and ACh (10^{-4} M)-induced contraction in rat detrusor muscle by 43.97 ± 6.32% and 39.27 ± 5.18%, respectively. However, nifedipine (Ca^{2+} channel blocker, 1 μM) reduced EFS and ACh (10^{-4} M)-induced contraction in rat detrusor muscle by 56.83 ± 4.53% and 55.45 ± 5.59%, respectively.

In the presence of TEA, the inhibitory effect of lead acetate on EFS (4 Hz)-induced contraction was potentiated significantly, reaching 51.52 ± 2.78% compared to 36.93 ± 3.81% in its absence. TEA also potentiated the inhibitory effect of lead acetate on ACh (10^{-4} M)-induced contraction from 38.33 ± 3.07% to 56.72 ± 6.72%. In the presence of nifedipine, the inhibitory effect of lead acetate on EFS and ACh (10^{-4} M)-induced contraction did not significantly change.

TEA (10^{-3} M) had no effect on the inhibitory effect of cadmium sulfate on both EFS and ACh. On the other hand, cadmium sulfate's inhibitory effect on EFS and ACh (10^{-4} M)-induced contraction was significantly decreased in presence of nifedipine to attain 38.21 ± 6.06% and 32.14 ± 5.23% relative to 58.73 ± 3.78% and 65.8 ± 2.54%, respectively, compared to the inhibitory effect of cadmium sulfate alone (Figs. 7 & 8).

Trifluoperazine (calmodulin-dependent stimulation inhibitor, 10^{-5} M) decreased EFS and ACh (10^{-4} M)-induced contraction in rat detrusor muscle by 39.89 ± 3.98% and 35.89 ± 3.36%, respectively. Trifluoperazine causes a significant decrease in the inhibitory effect of cadmium sulfate on EFS and ACh (10^{-4} M)-induced contraction from 58.73 ± 3.78% and 65.8 ± 2.54%, for cadmium sulfate alone, to 29.18 ± 2.26% and 35.50 ± 2.78%, respectively. On the other hand, trifluoperazine had no effect on the inhibitory effect of lead acetate (Figs. 7 & 8).

L-NAME (N^G-nitro-L-arginine methyl ester, NOS inhibitor, 10^{-4} M) increased EFS-induced contraction in rat detrusor muscle by 21.94 ± 3.67%. Furthermore, pretreatment of the tissues with the sGC inhibitor methylene blue (MB, 10^{-4} M) increased EFS-induced contractions by 19.45 ± 3.16%. In the presence of L-NAME or MB, the inhibitory effect of lead acetate on EFS-induced contraction was significantly potentiated to 51.03 ± 1.92% and 54.17 ± 3.95%, respectively, compared to 36.93 ± 3.81% as the inhibitory effect of lead acetate alone. On the other hand, neither L-NAME nor MB had a significant effect on cadmium sulfate's inhibitory effect on EFS-induced contraction (Figs. 7 & 8).

4. Discussion

The current experimental study reports on the role of heavy metals in disturbing detrusor muscle myogenic functions in rats. Because of the extensive use of metallic compounds and industrialization, the use of metals has been crucial to the development and prosperity of human society. Among environmental pollutants, metals are peculiar in that they all occur naturally and, often, are omnipresent in the human environment (Rahman and Singh, 2019). Epidemiological studies have shown possible associations between lead (Golabek et al., 2009), or cadmium (Feki-Tounsi and Hamza-Chaffai, 2014), and bladder cancer in patients. Furthermore, a recent study found that lead and cadmium blood concentrations in Egyptian bladder cancer patients were statistically higher than in controls (Awadalla et al., 2020).

For adults, the acceptable blood levels of lead and cadmium are <10 μg/dl and 0.03–0.12 μg/dl, respectively (World Health Organization, 2006). It has also been reported that normal concentrations of cadmium in non-smokers are <1 μg/L, and just <5 μg/L in smokers (Johri et al., 2010). Additionally, iron concentrations between 300 and 500 μg/dl (normal 50 to 150 μg/dl) are often associated with toxicity (Reynolds and Ventre, 2007). In the current study, selected lead and cadmium salt doses (30 mg/kg and 1 mg/kg, respectively) increased serum metal concentrations, nearly tripling the acceptable lead level and simulating smokers' cadmium levels. The metal serum levels also simulate the levels in occupationally exposed people, as reported in clinical studies (Alli, 2015; Singh et al., 2013). Iron overload toxicity has been attained in the current study by using ferrous sulfate (30 mg/kg).

The histopathological observations of the current study are consistent with those made by Romaniuk et al. (2017) in which heavy
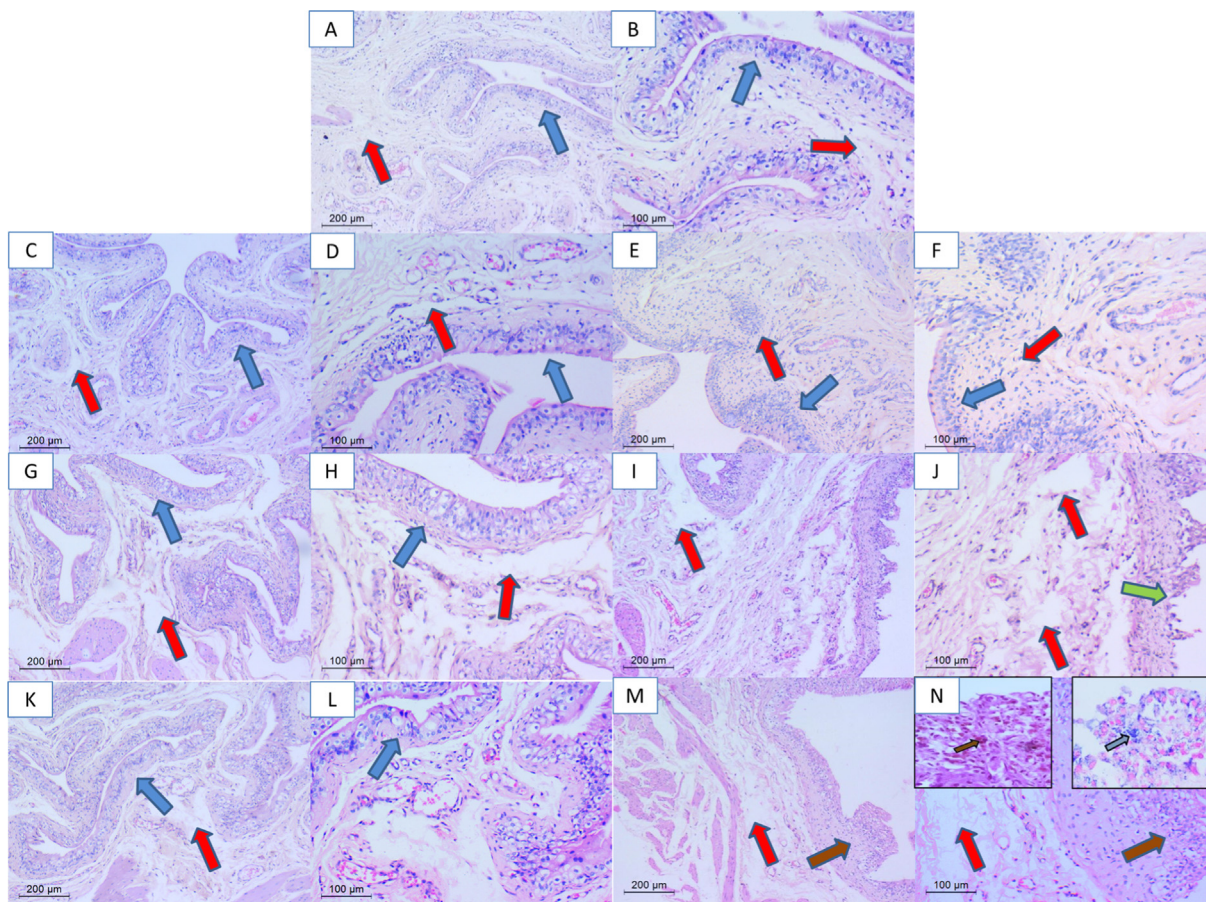
Fig. 5. **_Control_** urinary bladder showing intact urothelial lining (blue arrow) and lamina propria (red arrow) (H & E, x100 **(A)**, x200 **(B)** magnification). **_Low dose lead,_** urinary bladder showing mild inflammatory infiltrate with mild edema (red arrow) in the lamina propria with intact urothelial lining (H & E, x100 **(C)**, x200 **(D)** magnification), **_High dose lead_**, urinary bladder showing moderate inflammatory infiltrate with mild edema (red arrow) in the lamina propria with intact urothelial lining (blue arrow) (H & E, x100 **(E)**, x200 **(F)** magnification). **_Low dose cadmium,_** urinary bladder showing mild edema of the lamina propria (red arrow) with intact urothelial lining (blue arrow) (H & E, x100 **(G)**, x200 **(H)** magnification), **_High dose cadmium,_** urinary bladder showing moderate edema of the lamina propria (red arrow), with focal erosion and attenuation of the urothelial lining (green arrow), (H & E, x100 **(I)**, x200 **(J)** magnification). **_Low dose iron,_** urinary bladder showing mild to moderate edema of the lamina propria (red arrow) with intact urothelial lining (blue arrow) (H & E, x100 **(K)**, x200 **(L)** original magnification), **_High dose iron,_** urinary bladder showing brown pigment deposition (brown arrow) predominantly on the surface with mild inflammatory infiltrate and edema in the lamina propria (red arrow), (Inset iron pigment-stained blue by Prussian blue special stain) (H & E, x100 **(M)**, x200 **(N)** magnification.

Table 1
Effect of lead acetate, cadmium sulfate or ferrous sulfate on relative urinary bladder weight in subacute toxicity study (21 days, ip).

Group	Urinary bladder weight/Body weight, μg/g
Control	0.35 ± 0.02
Lead acetate 3 mg/kg	0.36 ± 0.02
Lead acetate 30 mg/kg	0.42 ± 0.04
Cadmium sulfate 0.1 mg/kg	0.38 ± 0.01
Cadmium sulfate 1 mg/kg	0.35 ± 0.03
Ferrous Sulfate 3 mg/kg	0.38 ± 0.03
Ferrous sulfate 30 mg/kg	0.38 ± 0.01

Data are expressed as mean ± SEM, (n = 6–8).

Table 2
Lead, cadmium, and iron concentrations in blood and urinary bladder tissues in subacute toxicity study (21 days, ip) of lead acetate, cadmium sulfate or ferrous sulfate.

Treated group	Concentration of metals	
	Urinary bladder (Wet weight) μg/g	Blood level
	Lead	μ**g/dl**
Control	0.09 ± 0.01	2.19 ± 0.17
Lead acetate 3 mg/kg	0.40 ± 0.09	9.30 ± 2.14
Lead acetate 30 mg/kg	2.03 ± 0.31*	37.64 ± 7.91*
	Cadmium	μ**g/l**
Control	0.15 ± 0.02	0.12 ± 0.05
Cadmium sulfate 0.1 mg/kg	0.35 ± 0.03	0.34 ± 0.14
Cadmium sulfate 1 mg/kg	1.75 ± 0.16*	6.12 ± 1.33*
	Iron	μ**g/dl**
Control	126.50 ± 6.57	146.20 ± 9.23
Ferrous Sulfate 3 mg/kg	152.33 ± 8.78	185.33 ± 13.67
Ferrous sulfate 30 mg/kg	200.51 ± 14.52*	320.82 ± 23.81*

Data are expressed as mean ± SEM, (n = 6–7). * denotes significant difference from corresponding control ($P < 0.05$).

metal salts induced profound morphologic alterations in tissue wall structures. Our results show an accumulation of lead, cadmium, or iron in urinary bladder tissue, which appears mainly after treatment with high doses of metal salts, and that explains the histological changes in the tissues. It has been reported that lead and cadmium concentrations reached statistically higher values in bladder cancer tissues compared to control volunteers (Feki-Tounsi et al., 2014; Golabek et al., 2009). Because the kidneys' role is to excrete toxic substances, including metal salts, in the urine,
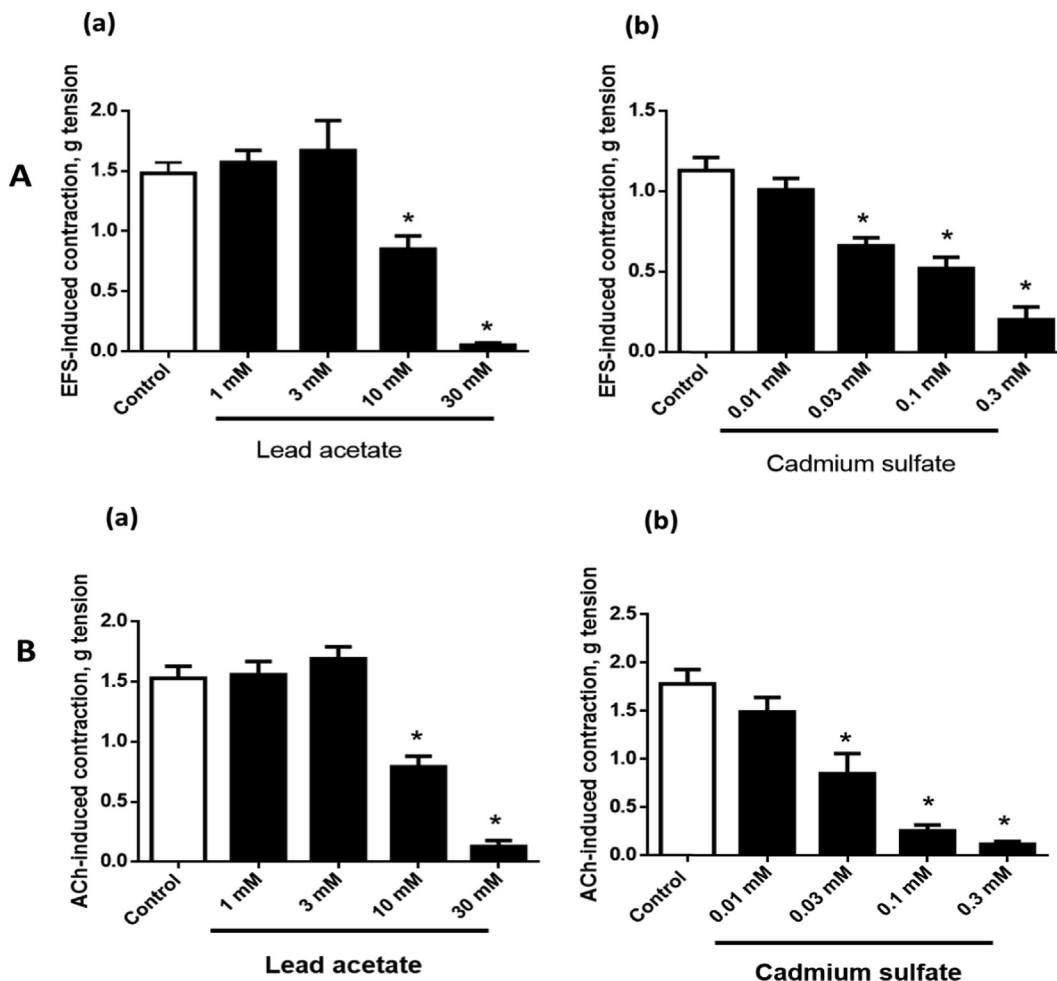
Fig. 6. Effect of directly added lead acetate, Panel a, or cadmium sulfate, Panel b, on electric field stimulation (EFS, 4 Hz, **A**) or ACh (10^{-4} M, **B**)-induced contraction (g tension) of isolated rat detrusor muscles. Data are expressed as mean ± SEM, (n = 6–7). * denotes significant difference from control (P < 0.05). Metal salts were incubated for 10 min.
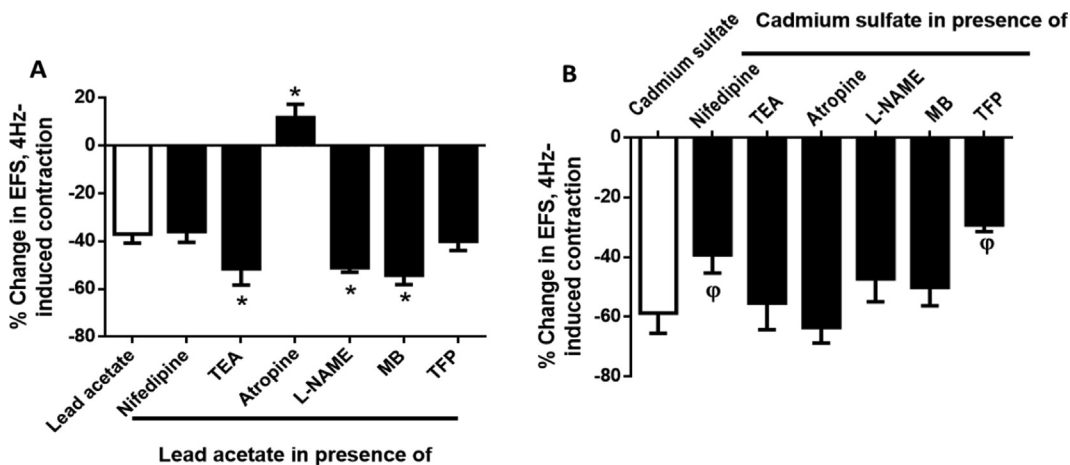

Fig. 7. Effect of lead acetate (10 mM, Panel **A**) or cadmium sulfate (0.1 mM, Panel **B**) on electrical field stimulation (EFS, 4 Hz)-induced contraction (g tension) of isolated rat detrusor muscles in male rats in the absence and presence of nifedipine (1 μM), TEA (10^{-3} M), atropine (1 μM), L-NAME (10^{-4} M), MB (10^{-4} M), or TFP (trifluoperazine, 10^{-5} M). Data are expressed as mean ± SEM, (n = 6–7). * Denotes significant difference from lead acetate values. $^{\phi}$ denotes significant difference from cadmium sulfate values (P < 0.05). The tested drugs were incubated for 20 min prior to lead acetate or cadmium sulfate. Metal salts were incubated for 10 min.

heavy metals are retained in the bladder and accumulate in its walls, potentially impairing its function. That proves that excessive exposure to heavy metals has a negative impact on the urinary bladder (Papagiannis et al., 2019).

In the current study, lead acetate and cadmium sulfate toxicity seem to induce hypoactive bladder through inhibiting EFS and ACh-induced contractions. Although there is little data regarding the effects of metals on the lower urogenital tract smooth muscle
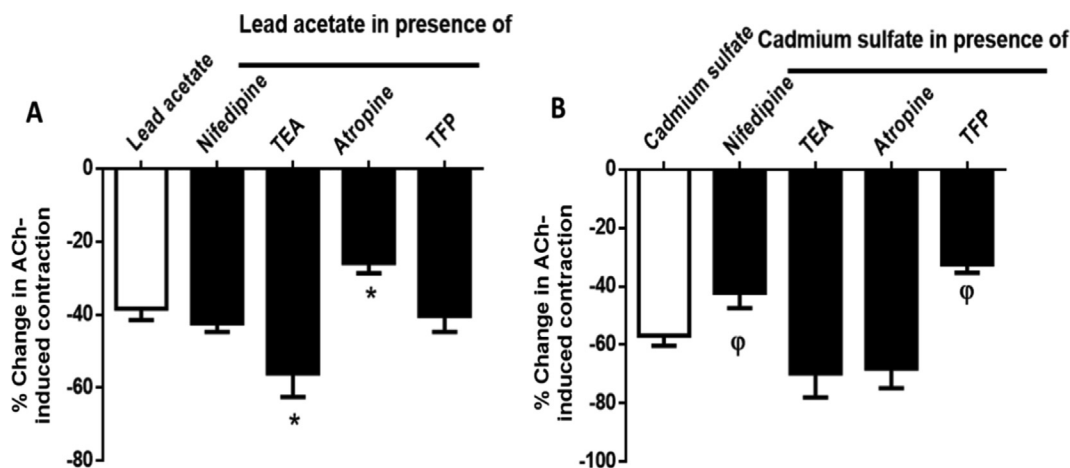
Fig. 8. Effect of lead acetate (10 mM, Panel **A**) or cadmium sulfate (0.1 mM, Panel **B**) on ACh (10^{-4} M)-induced contraction (g tension) of isolated rat detrusor muscles in male rats in the absence and presence of nifedipine (1 μM), TEA (10^{-3} M), atropine (1 nM), or TFP (trifluoperazine, 10^{-5} M). Data are expressed as mean ± SEM, (n = 6–7). * Denotes significant difference from lead acetate values. $^\phi$ denotes significant difference from cadmium sulfate values (P < 0.05). The tested drugs were incubated for 20 min prior to lead acetate or cadmium sulfate. Metal salts were incubated for 10 min.

contractility, the literature has reported an inhibitory effect of lead acetate on methacholine-induced contraction of ileal longitudinal smooth muscle strips *in-vitro* (Walsh and Harnett, 1986), which is consistent with our results. Similarly, Gupta and Fahim (2007) stated that ACh-induced contractile response in rat tracheal smooth muscle was inhibited after lead acetate exposure. Moreover, it has been reported that low GIT motility, and hence constipation, is a common feature of lead acetate toxicity (Gupta, 2016), which is in line with our results. Furthermore, it has been reported that difficulty with urination, increased frequency, or a weak stream are all symptoms that may be considered early signs of bladder cancer or other pathologic etiologies (Philyppov et al., 2020).

Concerning cadmium, the results are consistent with those previously reported on other examples of smooth muscles, as reported by Niwa et al. (1981), who proved the inhibitory effect of cadmium on ACh response in isolated vas deferens. Also, cadmium was stated to inhibit the response of the isolated ileum to various agonists, such as methacholine or histamine (Schnieden and Small, 1971). Additionally, it has been reported that cadmium had an antagonistic effect on ACh in the pyloric antrum of the rat's stomach (Shino, 1976). Furthermore, heavy smokers have been shown to be more prone to developing motor incontinence than non-smokers (Tampakoudis et al., 1995), which could be attributed to the increased cadmium levels. It seems that the previously reported effects of lead and cadmium on smooth muscle contractility of different tissues are also manifested in the rat's bladder, with dysfunctional consequences, as shown in the current study.

Subacute iron overload toxicity also reduced the maximal EFS and ACh-induced contraction of the detrusor muscle. This is in line with the theory stating that excess iron in the bladder or in the urine could exacerbate inflammation and increase urothelial cell death, mainly during infection and prolong bacterial burden (Bauckman et al., 2019; Bauckman and Mysorekar, 2016), which may affect the contractility of the detrusor muscle. This is especially intriguing because children who receive more iron are more prone to getting urinary tract infections (Collard, 2009; Mava et al., 2011). Additionally, the elderly have shown higher levels of urinary iron clearance in their urine than adults (Pfrimer et al., 2014), which may be linked to recurrent urinary tract infections with ageing (Cortes-Penfield et al., 2017).

In an attempt to explain the inhibitory effects of lead acetate and cadmium sulfate on detrusor muscle, the involvement of muscarinic activity in their effects was investigated. Our results suggest that lead, as a part of its action, may interfere with muscarinic signaling in the urothelium, which is consistent with previous studies which stated the inhibitory effect of lead on muscarinic receptors *in-vitro* and *in-vivo* (Bondy and Agrawal, 1980). The inhibitory effect of lead acetate on neurally-mediated contraction of isolated detrusor muscle was unexpectedly reversed in the presence of atropine. In that case, upon blocking the muscarinic receptor, the main part of the inhibitory effect of lead acetate on ACh may have been lost, giving the upper hand to other contractile mediators, including ATP and prostaglandins, which are also released upon neuronal stimulation (Andersson and Arner, 2004). The inhibitory effect of cadmium, on the other hand, may not be muscarinic dependent, which is consistent with previous studies (Koç et al., 2008).

ATP-induced contraction of detrusor muscle was significantly diminished in rats treated for 21 days with high doses of lead acetate and cadmium sulfate only. It was previously reported that cystitis in rats results in a decline in contraction in response to methacholine, ATP, and adenosine (Aronsson et al., 2014). It is also reported that in cats with interstitial cystitis, there was a significant reduction in P_2X_1 expression, which resulted in an alteration in purinergic mechanisms (Birder et al., 2004). It seems that bladder function is altered in a similar fashion by the effect of metal exposure, as observed in our study. Furthermore, a previous study proved that lead toxicity significantly reduced both the level of purines as well as the expression of purinergic receptors in the rat brain (Baranowska-Bosiacka et al., 2011).

Regarding Ca^{2+} channels, in the current study, the addition of nifedipine (Ca^{2+} channel blocker) or trifluoperazine (antipsychotic drug that is capable of inhibiting the intracellular effects of calmodulin) to the organ bath was found to significantly decrease only the inhibitory effect of cadmium sulfate on isolated rat detrusor muscles. Therefore, we can speculate that cadmium may interfere with the Ca^{2+} channels. Cadmium is well-known to participate in different Ca^{2+}-dependent pathways, attributable to its actions as a Ca^{2+} mimetic, with a pivotal role in calmodulin action (Choong et al., 2014). It may interact with a second messenger system involved in excitation–contraction coupling or interact directly with the contractile proteins (Dulhunty and Gage, 1989). The results of the cur-

rent study are consistent with the reports which suggest the ability of cadmium to block Ca^{2+} channels (Balaraman et al., 1989), and modulate several calmodulin-dependent functions (Sutoo et al., 1990; Vig et al., 1989). Although *in-vivo* studies suggest that lead interferes with Ca^{2+} channels in the brain (Rius et al., 1986), the inhibitory effect of lead, in the current study, was not affected significantly in the presence of either nifedipine, which is in line with Senbel et al. (2016), or trifluoperazine.

Concerning the role of the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) pathway in the bladder, there are various contradictory reports. In the current study, results showed that NO and cGMP contribute to the rat's detrusor muscle relaxation in default physiological conditions. Moreover, Gumrah et al. reported that sildenafil reduces contraction of the rat bladder, as it perpetuates the action of NO through inhibiting cGMP breakdown (Gumrah et al., 2017). In the current study, we investigated the role of lead acetate on NO pathway to confirm the previously reported role of lead in other tissues, such as blood vessels and the trachea. It has been found that the inhibitory effect of lead acetate was potentiated in the presence of L-NAME or MB in rat detrusor muscles. Bassiouni et al. reported a similar observation: the inhibitory effect of sildenafil on rat detrusor muscle contraction was potentiated by NOS and GC inhibitors, indicating the involvement of mediators other than NO in its effect (Bassiouni et al., 2019). It seems that, also in the current study, the absence of cGMP gives the upper hand to contractile components such as muscarinic and purinergic pathways, which are modulated by lead acetate as mentioned above, thus giving more targets for the action of lead acetate to be exhibited in an exaggerated fashion.

In complementation, we investigated the role of the K$^+$ channel modulation in the mechanism of action of either lead or cadmium in the detrusor muscle, by using TEA (K$^+$ channel blocker). The findings revealed that the inhibitory effect of lead acetate on isolated rat detrusor muscle was amplified in the presence of TEA. Therefore, it is supposed that the lead acetate inhibitory effect in this case is not K$^+$ channel dependent. Cadmium had no significant role in K$^+$/NO/cGMP pathway, which is consistent with previous studies (Senbel et al., 2016). Cadmium, on the other hand, was found to reduce the functional availability of NO in *in-vivo* studies (Skoczynska and Martynowicz, 2005).

Activation of β_3-receptors in response to NA induces an elevation in cAMP level, resulting in smooth muscle relaxation (Schena and Caplan, 2019). In addition, it was previously suggested that the degradation of cAMP results in adenosine, which acts at the A1 receptor (Silva et al., 2017), as well as adenosine itself directly induces relaxation *in-vitro* in precontracted detrusor muscle strips (Acevedo et al., 1992), which is in line with our results.

Lead acetate has been found to decrease detrusor muscle relaxation, either through β-adrenoceptors or purinoceptors, which is expected to be secondary to inflammation induced by lead as shown in the histopathological study. The findings are consistent with a study which stated that isoprenaline-and adenosine-induced relaxations in detrusor muscle were found to be smaller in inflamed strips (Giglio et al., 2007; Vesela et al., 2011), supporting the hypothesis that P1A1 purinoceptor expression is downregulated in inflamed bladders. Our findings are consistent with previous findings that lead reduces isoprenaline's relaxant effect in rat tracheal smooth muscle (Gupta and Fahim, 2007), as well as its role in β-adrenoceptor and cAMP decrement in the heart (Tsao et al., 2000). Another study, on the other hand, found no significant effect of directly added lead on isoprenaline-induced relaxation in rat thoracic aorta, which could be because it looked at the effect of directly adding lead to the organ bath rather than the subacute toxicity of lead (Shelkovnikov and Gonick, 2001). The same was observed with cadmium sulfate toxicity as it decreased adenosine-induced relaxation. Previous research suggests that cadmium ions may cause conformational changes in the A1 receptor molecule, resulting in a decrease in receptor affinity (Rosati and Traversa, 1999). There was no significant effect of iron overload on isoprenaline or adenosine-induced relaxation, which may be attributed to the degree of inflammation induced by the current used dose of ferrous sulfate.

Developing effective guidelines and detecting highly exposed areas has become a crucial medical and regulatory procedure to be adopted to avoid the aggravation of bladder problems, which are one of the most commonly reported incapacitation medical problems among the population over 60 years old. Studying the differential effects of the tested heavy metals on intravesical pressure by *in-vivo* cystometry study will be needed to confirm the current results. Testing some management options (besides metal elimination) for heavy metal toxicity on bladder functions based on the potential mechanisms of their toxic actions investigated in this study may be of great medical value.

5. Conclusions

Our data reveals that subacute toxicity of metal salts (lead, cadmium, or iron) results in their accumulation in the urinary bladder, which is not only associated with histopathological damage of the urinary bladder, but also with hypoactivity of the detrusor muscle, as an indication of a potential decrease in micturition responses. Lead and cadmium interfere with both contraction and relaxation of the detrusor muscle, disturbing urinary bladder function. To a lesser extent, iron overload toxicity induces detrusor muscle hypoactivity. The inhibitory effect of lead acetate on rat detrusor muscle contraction clearly involves interference with muscarinic signaling in the urothelium and is NO/cGMP independent. Cadmium, on the other hand, appears to modulate Ca^{2+}/calmodulin pathway in detrusor muscle.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Acevedo, C.G., Contreras, E., Escalona, J., Lewin, J., Huidobro-Toro, J.P., 1992. Pharmacological characterization of adenosine A1 and A2 receptors in the bladder: evidence for a modulatory adenosine tone regulating non-adrenergic non-cholinergic neurotransmission. Br. J. Pharmacol. 107 (1), 120–126.

Ahamed, M., Akhtar, M.J., Alaizeri, Z.M., Alhadlaq, H.A., 2020a. TiO(2) nanoparticles potentiated the cytotoxicity, oxidative stress and apoptosis response of cadmium in two different human cells. Environ. Sci. Pollut. Res. Int. 27 (10), 10425–10435.

Ahamed, M., Akhtar, M.J., Alhadlaq, H.A., 2019. Preventive effect of TiO(2) nanoparticles on heavy metal Pb-induced toxicity in human lung epithelial (A549) cells. Toxicol. In Vitro 57, 18–27.

Ahamed, M., Akhtar, M.J., Khan, M.A.M., 2020b. Single-Walled Carbon Nanotubes Attenuate Cytotoxic and Oxidative Stress Response of Pb in Human Lung Epithelial (A549) Cells. Int. J. Environ. Res. Public Health 17 (21), 8221.

Ahamed, M., Akhtar, M.J., Khan, M.A.M., Alhadlaq, H.A., 2020c. Reduced graphene oxide mitigates cadmium-induced cytotoxicity and oxidative stress in HepG2 cells. Food Chem. Toxicol. 143, 111515.

Alli, L.A., 2015. Blood level of cadmium and lead in occupationally exposed persons in Gwagwalada, Abuja, Nigeria. Interdiscip. Toxicol. 8 (3), 146–150.

Andersson, K.E., Arner, A., 2004. Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol. Rev. 84 (3), 935–986.

Aronsson, P., Vesela, R., Johnsson, M., 2014. Inhibition of nitric oxide synthase prevents muscarinic and purinergic functional changes and development of cyclophosphamide-induced cystitis in the rat. BioMed Res. Int. 2014.

Atieh, M.A., Ji, Y., Kochkodan, V., 2017. Metals in the Environment: Toxic Metals Removal. Bioinorg. Chem. Appl. 2017, 4309198.

Awadalla, A., Mortada, W.I., Abol-Enein, H., Shokeir, A.A., 2020. Correlation between blood levels of cadmium and lead and the expression of microRNA-21 in Egyptian bladder cancer patients. Heliyon 6, (12) e05642.

Balaraman, R., Gulati, O.D., Rathod, S.P., Bhatt, J.D., 1989. The acute pressor response to cadmium in rats. Arch. Int. Pharmacodyn. Ther. 301, 254–266.

Baranowska-Bosiacka, I., Dąbrowska-Bouta, B., Strużyńska, L., 2011. Regional changes in purines and selected purinergic receptors in immature rat brain exposed to lead. Toxicology 279 (1–3), 100–107.

Bassiouni, W., Senbel, A., Norel, X., Daabees, T., 2019. Sildenafil corrects the increased contractility of rat detrusor muscle induced by alprostadil in vitro. Pharmacol. Rep. 71 (4), 659–668.

Bauckman, K.A., Matsuda, R., Higgins, C.B., DeBosch, B.J., Wang, C., Mysorekar, I.U., 2019. Dietary restriction of iron availability attenuates UPEC pathogenesis in a mouse model of urinary tract infection. Am. J. Physiol.-Renal Physiol. 316 (5), F814–F822.

Bauckman, K.A., Mysorekar, I.U., 2016. Ferritinophagy drives uropathogenic Escherichia coli persistence in bladder epithelial cells. Autophagy 12 (5), 850–863.

Birder, L.A., Ruan, H.Z., Chopra, B., Xiang, Z., Barrick, S., Buffington, C.A., et al., 2004. Alterations in P2X and P2Y purinergic receptor expression in urinary bladder from normal cats and cats with interstitial cystitis. Am. J. Physiol.-Renal Physiol. 287 (5), F1084–F1091.

Bondy, S.C., Agrawal, A.K., 1980. The inhibition of cerebral high affinity receptor sites by lead and mercury compounds. Arch. Toxicol. 46 (3–4), 249–256.

Choong, G., Liu, Y., Templeton, D., 2014. Interplay of Calcium and Cadmium in Mediating Cadmium Toxicity. Chem. Biol. Interact. 211.

Collard, K.J., 2009. Iron homeostasis in the neonate. Pediatrics 123 (4), 1208–1216.

Cortes-Penfield, N.W., Trautner, B.W., Jump, R.L.P., 2017. Urinary Tract Infection and Asymptomatic Bacteriuria in Older Adults. Infect. Dis. Clin. North Am. 31 (4), 673–688.

de Groat, W.C., Yoshimura, N., 2015. Anatomy and physiology of the lower urinary tract. Handb Clin Neurol 130, 61–108.

Ding, H.-L., Ryder, J.W., Stull, J.T., Kamm, K.E., 2009. Signaling processes for initiating smooth muscle contraction upon neural stimulation. J. Biol. Chem. 284 (23), 15541–15548.

Dulhunty, A.F., Gage, P.W., 1989. Effects of cobalt, magnesium, and cadmium on contraction of rat soleus muscle. Biophys. J. 56 (1), 1–14.

Feki-Tounsi, M., Hamza-Chaffai, A., 2014. Cadmium as a possible cause of bladder cancer: a review of accumulated evidence. Environ. Sci. Pollut. Res. Int. 21 (18), 10561–10573.

Feki-Tounsi, M., Olmedo, P., Gil, F., Mhiri, M.N., Rebai, A., Hamza-Chaffai, A., 2014. Trace metal quantification in bladder biopsies from tumoral lesions of Tunisian cancer and controls subjects. Environ. Sci. Pollut. Res. Int. 21 (19), 11433–11438.

Fowler, C.J., Griffiths, D., de Groat, W.C., 2008. The neural control of micturition. Nat. Rev. Neurosci. 9 (6), 453–466.

Giglio, D., Aronsson, P., Eriksson, L., Tobin, G., 2007. In vitro characterization of parasympathetic and sympathetic responses in cyclophosphamide-induced cystitis in the rat. Basic Clin. Pharmacol. Toxicol. 100 (2), 96–108.

Golabek, T., Darewicz, B., Borawska, M., Markiewicz, R., Socha, K., Kudelski, J., 2009. Lead concentration in the bladder tissue and blood of patients with bladder cancer. Scand. J. Urol. Nephrol. 43 (6), 467–470.

Gumrah, A., Tanidir, Y., Tinay, I., Ozyurek, M., Tarcan, T., 2017. The effect of doxazosin and sildenafil citrate combination on bladder tissue contractility, alpha adrenergic receptor, and iNOS subtype expression in a male rat model of partially bladder outlet obstruction. Neurourol. Urodyn. 36 (6), 1479–1487.

Gupta, N., Fahim, M., 2007. Lead acetate induced contraction in rat tracheal smooth muscle is independent of epithelium. Indian J. Physiol. Pharmacol. 51 (1), 49–54.

Gupta, P.K., 2016. Chapter 18 - Toxic effects of metals. In: Gupta, P.K. (Ed.), Fundamentals of Toxicology. Academic Press, pp. 203–213.

Hamid, A., Riaz, H., Akhtar, S., Ahmad, S., 2016. Heavy Metal Contamination in Vegetables, Soil and Water and Potential Health Risk Assessment.

Hill, W.G., 2015. Control of urinary drainage and voiding. Clinical J. Am. Soc. Nephrol.: CJASN 10 (3), 480–492.

Johri, N., Jacquillet, G., Unwin, R., 2010. Heavy metal poisoning: the effects of cadmium on the kidney. Biometals 23 (5), 783–792.

Koç, E., Koçak, M., Akçil, E., 2008. Cadmium reduces contractile responses of rat duodenum, in vitro. Biol. Trace Elem. Res. 123 (1–3), 154–160.

Luheshi, G.N., Zar, M.A., 1991. The effect of streptozotocin-induced diabetes on cholinergic motor transmission in the rat urinary bladder. Br. J. Pharmacol. 103 (3), 1657–1662.

Mava, Y., Ambe, J.P., Bello, M., Watila, I., Nottidge, V.A., 2011. Urinary tract infection in febrile children with sickle cell anaemia. West Afr. J. Med. 30 (4), 268–272.

Michel, M.C., Barendrecht, M.M., 2008. Physiological and pathological regulation of the autonomic control of urinary bladder contractility. Pharmacol. Ther. 117 (3), 297–312.

Niwa, A., Miyazato, T., Murakami, N., Suzuki, A., 1981. Effect of cadmium on the isolated vas deferens from guinea-pig. Nihon Heikatsukin Gakkai Zasshi 17 (2), 53–58.

Papagiannis, I., Vezyraki, P., Simos, Y.V., Kontargiris, E., Giannakopoulos, X., Peschos, D., Sofikitis, N., Evangelou, A., Kalfakakou, V., 2019. Effects of secondary biological treatment plant effluent administration, as drinking water, to rats' urogenital system in relation to cadmium and lead accumulation. Environ. Sci. Pollut. Res. 26 (36), 36434–36440.

Parker, M.M., Humoller, F.L., Mahler, D.J., 1967. Determination of copper and zinc in biological material. Clin. Chem. 13 (1), 40–48.

Petkov, G.V., 2014. Central role of the BK channel in urinary bladder smooth muscle physiology and pathophysiology. Am. J. Physiol. Regul. Integr. Comp. Physiol. 307 (6), R571–R584.

Pfrimer, K., Micheletto, R.F., Marchini, J.S., Padovan, G.J., Moriguti, J.C., Ferriolli, E., 2014. Impact of aging on urinary excretion of iron and zinc. Nutr Metab Insights 7, 47–50.

Philyppov, I.B., Sotkis, G.V., Rock, A., Roudbaraki, M., Bonnal, J.L., Mauroy, B., et al., 2020. Alterations in detrusor contractility in rat model of bladder cancer. Sci. Rep. 10 (1), 19651.

Rahman, Z., Singh, V.P., 2019. The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. Environ. Monit. Assess. 191 (7), 419.

Reynolds, M.E., Ventre, K.M., 2007. Iron Overdose. In: Garfunkel, L.C., Kaczorowski, J.M., Christy, C. (Eds.), Pediatric Clinical Advisor. second ed. Mosby, Philadelphia, pp. 318–319.

Rius, R.A., Govoni, S., Trabucchi, M., 1986. Regional modification of brain calcium antagonist binding after in vivo chronic lead exposure. Toxicology 40 (2), 191–197.

Romaniuk, A., Sikora, V., Lyndin, M., Smiyanov, V., Sikora, V., Lyndina, Y., et al., 2017. The features of morphological changes in the urinary bladder under combined effect of heavy metal salts. Intervent. Med. Appl. Sci. 9 (2), 105–111.

Rosati, A.M., Traversa, U., 1999. Mechanisms of inhibitory effects of zinc and cadmium ions on agonist binding to adenosine A1 receptors in rat brain. Biochem. Pharmacol. 58 (4), 623–632.

Schena, G., Caplan, M.J., 2019. Everything You Always Wanted to Know about β(3)-AR * (* But Were Afraid to Ask). Cells 8 (4), 357.

Schnieden, H., Small, R.C., 1971. Spasmolytic effects of cadmium and zinc ions upon the guinea-pig isolated ileum preparation. Br. J. Pharmacol. 41 (3), 488–499.

Senbel, A.M., Saad, E.I., Taha, S.S., Mohamed, H.F., 2016. Different mechanisms for lead acetate, aluminum and cadmium sulfate in rat corpus cavernosum. Toxicology 340, 27–33.

Shelkovnikov, S.A., Gonick, H.C., 2001. Influence of lead on rat thoracic aorta contraction and relaxation. Am. J. Hypertens. 14 (9 Pt 1), 873–878.

Shino, H., 1976. Mechanism of the contracting actions of K, acetylcholine and Ba and of the antispasmodic actions of Cd and Mn in the pyloric antrum strip of the rat, with special reference to their relationship to Ca. Nihon Yakurigaku Zasshi 72 (1), 95–104.

Silva, I., Costa, A.F., Moreira, S., Ferreirinha, F., Magalhães-Cardoso, M.T., Calejo, I., et al., 2017. Inhibition of cholinergic neurotransmission by β(3)-adrenoceptors depends on adenosine release and A(1)-receptor activation in human and rat urinary bladders. Am. J. Physiol.-Renal Physiol. 313 (2), F388–f403.

Singh, Z., Chadha, P., Sharma, S., 2013. Evaluation of oxidative stress and genotoxicity in battery manufacturing workers occupationally exposed to lead. Toxicol. Int. 20 (1), 95–100.

Skoczynska, A., Martynowicz, H., 2005. The impact of subchronic cadmium poisoning on the vascular effect of nitric oxide in rats. Hum. Exp. Toxicol. 24 (7), 353–361.

Sulaiman, F.R., Hamzah, H.A., 2018. Heavy metals accumulation in suburban roadside plants of a tropical area (Jengka, Malaysia). Ecol. Processes 7 (1), 28.

Sutoo, D., Akiyama, K., Imamiya, S., 1990. A mechanism of cadmium poisoning: the cross effect of calcium and cadmium in the calmodulin-dependent system. Arch. Toxicol. 64 (2), 161–164.

Tampakoudis, P., Tantanassis, T., Grimbizis, G., Papaletsos, M., Mantalenakis, S., 1995. Cigarette smoking and urinary incontinence in women–a new calculative method of estimating the exposure to smoke. Eur. J. Obstet. Gynecol. Reprod. Biol. 63 (1), 27–30.

Tsao, D.A., Yu, H.S., Cheng, J.T., Ho, C.K., Chang, H.R., 2000. The change of beta-adrenergic system in lead-induced hypertension. Toxicol. Appl. Pharmacol. 164 (2), 127–133.

Vesela, R., Aronsson, P., Tobin, G., 2011. Functional and morphological examinations of P1A1 purinoceptors in the normal and inflamed urinary bladder of the rat. Auton. Neurosci. 159 (1–2), 26–31.

Vig, P.J., Bhatia, M., Gill, K.D., Nath, R., 1989. Cadmium inhibits brain calmodulin: in vitro and in vivo studies. Bull. Environ. Contam. Toxicol. 43 (4), 541–547.

Walsh, C.T., Harnett, K.M., 1986. Inhibitory effect of lead acetate on contractility of longitudinal smooth muscle from rat ileum. Toxicol. Appl. Pharmacol. 83 (1), 62–68.

World Health Organization. 2006. "The world health report : 2006 : working together for health" Geneva: World Health Organization.