RESEARCH PAPER

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The effects of co-exposure to caffeine and heavy metals on learning and oxidative stress in mice

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Caffeine, a widely consumed psychoactive substance, may affect the neurotoxicity of environmental toxins. The study aimed to investigate the effects of the combined administration of caffeine and heavy metal compounds (cadmium chloride and lead acetate) on brain tissue function, focusing on memory processes and oxidative stress parameters. Adult male Swiss mice were administered substances intraperitoneally for 10 days. A passive avoidance test evaluated long-term memory, while the Y-maze assessed spatial working memory. In addition, lipid peroxidation and glutathione levels, as well as superoxide dismutase, catalase, and acetylcholinesterase activity, were determined in mouse brains. The results showed that exposure to caffeine and cadmium or lead caused different neurotoxic effects. Unlike lead, cadmium interacted with caffeine in memory tests. Caffeine protected mice against cadmium-induced spatial working memory impairment. The combination of caffeine and cadmium impaired learning in the passive avoidance test. Cadmium and lead caused oxidative stress in the brain, and caffeine had a preventive effect against it. The study showed that co-exposure to caffeine and cadmium could affect learning and memory. The antioxidant activity of caffeine may play a protective role in cadmium-induced spatial memory impairment.

Key words: caffeine, heavy metals, learning, memory, oxidative stress, mice

INTRODUCTION

Caffeine (CAF; 1,3,7-trimethylxanthine), found in popular drinks such as coffee, tea, and energy drinks, is the most frequently consumed psychoactive drug globally (Temple et al., 2017). The effects of CAF, especially those improving concentration, memory, and physical performance, are behind the worldwide CAF consumption increase (Cappelletti et al., 2015). On the other hand, high doses of CAF cause numerous impairments related to the central nervous system. For example, animal studies have shown that, in addition to its pro-cognitive effect, high CAF doses can have an amnesic effect on some types of memory (Silva & Frussa-Filho, 2000; Dubroqua et al., 2015). CAF-induced effects at the cellular level may be due to adenosine receptor (A_1 and A_{2A}) antagonism, intracellular calcium storage mobilization, and phosphodiesterase inhibition (Cappelletti et al., 2015; van Koert et al., 2018). Additionally, CAF is an acetylcholinesterase (AChE) inhibitor and affects oxidative stress in the brain (Fabiani et al., 2018; Ikram et al., 2020).

Consumed CAF can interact pharmacologically with medications, with CAF-drug interactions described for antiepileptic drugs, for example (van Koert et al., 2018). CAF effects on the central activity of environmental toxins are also possible, with experimental studies in mice showing that CAF may affect pesticide neurotoxicity (Łukawski et al., 2021). Furthermore, recent research demonstrated that CAF attenuated cadmium (Cd)-induced oxidative stress, neuronal loss, synaptic dysfunction, and learning and cognitive deficits *in vitro* and *in vivo* (Khan et al., 2019).

Heavy metals such as Cd and lead (Pb) are widely recognized environmental toxins (Rahman et al.,

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2019). The primary anthropogenic sources of Cd are non-ferrous metal mining and refining, phosphate fertilizer production and application, fossil fuel combustion, and waste incineration and disposal (ATSDR, 2012). Plants and other organisms can acquire Cd from contaminated soil and water, which can then pass into the food supply (ATSDR, 2012), the principal source of Cd intake in the general population (Järup & Akesson, 2009). Occupational exposure primarily involves inhaling dust and fumes or incidental ingestion from contaminated hands (ATSDR, 2012).

Pb is primarily released into the environment through mining, ore processing, and Pb-acid battery recycling, with other sources of contamination including petrol, pipes, ammunition, pesticides, paint pigments, electronic waste, dyes, and ceramic glazes (Rahman et al., 2019). Pb exposure is predominantly oral in the general population, with a minor contribution from inhalation. However, inhalation exposures may be more significant in the workplace, depending on particle size. Furthermore, exposure to organic Pb compounds through the dermal route may be important in occupational settings (ATSDR, 2020).

Epidemiological and animal studies have shown the occurrence of neurological symptoms, including memory deficits, as a result of Cd or Pb exposure (Schwartz et al., 2005; Łukawski & Sieklucka-Dziuba, 2007; Khan et al., 2019; Wang et al., 2022; Zhu et al., 2023). Animal studies have also demonstrated that Pb or Cd intoxication leads to biochemical changes in the brain, such as oxidative stress (Gurer & Ercal, 2000; Khan et al., 2019). Considering that CAF has antioxidant properties and affects memory, this study investigated the effects of the combined administration of CAF and Cd or Pb on the function of brain tissue, with particular emphasis on memory processes and oxidative stress parameters in mice.

METHODS

Animals

The study used 144 adult male Swiss mice weighing 25-32 g (around eight-week-old) obtained from a licensed dealer (J. Kolacz, Warsaw, Poland). The animals were grouped in cages specially adapted for this purpose, with constant access to food and water, under standard laboratory conditions (a 12-hour light-dark cycle, room temperature [22 ± 2°C], and relative humidity [55 ± 10 %]). After a minimum seven-day adaptation to laboratory conditions, the animals were randomized into experimental groups (n=8/group), with mice from the same cage grouped together. The experiments were carried out between 08:00 and 15:00 hours under constant environmental conditions (temperature, lighting, and noise level), and each animal was used only once. Two mice were excluded from behavioral testing due to very low weight, and no mice died during the experiments. The Local Ethics Committee at the University of Life Sciences in Lublin approved all experimental procedures (license no.: 10/2019), which complied with the EU Directive 2010/63/EU for animal experiments.

Substances

Cd chloride ($CdCl_2 \cdot 2½H_2O$, Chempur, Piekary Śląskie, Poland), Pb acetate ($(CH_3COO)_2 \cdot 3H_2O$, Chempur, Piekary Śląskie, Poland), and caffeine-sodium benzoate (Sigma-Aldrich, MO, USA) were used in this study. The heavy metals and CAF were dissolved in saline (0.9% NaCl) and administered intraperitoneally (IP) (5 ml/kg body weight) for 10 days. Mice not exposed to heavy metals and/or CAF received an equivalent volume of saline. Heavy metals doses did not exceed 5% of the median lethal dose (LD_{50}), based on previous publications using Cd chloride and Pb acetate in Swiss mice (Łukawski et al., 2005; 2007). Substances were used at 10–20 mg/kg (CAF), 0.035–0.7 mg/kg (Cd chloride), and 29.3 mg/kg (Pb acetate), doses which were applied in earlier studies (Nehlig et al., 1992; Łukawski et al., 2005; 2007). Propionylthiocholine iodide (PTC), eserine (physostigmine salicylate salt), and 5.5-dithiobis-2-nitro-benzoic acid (DTNB) were purchased from Merck (Merck Life Sciences, Darmstadt, Germany). All substances used were of the highest available chemical purity.

Passive avoidance task

A single-trial step-through passive avoidance test assessed the effects of prolonged treatment (10 days) with the test substances on long-term memory (Maurice et al., 2019; Łukawski et al., 2024). On the 10th day, 30 minutes after heavy metal, CAF, or a combination of heavy metals and CAF injections, animals were individually placed in an illuminated box (12 × 20 × 15 cm) connected *via* a 4 × 7 cm doorway to a dark box (24 × 20 × 15 cm) with an electric grid floor linked to a generator. When a mouse entered the dark box, it was immediately subjected to an electric foot shock (0.3 mA for 3 seconds) and removed from the box. The next day (24 hours later), a retention test was conducted in which the same mice (without any treatment) were put into the illuminated box, and the time taken (latency) to enter the dark box was recorded. The exper-

iment ended when the mouse entered the dark box or after 300 seconds of observation. Animals that avoided the dark box for 300 seconds were considered to have remembered the task. After the retention test, mouse brains were collected for biochemical assays (oxidative stress parameters and AChE activity).

Y-maze

The Y-maze evaluated spatial working memory by recording spontaneous alternation behavior (Sarter, 1988). The Y-maze consisted of three 10 × 10 × 10 cm chambers, resembling the letter Y, connected by 4 cm long corridors so that each corridor was connected to only one chamber. Mice tend to explore the maze by systematically entering each arm in turn. Before Y-maze testing, mice were injected with heavy metals and/or CAF for 10 days. On the 10th day, 30 minutes after the injections, the animals were placed separately in the maze for eight minutes. During this period, locomotor activity (total number of arm entries) and spontaneous alternation, defined as entries into the other arms of the maze without repetitions in overlapping triplet sets, were scored. The percent alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus 2 × 100). The ability to alternate means that the mouse knows which arm it has previously explored. Therefore, spontaneous alternation is a measure of spatial working memory (Sarter, 1988).

Oxidative stress parameters

Biochemical tests were performed on mouse brains collected after the passive avoidance test (n=7/treatment group). Commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical Company, MI, USA) measured lipid hydroperoxide as a marker of lipid peroxidation (LPO), reduced glutathione (GSH), and the activities of superoxide dismutase (SOD) and catalase (CAT) in mouse brains. All tests were carried out in accordance with the manufacturer's standard procedures. A BioTek ELX800 Absorbance Microplate Reader (BioTek Instruments, VT, USA) measured the absorbance of the samples.

Acetylcholinesterase activity

A modified version of Ellman's colorimetric method (Ellman, 1961) evaluated AChE activity. After being removed from the deep freezer, mouse brains were washed, rinsed with cooled phosphate buffer (pH 7.8), dried, and weighed. The fresh, non-frozen tissue was homogenized at a 1 g/5 ml ratio in ice-cold 0.05 M sodium phosphate buffer (pH 7.8). The sample (4 ml) was used to determine AChE activity after a 50 µl homogenate aliquot was added to a 20 ml buffer containing DTNB (10 mg/100 ml). The samples were then mixed with 20 mM PTC (50 µl). PTC was hydrolyzed by AChE to create thiocholine, which reacted with DTNB to form yellow 5-thio-2-nitrobenzoate. Subsequently, the samples were centrifuged at 1850 g for five minutes. AChE activity was measured by evaluating changes in absorbance, which are directly proportional to its activity, using a Specol 10 spectrophotometer (Carl Zeiss, Jena, Germany) at 412 nm. AChE measurements were repeated after pre-incubation with 50 µl 10 mM eserine for 15 minutes at 30°C.

Statistical analysis

Kruskal-Wallis's non-parametric analysis of variance (ANOVA), followed by Dunn's multiple comparisons, analyzed the passive avoidance test data. The data were non-parametric because the upper cut-off time was set, in contrast to the parametric data collected from the Y-maze. As such, one-way ANOVA and Tukey's *post hoc* test compared the results from the Y-maze. The levels and activities of oxidative stress parameters and AChE were analyzed using ANOVA and Tukey's test. Statistical evaluation employed GraphPad InStat and GraphPad Prism 8.0 software (GraphPad Software, CA, USA), with group differences considered statistically significant at $p<0.05$.

RESULTS

Passive avoidance task

The Kruskal-Wallis test revealed a significant overall group effect ($H=24.888$, $p=0.0008$). The combined prolonged IP administration of CAF (20 mg/kg) and Cd chloride (0.7 mg/kg) for 10 days impaired passive avoidance task acquisition ($p<0.05$; Dunn's test). This effect was not observed when mice were exposed to CAF and Cd at lower doses or when CAF (20 mg/kg) and Cd chloride (0.7 mg/kg) were given separately. Administration of Pb acetate (29.3 mg/kg) alone or in combination with CAF (20 mg/kg) did not significantly alter latency (Fig. 1). Fig. 1 presents all statistically significant between-group differences.

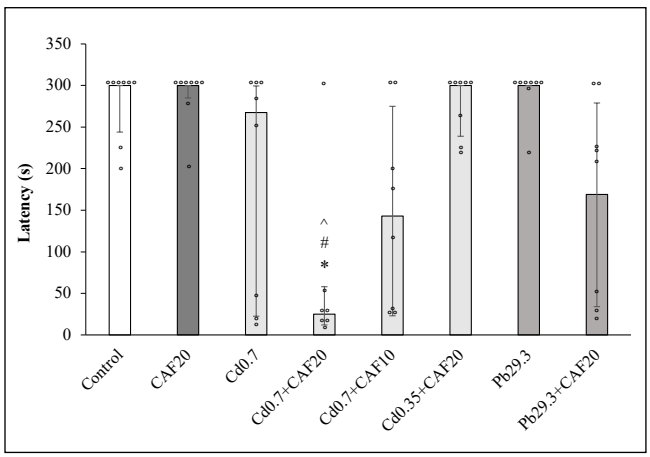

Fig. 1. The effects of prolonged heavy metal (0.35–0.7 mg/kg cadmium [Cd] and 29.3 mg/kg lead [Pb]) and caffeine (CAF 10 or 20 mg/kg) administration on the passive avoidance test in mice. All substances were given intraperitoneally (IP) for 10 days in eight-week-old mice. The number of mice used in this experiment was 64. Each group contained eight mice, except for the 0.7 mg/kg Cd plus 20 mg/kg CAF group (n=7; one mouse was excluded from testing). Data are shown as median values with the 25th and 75th percentiles (vertical lines). *p<0.05 *vs.* control; #p<0.05 *vs.* CAF 20; ^p<0.05 *vs.* Pb 29.3 (Dunn's test).

Y-maze

The ANOVA results from the Y-maze revealed overall group effects for alternation behavior ($F_{8,70}$=4.236; p=0.0003) and locomotor activity ($F_{8,70}$=10.233; p<0.0001). The prolonged IP administration of 0.7 mg/kg Cd chloride for 10 days impaired spontaneous alternation (p<0.001; Tukey's test, compared to the control group) (Fig. 2A) and had no effect on locomotor activity (Fig. 2B). At the lower dose of 0.035 mg/kg, Cd chloride did not cause memory impairment. No effect on spatial working memory was observed for prolonged Pb acetate intoxication with 29.3 mg/kg. CAF administered for 10 days at doses of 10 mg/kg and 20 mg/kg did not affect spontaneous alternation, but 20 mg/kg had a protective effect in mice given 0.7 mg/kg Cd (Cd 0.7 mg/kg + CAF 20 mg/kg *vs.* Cd 0.7 mg/kg, p<0.01; Tukey's test) (Fig. 2A). Moreover, 20 mg/kg CAF alone or in combination with Cd chloride (0.7 mg/kg) increased locomotor activity compared to the control group (p<0.001 for both groups; Tukey's test). In the remaining groups administered Cd chloride (0.035 mg/kg) alone, CAF (10 mg/kg) alone or combined with Cd (0.7 mg/kg), or Pb acetate (29.3 mg/kg) with or without CAF (20 mg/kg), there was no motor activity disturbances compared with the control group (Fig. 2B). All statistically significant between-group differences in alternation behavior and locomotor activity are shown in Fig. 2A and Fig. 2B, respectively.

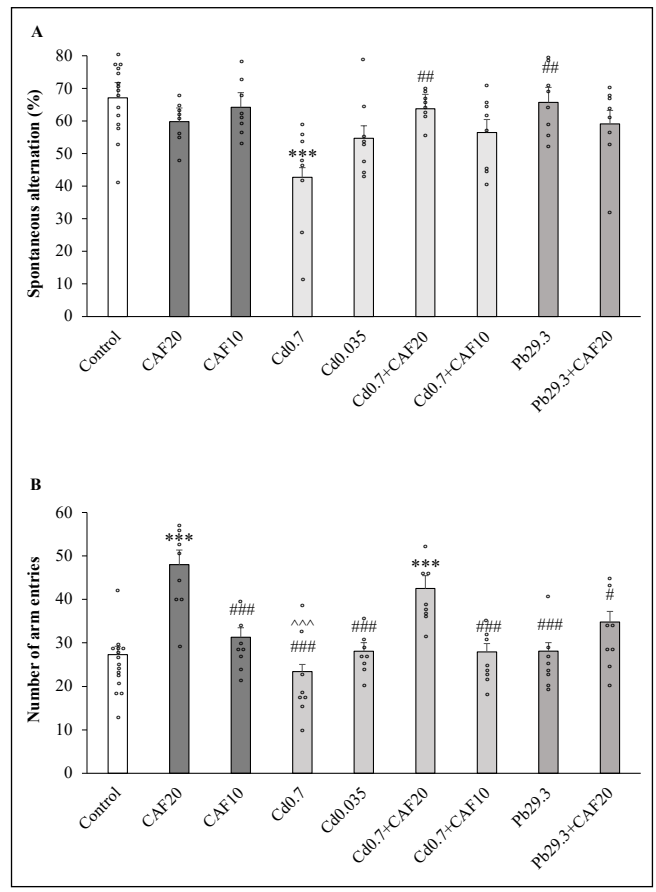

Fig. 2. The effects of prolonged heavy metal (0.35–0.7 mg/kg cadmium [Cd] and 29.3 mg/kg lead [Pb]) and caffeine (CAF 10 or 20 mg/kg) administration on spontaneous alternation (A) and locomotor activity (B) in the Y-maze in mice. All substances were injected intraperitoneally (IP) for 10 days in eight-week-old mice. The number of mice used in this experiment was 80. Data are expressed as the mean ± standard error of the mean (SEM). Each group contained eight mice, except the control group (n=15; one mouse was excluded from testing). ***p<0.001 *vs.* control; ##p<0.01 *vs.* Cd 0.7 (Fig. 2A; Tukey's test); ***p<0.001 *vs.* control; #p<0.05, ###p<0.001 *vs.* CAF 20; ^^^p<0.001 *vs.* Cd 0.7 + CAF 20 (Fig. 2B; Tukey's test).

Brain oxidative stress parameters

The ANOVA revealed overall group effects for LPO ($F_{5,36}$=8.703; p<0.0001) and GSH ($F_{5,36}$=4.048; p=0.0051) levels, as well as SOD ($F_{5,36}$=8.124; p<0.0001) and CAT ($F_{5,36}$=6.509; p=0.0002) activity. Compared to the control group, prolonged IP administration (10 days) of 0.7 mg/kg Cd chloride or 29.3 mg/kg Pb acetate increased LPO in brain tissue (p<0.01 and p<0.001, respectively; Tukey's test). This effect of Cd (p<0.01) and Pb (p<0.001) was also substantial when compared to the 20 mg/kg CAF group. Prolonged CAF (20 mg/kg) administration did not affect LPO but had a protective effect in Cd and Pb-exposed groups, as LPO levels did not differ from the control (Fig. 3A). The same effects of heavy

metals and CAF were observed on GSH levels, with Cd chloride (0.7 mg/kg) and Pb acetate (29.3 mg/kg) reducing GSH levels (p<0.05 and p<0.01, respectively). CAF (20 mg/kg) alone did not alter GSH but increased its level in the groups exposed to Cd and Pb (Fig. 3B).

Prolonged administration of 0.7 mg/kg Cd chloride or 29.3 mg/kg Pb acetate decreased SOD activity in the brain tissue (p<0.01 for both groups, compared to the control group). This effect was not observed in experimental groups receiving CAF (20 mg/kg) alone or CAF alongside heavy metals (Fig. 4A). Compared to the 20 mg/kg CAF group, four experimental groups showed marked changes (Fig. 4A). Cd chloride (0.7 mg/kg), injected subchronically, caused a decrease in CAT activity (p<0.001), which was not observed after the prolonged administration of CAF (20 mg/kg), Pb acetate (29.3 mg/kg), or heavy metals combined with CAF. This effect of Cd was also statistically significant compared to the CAF 20 mg/kg group (p<0.001) (Fig. 4B).

Acetylcholinesterase activity

The ANOVA revealed overall group effects for AChE activity ($F_{5,36}$=10.013; p<0.0001). As shown in Fig. 5, subchronic IP administration of Cd chloride (0.7 mg/kg) or Pb acetate (29.3 mg/kg) decreased brain AChE activity (p<0.01 and p<0.001, respectively). Reduced AChE activity was also detected in the mice receiving Pb acetate (29.3 mg/kg) with CAF (20 mg/kg) (p<0.01). CAF (20 mg/kg) administration increased AChE activity in the 0.7 mg/kg Cd group. However, CAF alone did not affect AChE activity (Fig. 5).

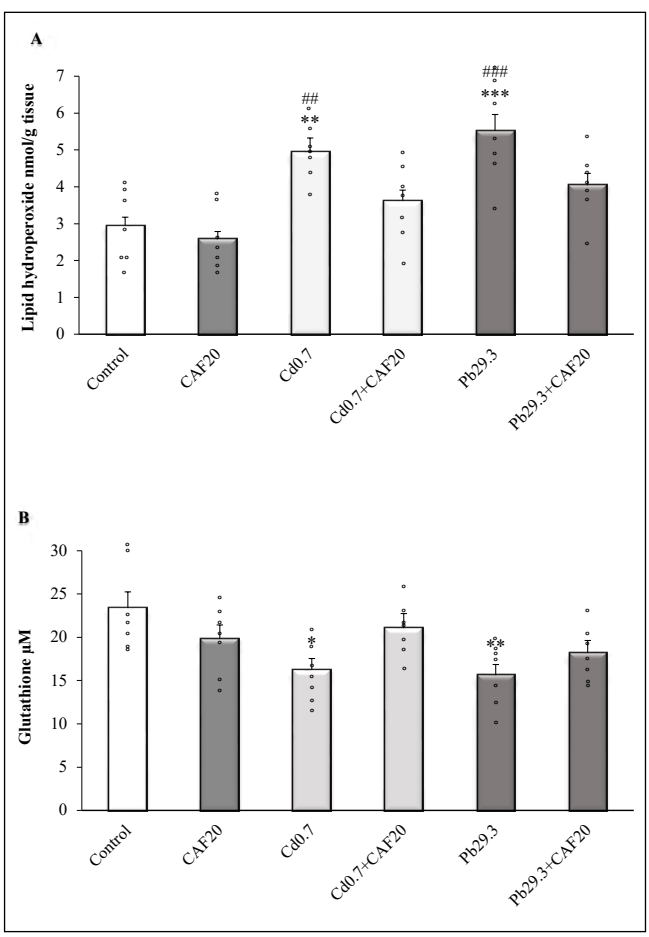

Fig. 3. The effects of prolonged heavy metal (0.35–0.7 mg/kg cadmium [Cd] and 29.3 mg/kg lead [Pb]) and caffeine (CAF 10 or 20 mg/kg) administration on levels of lipid hydroperoxide (A) and reduced glutathione (B) in mouse brains. Results are shown as the mean ± standard error of the mean (SEM) of seven measurements. ***p<0.001, **p<0.01, *p<0.05 *vs.* respective control group; ###p<0.001, ##p<0.01 *vs.* CAF 20 (Tukey's test).

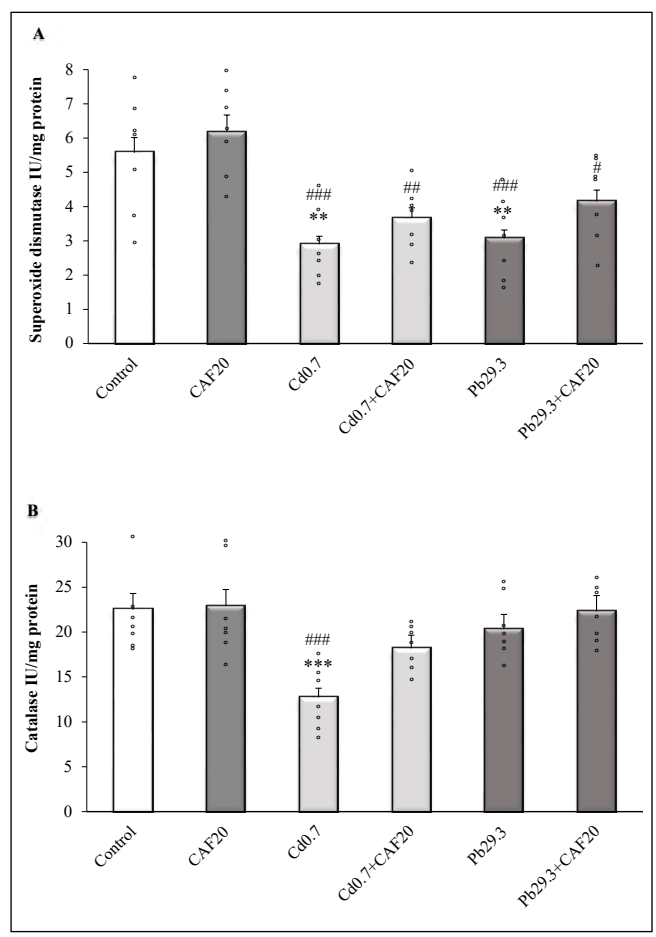

Fig. 4. The effects of prolonged heavy metal (0.35–0.7 mg/kg cadmium [Cd] and 29.3 mg/kg lead [Pb]) and caffeine (CAF 10 or 20 mg/kg) administration on the activity of superoxide dismutase (A) and catalase (B) in mouse brains. Data are presented as the mean standard error of the mean (SEM) of seven measurements. ***p<0.001, **p<0.01 *vs.* respective control group; ###p<0.001, ##p<0.01, #p<0.05 *vs.* respective CAF 20 group (Tukey's test).

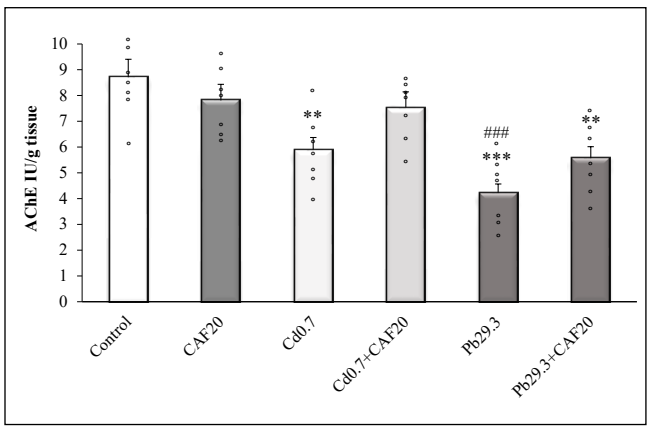

Fig. 5. The effects of prolonged heavy metal (0.35–0.7 mg/kg cadmium [Cd] and 29.3 mg/kg lead [Pb]) and caffeine (CAF 10 or 20 mg/kg) administration on brain acetylcholinesterase (AChE) activity in mice. Results are shown as the mean ± standard error of the mean (SEM) of seven measurements and expressed in IU/g of wet brain tissue. ***p<0.001, **p<0.01 *vs.* control; ###p<0.001 *vs.* CAF 20 (Tukey's test).

DISCUSSION

The current study used CAF doses capable of causing behavioral effects in rodents and interacting with the pharmacological effects of other substances (Nehlig et al., 1992; Łukawski et al., 2021). The main findings are that simultaneous exposure to CAF and Cd (not Pb) affects learning and memory, and CAF antioxidant action may protect against Cd-induced spatial memory impairment.

IP Cd administration can cause different behavioral alterations in rodents, including memory deficits (Lamtai et al., 2018; Khan et al., 2019). In the current experiment, prolonged Cd intoxication (0.7 mg/kg) impaired spontaneous alternation in the Y-maze without disturbing the locomotor activity of mice. However, there was no effect of Cd administered alone on learning in the passive avoidance test, which could imply that spatial working memory is more vulnerable to Cd intoxication than long-term memory. Spontaneous alternation impairments in the Y-maze induced by the administration of Cd compounds have also been reported by other authors (Oboh et al., 2020; Lamtai et al., 2021; Yang et al., 2022).

Pb exposure may impair human learning and memory processes (Schwartz et al., 2000). Animal studies confirm that Pb has strong neurotoxic properties, with Pb exposure in rodents causing numerous behavioral alterations, including memory impairment in the passive avoidance task (Rashno et al., 2022) and the Y-maze (Wang et al., 2024). Pb acetate (and Cd chloride) was used at a 5% LD_{50} dose, which was based on our earlier studies (Łukawski et al., 2005; 2007). The passive avoidance test revealed no effect of Pb acetate on learning. Furthermore, unlike Cd intoxication, Pb treatment did not alter spatial working memory in the Y-maze. These results suggest that when Pb and Cd are administered at similar concentrations (5% of their respective LD_{50} values), Pb demonstrates less toxicity on spontaneous alternation in the Y-maze.

However, when it comes to heavy metal effects on oxidative stress, this was not the case. Indeed, subchronic administration of both metals caused oxidative stress in mouse brains, but Pb produced more intense LPO and GSH reduction in the brain tissue. This finding suggests that mechanisms other than oxidative stress may be responsible for the spontaneous alternation impairment induced by Cd, as the impairment was not observed after Pb administration. Furthermore, it is hypothesized that CAT rather than SOD is more affected when comparing Cd-induced and Pb-induced oxidative stress in the brain. In addition, prolonged Pd intoxication led to greater AChE activity inhibition than Cd administration. Since Pb intoxication did not impair spatial working memory, the role of AChE in Cd-induced memory impairment seems to be of little importance.

The potential role of oxidative stress in memory impairment induced by Cd or Pb was presented in previous studies. Lamtai et al. (2021) reported that adult male and female rats exposed to chronic IP Cd exhibited impaired spontaneous alternation in the Y-maze. This was associated with a significant increase in LPO (thiobarbituric acid reactive substances [TBARS] level) and decreases in SOD and CAT activities in the hippocampus. Our findings in adult male mice are consistent with those reported by Lamtai et al. (2021). Also, similar to our results, Naïla et al. (2021) reported that Pb-induced oxidative stress in the rat hippocampus, as demonstrated by an increase in LPO and a reduction in SOD, was not accompanied by alternation behavior impairment in the Y-maze. On the other hand, impaired spontaneous alternation after Pb exposure was reported in other studies in rodents (Zhou et al., 2020). As such, it is thought that the effects of Pb in memory tests are dependent on the metal concentration and the duration and stage of exposure (Naïla et al., 2021). However, based on the results obtained, using comparable doses of Pb and Cd (5% LD_{50}) does not always result in behavioral effects after exposure to each metal.

One of the purposes of the current study was to examine the effects of CAF on the neurotoxic effects of Cd and Pb in mouse memory tests. The effects of CAF on the central nervous systems of humans and animals appear to be dependent on the amount consumed or used. Generally, consuming CAF in moderate amounts

can be beneficial for the human body. However, the higher the dose of CAF, the greater the likelihood of developing neurological symptoms. Consuming moderate amounts of CAF, for example, reduces the feeling of fatigue, improves physical, motor, and cognitive performance, and increases alertness and concentration (Glade, 2010). Although CAF is commonly believed to improve learning and memory, increasing evidence suggests that it does not have a procognitive effect on all types of memory in animals and may even cause impairment (Angelucci et al., 1999). In the passive avoidance test, CAF affects different stages of memory processing, and it has been suggested that dosing range from 10–100 mg/kg may impair learning when administered before a training session. On the other hand, CAF may improve memory consolidation (0.25-30 mg/kg) and recall (3-10 mg/kg) in mice (Angelucci et al., 1999; Dubroqua et al., 2015). Other studies have shown that CAF (20-46.2 mg/kg) does not affect learning in the passive avoidance test (Chrościńska-Krawczyk et al., 2009; Sanday et al., 2013). Similarly, subchronic CAF administration (20 mg/kg) did not impair learning in the current experiment. In previous Y-maze studies, CAF improved (Cox, 1970) or had no impact (Dall'Igna et al., 2003) on spontaneous alternation in animals. Dall'Igna et al. (2003) reported that acute IP CAF treatment (30 mg/kg) and chronic administration for seven days in a drinking solution (1 mg/ml) did not impair alternation behavior in mice. Similarly, in the current study, prolonged IP CAF (10-20 mg/kg) had no effect on spatial working memory in the Y-maze. However, mice showed an increase in locomotor activity after receiving CAF at a dose of 20 mg/kg. It is believed that the stimulating effect of CAF on locomotor activity is mediated by the blockade of ventral and dorsal striatum adenosine A_{2A} receptors (El Yacoubi et al., 2000).

Despite the lack of effect of CAF on learning in the passive avoidance test and spontaneous alternation in the Y-maze, CAF administered together with Cd caused behavioral effects in these tests. Specifically, the combined administration of both compounds impaired learning in the passive avoidance test, and CAF was protective against Cd-induced spatial working memory impairment in the Y-maze. In the current study, we used doses of Cd and CAF that do not impair learning in the passive avoidance task and spontaneous alternation in the Y-maze, administered as single doses 30 min before testing. The lack of effect of acute doses was documented in a preliminary study (results not shown) and previous reports (Łukawski et al., 2005). Therefore, the observed findings are long-term effects of exposure to Cd and CAF.

CAF had a protective action against oxidative stress induced by Cd and Pb, though no effects were observed in memory tests after the combined administration of CAF and Pb. The data from the Y-maze are consistent with the results of Khan et al. (2019), in which chronic CAF administration (30 mg/kg) rescued Cd (5 mg/kg)-induced oxidative stress-mediated memory impairment in the Y-maze in mice. The neuroprotective action of CAF against Cd-induced neurotoxicity was also demonstrated in the Morris water maze (Khan et al., 2019). Since reduced latency was only observed in the mice administered 0.7 mg/kg Cd and 20 mg/kg CAF in the passive avoidance test, it cannot be ruled out that the observed effect is task-dependent. As such, it would be of interest to conduct a similar experiment using an analogous behavioral task.

In summary, our study supports the suggestion that CAF might be an antioxidant and neuroprotective agent against Cd-induced impairment in spatial memory (Khan et al., 2019). The opposite effect of CAF in the passive avoidance test (impaired learning after combined administration with Cd) may result from the different effects of CAF in this test compared to the Y-maze. As mentioned earlier, CAF may impair learning in the passive avoidance test. Therefore, it may potentiate the neurotoxic effect of Cd on passive avoidance behavior (Goncalves et al., 2010), which could lead to the development of the impairment. Further research is required to clarify the mechanisms of this phenomenon. However, it is interesting that the impairment occurred despite the antioxidant properties of CAF and its inhibition of Cd-induced oxidative stress.

CONCLUSIONS

Simultaneous exposure to heavy metals (Cd and Pb) and CAF may provide different effects on cognition. Unlike Pb acetate, Cd chloride combined with CAF influenced memory in mice exposed to similar doses (5% of LD_{50} value). Furthermore, the current study confirms prior findings that CAF protects against Cd-induced spatial memory impairments, but (as shown here) it may have an amnesic effect in combination with Cd on other types of memory (long-term memory). CAF protects against oxidative stress induced by Cd or Pb, which may protect against Cd-induced spatial working memory impairment.

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