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The Effect of Lead (Pb) on Mice's Parathyroid Gland's Regulation of Calcium Level

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Abstract:

This study will use biochemical markers that include parathyroid hormone (PTH) levels, calcium-sensing receptor (CaSR) expression, Vitamin D levels, and glutathione (GSH) activity to study the effects of lead (Pb) exposure on mice. We can understand how Pb affects calcium regulation, oxidative stress response, and calcium homeostasis using these biomarkers. The experiment is conducted using ELISA, flow cytometry (FACS), and GSH assays with mice exposed to Pb. Our results suggest that lead exposure can lead to several outcomes: biomarkers are affected significantly, biomarkers are partially affected with some unchanged, and biomarkers are completely unaffected. These results are relevant to real world environmental health crises like the Flint water crisis because Pb contamination led to health problems. The goal of understanding the mechanisms of Pb toxicity is to help inform people to mitigate the negative effects of Pb exposure.

Keywords: Lead (Pb) Exposure, Parathyroid Hormone (PTH), Calcium-Sensing Receptor (CaSR), Vitamin D, Glutathione (GSH) Activity, Oxidative Stress, Flint Water Crisis, Calcium Homeostasis.

1. Introduction

The Flint Water Crisis is considered to be one of the most devastating environmental contamination events happened in United States. In April 2014, the city of Flint in Michigan switched its drinking water supply from Lake Huron to the Flint River[1]. However, due to the failure of treating water properly, systemic lead contamination—revealed through a collaborative sampling effort by local activists and engineers in the summer of 2015—resulted from corrosive water eating into lead pipes and other lead-bearing components of Flint's water infrastructure. Studies have found that the abnormal lead (Pb) level in the Flint water supply has caused elevated blood Pb levels increased from 2.4% to 4.9% ($P < .05$) after water source change, and neighborhoods with the highest water lead levels experienced a 6.6% increase after the water supply switched[2].

Lead (Pb) is a heavy metal toxin for both humans

and animals and has high affinity for biological tissues that are rich in calcium such as bones and teeth[3]. It has a molecular weight of 207.2 Da and often is expressed as Pb^{2+} in biological systems. Lead is highly toxic to the nervous system, kidneys, and cardiovascular system and is able to disrupt enzyme function, neurotransmitter release, and cellular signaling pathways[4]. However, it also has a significant impact on parathyroid gland since it is often overlooked and the effect caused by Pb is credited to the dysfunction of thyroid gland. The parathyroid glands can be affected by Pb exposure due to the metal's interaction with calcium signaling pathways and thus disrupting the calcium homeostasis. Disruption of parathyroid function can lead to dysregulated calcium levels and results to hyperparathyroidism or hypocalcemia. Although more focus has been on neurological and renal diseases during the Flint water Crisis, there is a growing concern about the potential impact on parathyroid function and calcium homeostasis among the affected population[5]. Lead exposure is known to disrupt calcium homeostasis and can affect bone metabolism[6], which might indirectly affect the parathyroid glands since they play an important role in regulating calcium levels in the blood. Some studies suggest that lead exposure could affect bone turnover and calcium metabolism, which is relating to parathyroid hormone (PTH) secretion and function[7]. However, there is only limited research on how lead exposure affects the parathyroid. Hence, the study aims to see whether Pb induces a disruption in calcium regulation and resulted in more parathyroid hormone (PTH) secretion(PTH Increase calcium level by acting on bones, kidneys, and intestines); interference on the expression of calcium-sensing receptors (CaSR)(CaST Inhibits the release of PTH); decreased vitamin D levels(Vitamin D Converts to Calcitriol to maintain calcium level); and decreased GSH activity(GSH is an antioxidant and protect body from oxidative stress), which is an antioxidant that serves as marker of oxidative stress.

Hypothesis

I predict that increasing concentrations and treatment durations of lead exposure in mice increases parathyroid hormone (PTH), decreased expression of calcium-sensing receptors
(CaSR) leading to decreased vitamin D levels and decreased GSH.

2. Methods

Toxins

Lead(Pb) needs to be prepared by dissolving lead acetate ($Pb(C_2H_3O_2)_2$) in distilled water to achieve the desired concentration.

Animals

Male albino mice weighing 30-35g were obtained from the laboratory

PTH Measurement by ELISA

Serum samples were collected from mice subjected to: lead exposure (0.1, 0.5, 1.0 ppm in drinking water for 1, 2, 4 weeks), no lead exposure, and Parathyroidectomized (PTX) mice treated with recombinant PTH (10-80 ng/kg body weight subcutaneously, serum collected after 1-2 hours). The serum samples were diluted according to the ELISA kit instructions. The ELISA plate was coated with the capture antibody (if not pre-coated), and 50-100 µL of standards, controls, and samples were added to the appropriate wells. The well is then incubated at room temperature for 1-2 hours. The wells were washed 3-5 times with the provided wash buffer before the detection antibody was added and incubate it again. After color change occurred, the reaction was stopped with the stop solution and absorbance was measured at 450 nm[8]. The positive control was serum from PTX mice treated with recombinant PTH. Negative control was serum from untreated mice (no lead exposure). Each of the treatment conditions was repeated with three separate experiments. All treatment groups were compared with the control group. Two-tailed P values were calculated through paired T-tests and P value< 0.05 was considered statistically significant.

CaSR Surface Expression by FACS

Cells were isolated from the tissues of mice subjected to: lead exposure (0.1, 0.5, 1.0 ppm in drinking water for 1, 2, 4 weeks), untreated negative controls, and positive controls treated with 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3) at 10 nM for 24-48 hours to upregulate CaSR expression. The isolated cells were washed with PBS and resuspended in FACS buffer. The cells were then incubated with fluorescently labeled anti-CaSR antibodies for 30-60 minutes on ice or at 4°C in the dark. The well is then washed with FACS buffer to remove unbound antibodies. If necessary, cells were fixed before FACS analysis. The stained cells were analyzed using a flow cytometerby recording the fluorescence intensity and comparing it across the different treatment groups[9]. The positive control was cells treated with 1α,25(OH)2D3. Negative control was cells from untreated mice. Each of the treatment conditions was repeated with three separate experiments. All treatment groups were compared with the control group. Two-tailed P values were calculated through paired T-tests and P value< 0.05 was considered statistically significant.

GSH Activity by GSH Assay Kit

Tissues were homogenized or cell lysates were prepared from mice treated with: lead exposure (0.1, 0.5, 1.0 ppm in drinking water for 1, 2, 4 weeks), untreated negative

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controls, and positive controls treated with N-acetylcysteine (NAC) at 1-10 mM for 24 hours to increase GSH levels. The samples were centrifuged to obtain the supernatant. GSH standards were prepared according to the kit instructions, and 50-100 µL of standards, controls, and samples were added to the microplate. The reaction mix was added before the plate was incubated at room temperature for 15 minutes. Absorbance was measured at the wavelength of 405-420 nm[10]. The positive control was samples treated with NAC (N-Acetyl Cysteine). Negative control was samples from untreated mice. Each of the treatment conditions was repeated with three separate experiments. All treatment groups were compared with the control group. Two-tailed P values were calculated through paired T-tests and P value< 0.05 was considered statistically significant.

Vitamin D Measurement by ELISA

Serum was collected from mice treated with: lead exposure (0.1, 0.5, 1.0 ppm in drinking water for 1, 2, 4 weeks), untreated negative controls, and positive controls treated with high doses of Vitamin D (1000-2000 IU/day for 1-2 weeks). The serum samples were diluted according to the ELISA kit instructions. The ELISA plate was coated with the capture antibody (if not pre-coated), and 50-100 µL of standards, controls, and samples were added to the appropriate wells. The well is then incubated at room temperature for 1-2 hours. The wells were washed 3-5 times with the provided wash buffer before the detection antibody was added and the wells were incubated again. After color change occurred, the reaction was stopped with the stop solution and absorbance was measured at 450 nm[8]. The positive control is serum from mice treated with high doses of Vitamin D. Negative control is serum from untreated mice. Each of the treatment conditions was repeated with three separate experiments. All treatment groups were compared with the control group. Two-tailed P values were calculated through paired T-tests and P value< 0.05 was considered statistically significant.

3. Results

CR1: If I obtain this result, I would see that lead exposure increases PTH levels, decreases CaSR surface expression, decreases Vitamin D levels, and decreases GSH levels. This result fully supports the hypothesis that lead exposure affects calcium homeostasis, Vitamin D metabolism, and antioxidant defenses.

CR2: If I obtain this result, I would see that lead exposure increases PTH levels, decreases CaSR expression and Vitamin D levels, and no change in GSH levels. This result partially supports the hypothesis and suggests that while lead affects calcium regulation, its impact on antioxidant defenses might not be true or requires more complex study.

CR3: If I obtain this result, I would see that lead exposure increases PTH levels, decreases CaSR expression and GSH levels, and no change in Vitamin D levels. This result partially supports the hypothesis and suggests that while lead affects PTH, CASR expression, and oxidative stress, its impact on Vitamin D might not be true or requires more complex study.

CR4: If I obtain this result, I would see that lead exposure increases PTH levels, decreases Vitamin D and GSH levels, and no change in CaSR expression. This result partially supports the hypothesis and suggests that while lead affects PTH, Vitamin D, and oxidative stress, its impact on CaSR expression might not be true or requires more complex study.

CR5: If I obtain this result, I would see that lead exposure decreases CaSR expression, Vitamin D, and GSH levels, and no change in PTH levels. This result partially supports the hypothesis and suggests that while lead affects CaSR expression, Vitamin D, and oxidative stress, its impact on PTH levels might not be true or requires more complex study.

CR6: If I obtain this result, I would see that lead exposure increases PTH levels, decreases CaSR expression, and no change in Vitamin D or GSH levels. This result partially supports the hypothesis and suggests that while lead affects PTH levels and CaSR expression, its impact on Vitamin D metabolism and antioxidant defenses might not be true or requires more complex study.

CR7: If I obtain this result, I would see that lead exposure increases PTH levels, decreases GSH levels, and no changes in CaSR expression or Vitamin D levels. This result partially supports the hypothesis and suggests that while lead affects PTH levels and antioxidant defenses, its impact on Vitamin D metabolism and CASR expression might not be true or requires more complex study.

CR8: If I obtain this result, I would see that lead exposure decreases Vitamin D and GSH levels, but no change in PTH levels and CaSR expression. This result partially supports the hypothesis and suggests that while lead affects Vitamin D metabolism and antioxidant defenses, its impact on calcium homeostasis might not be true or requires more complex study.

CR9: If I obtain this result, I would see that lead exposure increases PTH levels, decreases Vitamin D levels, and no change in CaSR expression or GSH levels. This result partially supports the hypothesis and suggests that while lead affects PTH levels and Vitamin D metabolism, its impact on CaSR expression and antioxidant defenses might not be true or requires more complex study.

CR10: If I obtain this result, I would see that lead expo-

sure decreases CaSR expression and GSH levels, and no change in PTH and Vitamin D levels. This result partially supports the hypothesis and suggests that while lead affects CaSR expression and antioxidant defenses, its impact on PTH levels and Vitamin D metabolism might not be true or requires more complex study.

CR11: If I obtain this result, I would see that lead exposure decreases CaSR expression and Vitamin D levels, and no change in PTH or GSH levels. This result partially supports the hypothesis and suggests that while lead affects CaSR expression and Vitamin D metabolism, its impact on PTH levels and antioxidant defenses might not be true or requires more complex study.

CR12: If I obtain this result, I would see that lead exposure increases PTH levels and no change in CaSR expression, Vitamin D, or GSH levels. This result partially supports the hypothesis and suggests that while lead affects PTH levels, its impact on CaSR expression, Vitamin D metabolism, and antioxidant defenses might not be true or requires more complex study.

CR13: If I obtain this result, I would see that lead exposure decreases CaSR expression, and no change in PTH, Vitamin D, or GSH levels. This result partially supports the hypothesis and suggests that while lead affects CaSR expression, its impact on PTH levels, Vitamin D metabolism, and antioxidant defenses might not be true or requires more complex study.

CR14: If I obtain this result, I would see that lead exposure decreases Vitamin D levels, and no change in PTH levels, CaSR expression, or GSH levels. This result partially supports the hypothesis and suggests that while lead affects Vitamin D metabolism, its impact on calcium homeostasis and antioxidant defenses might not be true or requires more complex study.

CR15: If I obtain this result, I would see that lead exposure decrease GSH levels, and no change in PTH levels, CaSR expression, or Vitamin D levels. This result partially supports the hypothesis and suggests that while lead affects antioxidant defenses, its impact on calcium homeostasis and Vitamin D metabolism might not be true or requires more complex study.

CR16: If I obtain this result, I would see that lead exposure had no effect on PTH levels, CaSR expression, Vitamin D, or GSH levels. This result fully contradicts the hypothesis and suggests that lead exposure under the tested conditions does not affect calcium homeostasis, Vitamin D metabolism, or antioxidant defenses.

Possible Results for Concentration and Treatment Duration

PTH by ELISA

Higher lead concentrations (closer to 1.0 ppm) may lead to greater increase in PTH levels. Lower concentrations might show no change. Longer treatment durations (4 weeks) are likely to show greater increase in PTH, while shorter duration may show a reduced response or no change.

CaSR by FACS

Higher lead concentrations may lead to a greater decrease in CaSR surface expression. Lower concentrations might show no change. Longer treatment durations are likely to show greater decrease in CaSR expression, while shorter duration may show a reduced response or no change.

Vitamin D by ELISA

Higher lead concentrations may lead to a greater decrease in Vitamin D levels. Lower concentrations might show no change. Longer treatment durations are likely to show greater decrease in Vitamin D levels, while shorter duration may show a reduced response or no change.

GSH by GSH Assay Kit

Higher lead concentrations may lead to a greater decrease in GSH levels. Lower concentrations might show no change. Longer treatment durations are likely to show greater decrease in GSH levels, while shorter duration may show a reduced response or no change.

Table 1: Effects of Lead (Pb) on Various Biological Markers and Hypothesis Support in Combination Results (CR#)

Combination Result # (CR#)					
	Pb increases PTH by ELISA	Pb decreases surface CaSR by FACS	Pb decreases vitamin D by ELISA	Pb decreases GSH by GSH assay kit	Support of hypothesis
1	+	+	+	+	Full
2	+	+	+	-	Partial
3	+	+	-	+	Partial
4	+	-	+	+	Partial
5	-	+	+	+	Partial
6	+	+	-	-	Partial
7	+	-	-	+	Partial

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8	-	-	+	+	Partial
9	+	-	+	-	Partial
10	-	+	-	+	Partial
11	-	+	+	-	Partial
12	+	-	-	-	Partial
13	-	+	-	-	Partial
14	-	-	+	-	Partial
15	-	-	-	+	Partial
16	-	-	-	-	Fully Contradicts

Table legend: "+" indicates alignments with positive control(increase in PTH, decrease in CaSR, Vitamin D, and GSH levels), "-" indicates no change

4. Discussion

Possible results 1,2,3,4,6,7,9,12 showcased an increased PTH levels and it is likely to suggest lead's ability to disrupt calcium regulation due to lead's interference with calcium absorption or bone resorption. A possible explanation is that it can mimic calcium in various biological processes but does not support normal calcium function and thus leading to disturbances in calcium homeostasis[11]. When calcium levels in the blood decrease (a condition known as hypocalcemia), the parathyroid glands secrete more PTH to help restore normal calcium levels. This is because PTH increases the release of calcium from bones and therefore enhances calcium absorption in the intestines as well as reduces calcium loss in the urine[12].

Possible results 1,2,3,5,6,10,11,13 showcased the decrease in CaSR surface expression and it is likely to suggest lead's ability to disrupt the parathyroid gland's ability to sense calcium levels. A possible explanation is that lead disrupts the cellular processes in the trafficking and stability of membrane proteins. This can lead to a reduction in the number of CaSR receptors on the cell surface[13]. Furthermore, lead can also affect the folding and processing of proteins within the endoplasmic reticulum to result in a decrease of CaSR surface expression since CaSR is a membrane protein that requires proper folding and trafficking to reach the cell surface[13].

Possible results 1,2,4,5,8,9,11,14 showcased a decrease in Vitamin D levels and it is likely to suggest lead's ability to disrupt Vitamin D metabolism. A possible explanation for this is that lead can disrupt the enzymatic processes in Vitamin D metabolism. Vitamin D is metabolized to its active form (calcitriol) in this process and the active Vitamin D level would be lowered if lead affects the expression of Vitamin D's metabolizing enzymes[14]. Another possible explanation is Lead's disruption with the gastrointestinal absorption of Vitamin D nutrients and caused the decrease in levels of vitamin D intake from outside of body and ultimately caused the failure to fulfill the body's needs[15].

Possible results 3,4,5,7,8,10,15 showcased a decrease in GSH levels and it is likely to suggest the role of oxidative stress in lead toxicity since GSH is an oxidative marker. A possible explanation is the causation of generating reactive oxygen species (ROS) by lead. The role of GSH is to help neutralize harmful molecules like ROS. The increased ROS would overwhelm the body's ability to regenerate GSH and thus leading to lower levels of GSH[16]. The decreased GSH level might also be caused by the interference with the synthesis of GSH by inhibiting the enzymes involved in its production like γ-glutamylcysteine synthetase[17]. This inhibition decreases the overall availability of GSH and thus inducing the oxidative stress.

The combination of result 1 indicate that lead exposure significantly disrupts calcium homeostasis and oxidative stress balance, which fully supports the hypothesis. Future experiments to further testify the hypothesis includes examining lead's impact on calcium signaling pathways and oxidative stress response genes.

The combination of results 2-15 partially supports the hypothesis. These combinations suggest that the effects of lead exposure might not be uniform across all markers (PTH, CaSR, Vitamin D, and GSH) or may be influenced by compensatory mechanisms, genetic mutations, or other mitigating factors. Future experiments should be designed to understand why certain pathways are more resilient or susceptible to lead exposure and examining factors like gene expression changes, enzyme activity, or involvement of secondary signaling pathways.

Combination of result 2 specifically support the hypothesis partially and indicates that Pb disrupts calcium regulation but not oxidative stress balance. One possible explanation of it is that the body may activate compensatory mechanisms to maintain GSH levels despite lead exposure. For instance, the upregulation of enzymes involved in GSH synthesis like γ-glutamylcysteine synthetase may not be affected by Lead and could help sustain GSH lev-

els even in the presence of oxidative stress induced by lead[17]. Another possible reason for this is the presence of other antioxidants in the system like superoxide dismutase (SOD) or catalase could mitigate the oxidative stress caused by lead and therefore not affecting GSH level since GSH might not be as heavily relied upon or depleted.

Combination of result 3 partially supports the hypothesis that Pb disrupts calcium regulation partially and the oxidative stress balance. One possible explanation for this could be that the body has strong homeostatic mechanisms to regulate vitamin D levels and thus has to adjust calcium absorption and renal conversion of vitamin D. For example, the body is able to mobilize calcium from the bones and reabsorb the filtrate of kidney before they are excreted in urine[18]. Combination of result 6 partially supports the hypothesis. It suggests that Pb disrupts calcium regulation almost entirely but not the oxidative stress balance. A possible explanation is that the body's reserves and dietary intake of vitamin D are sufficient and Vitamin D is readily available to convert to its active form Calcitriol. Calcitriol plays a role in helping antioxidant defense and would mitigate the depletion of GSH level thus GSH levels are also maintained. Despite this, Calcitriol also plays a role in helping antioxidant defense and would mitigate the depletion of GSH level thus GSH levels are also maintained. Vitamin D involves in the regulation of antioxidant enzymes and deploys antioxidant property via stimulating the expression of several antioxidant defense system molecules including Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Catalase (CAT), Peroxidase (POD), and suppressing the NADPH oxidase expression. Various studies have also shown that Vitamin D enhances the strength of the antioxidant defense system by increasing antioxidant capacity and controlling ROS[19]. However, the VDR is inhibited and Calcitriol is not able to promote calcium absorption therefore PTH and CaSR levels are not restored [20].

Combination of results 8 partially supports the hypothesis. It suggests that Pb disrupts the oxidative stress balance but not all the calcium regulation. A possible explanation for this is the presence of pro-inflammatory cytokines. Lead exposure can lead to inflammation and an increase in pro-inflammatory cytokines. Studies have found that pro-inflammatory cytokines are able to promote CaSR expression by activating the JAC-STAT pathway that targets the STAT1/3 and the Sp1/3 regulatory elements located in the first and second promoters. Due to the recovery of CaSR level, PTH level is controlled respectively[21].

Combination of result 14 partially supports the hypothesis. It suggests that Pb disrupts part of calcium regulation but not the oxidative stress balance. A possible explanation is the presence of 1α-hydroxylase. This enzyme is crucial in the synthesis of the active form of vitamin D (calcitriol). Calcitriol plays a significant role in calcium regulation by enhancing the absorption of calcium from the gut and modulating CaSR activity. If 1α-hydroxylase activity is maintained despite lead exposure, it can help sustain adequate levels of calcitriol, which in turn supports the regulation of CaSR and other parts of the calcium homeostasis. GSH level is maintained because Calcitriol could enhance the activities of several antioxidant enzymes by inducing the translocation of NRF2 to the nucleus, therefore GSH level is maintained.[22]

Combination of result 15 partially supports the hypothesis. It suggests that Pb disrupts oxidative stress balance only without affecting calcium regulation. A possible explanation that Pb target specifically on Vitamin D which impair the activity of key enzymes like 24-hydroxylase and cause the restoration of other biomarkers in return of the depletion of Vitamin D. 24-hydroxylase is responsible for the hydroxylation and subsequent degradation of both active vitamin D (calcitriol) and its precursors. This process helps to modulate the effects of vitamin D on calcium metabolism, ensuring that calcium levels in the blood remain within a narrow range. By degrading vitamin D, 24-hydroxylase helps balanced the calcium levels in the blood. This balance ensures that the CaSR levels can maintain appropriate calcium sensing and signaling which then helps regulate PTH level. Because the Calcitriol level is degraded and decreased, GSH level can no longer maintained.[23]

The combination of result 16 indicate that lead exposure has no effect on Calcium homeostasis and oxidative stress balance, which fully contradicts the hypothesis. Future experiments to further testify the result includes investigating whether other environmental toxins affect calcium homeostasis and oxidative stress balance in a way that Pb does not and if calcium homeostasis and oxidative stress balance can be affected by environmental toxins.

Discussion of concentration and treatment duration data

PTH by ELISA

Longer treatment durations (4-6 weeks) are likely to show greater increase in PTH because there is an ongoing stimulation of the parathyroid gland and thus affected more PTH. This would support the hypothesis that longer treatment affects the secretion of PTH and the Calcium metabolism. In future experiments, different dosages or combinations of treatment to modulate this response or investigation of the cellular mechanisms driving this increase should be explored. If the PTH levels show no change or only a slight increase, it suggests that the treatment duration is insufficient to cause a strong response. This would suggest that extension of experiment length is needed in order to cause a measurable effect. Future ex-

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periments might focus on understanding the time-dependent kinetics of PTH secretion or identifying the minimal treatment duration required for a measurable effect.

CaSR by FACS

Longer treatment durations are likely to show a decrease in CaSR expression. It suggests that downregulation as a response to prolonged stimulus is linked to homeostatic imbalances in calcium signaling. This would support the hypothesis that longer treatment affects calcium-sensing pathways. Future experiments could focus on the mechanism of this downregulation, perhaps looking at molecules other than calcium. If no significant change is observed in CaSR expression during shorter treatments, it would suggest that the treatment is either too brief to affect receptor expression or that CaSR is resistant to short-term modulation. Future experiments might increase the treatment frequency or combine shorter treatments with a higher concentration to assess the threshold at which CaSR expression begins to change.

Vitamin D by ELISA

Longer treatment durations are likely to show greater decrease in Vitamin D level because stimulation interferes with the synthesis or metabolism of Vitamin D. This would support the hypothesis that longer treatment affects the affects Vitamin D pathways. In future experiments, different dosages or combinations of treatment to modulate this response or investigation of the cellular mechanisms driving this decrease should be explored and other related biomarkers such as calcium and phosphate should be tested. If the Vitamin D levels show no change or only a slight increase, it suggests that the treatment duration is insufficient to cause a strong response or body's regulatory mechanisms are slow to respond. This would suggest that extension of experiment length is needed in order to cause a measurable effect. Future experiments might focus on increasing the concentration or frequency of treatment to determine how quickly Vitamin D levels can be influenced, or examine whether other hormonal or metabolic factors are buffering the Vitamin D response.

GSH by GSH assay kit

Longer treatment durations are likely to show greater decrease in GSH because the oxidative stress or cellular redox balance is being disrupted. This would support the hypothesis that longer treatment depletes antioxidant defenses over time. Future experiments could look at antioxidant supplementation as a way to mitigate this effect or investigate other markers of oxidative stress to see if they follow the same trend. If the GSH levels show no change or only a slight increase, it suggests that the oxidative stress may take time to accumulate, or that the body's natural antioxidant defenses are sufficient to counterbalance any early changes. This would suggest that extension of experiment length is needed in order to cause a measurable effect. Future experiments might focus on more frequent GSH monitoring or combine shorter durations with higher concentrations of treatment to understand the threshold at which oxidative stress begins to manifest.

5. Conclusion

In conclusion, this study explores the effect of lead (Pb) exposure on PTH levels, CaSR expression, Vitamin D levels, and GSH activity in mice. The results of this study will provide an idea on whether lead exposure can induce disruption in Vitamin D metabolism, oxidative stress, and disruption in calcium homeostasis. This study is relevant to environmental public health problems like the Flint water crisis where lead contamination led to human health issues. This study would be able to provide a suitable indication for future clinical and pharmaceutical development against lead poisoning and raise concerns about monitoring and regulation of lead levels in environment as well as daily bases.

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