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IN VITRO ASSESSMENT OF DIMERCAPTOSUCCINIC ACID AND CALCIUM DISODIUM EDTA ON VIABILITY AND FUNCTIONALITY OF ISLET CELLS EXPOSED TO LEAD TOXICITY

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Abstract

This study investigates the effect of Dimercaptosuccinic Acid (DMSA) and Calcium Disodium EDTA ($CaNa_2EDTA$) on viability and functionality of Islet cell-exposed to lead poison. Nine (9) mL of Islet cell culture media was divided into four (4) part and designated as Set 1 to 4 (n=3): Set 1 (Normal control): Received 1 mL of 0.1 % DMSO (vehicle); Set 2 (Negative control): Received 1 mL of 0.2 mg/dL of lead acetate in 0.1 % DMSO; Set 3 (DMSA): Received 0.9 mL of lead acetate (0.2 mg/dL) + 0.1 mL of 15 mg/mL DMSA after 1 hour; Set 4 ($CaNa_2EDTA$): Received 0.9 mL of lead acetate (0.2 mg/dL) + 0.1 mL of 15 mg/mL $CaNa_2EDTA$ after 1 hour. All the cell media were incubated for 24, 504 and 1008 hours, with glucose (3 mg) constantly added once per week. At the end of the experiment, glucose uptake and cell viability were assessed using a glucometer and haemocytometer counting of trypan blue-stained cells, respectively. Our results showed that 24 h Islet lead-exposed cell (at 0.2 mg/dL) can cause cell proliferation of 119,000±4.56 (cells/mL) healthy cells and 6,200±3.22 (cells/mL) death cells as well as impaired glucose uptake of 2.70±0.18 (mg/dL). These alterations were significantly mitigated when incubated with chelation therapies namely; DMSA and $CaNa_2EDTA$. Interestingly, $CaNa_2EDTA$ exhibited greater efficacy. In conclusion, this study suggests that lead poisoning impairs Islet cell viability and function, contributing to diabetes, while $CaNa_2EDTA$ chelation therapy shows promise in mitigating these effects.

Key words: In vitro; Pancreas; Islet cell; Viability; Functionality; Chelation therapy

1.0 introduction

In 2010, a devastating lead poisoning outbreak occurred in Zamfara State, Nigeria, resulting from illegal gold mining activities that released high concentrations of lead into the environment (MSF, 2012; WHO, 2010; Ekstrand *et al*., 2008). Investigation revealed that three hundred and fifty-five (355) villagers were exposed to lead through contaminated soil, water, and consumer products between March and June, leading to over one hundred and sixty-three (163) deaths, predominantly among children (CDC, 2010; WHO, 2010). In response, the Nigerian Federal Ministry of Health, in collaboration with the World Health Organisation (WHO), Centre for Disease Control (CDC) and Médecins Sans Frontières (MSF), developed an emergency medical protocol that included chelation therapy as the most effective treatment option for lead poisoning (Brown and Woolf, 2022; Udiba *et al*., 2019; MSF, 2012; CDC, 2010; WHO, 2010).

Chelation therapy, primarily using Calcium Disodium EDTA ($CaNa_2EDTA$) and Dimercaptosuccinic Acid (DMSA) is currently the best available option for treating lead poisoning, but it is not without adverse effects (Graziano, 2006; Wilhelm *et al*., 2006). For instance, its impact on glucose metabolism in treated patients remains unclear, and reports suggest that some chelation therapy may cause increased blood sugar levels due to damage to Islet cells responsible for insulin production, potentially leading to diabetes development (Lund *et al.*, 2014; Holzer *et al.*, 2012; Hornblad *et al.*, 2011; Hermansen *et al*., 2010). Therefore, it is crucial to consider the potential consequences of chelation therapy alongside its benefits before making treatment decisions, especially for individuals with a history of diabetes mellitus or those at risk of developing diabetes.

This study investigates the in vitro effect of Dimercaptosuccinic Acid (DMSA) and Calcium Disodium EDTA ($CaNa_2EDTA$) on viability and functionality of Islet cell-exposed to lead poison; providing valuable insights into metabolic consequences of this treatment approach in patients with lead toxicity.

2.0 Materials and methods

2.1 Chemicals and drugs

Calcium chloride anhydrous, RPMI 1640, Hanks Balance Salt Solution (HBSS), Fetal Bovine Serum (FBS), Collagenase Type V, Ethanol, Ficol and Lead acetate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA); Ketamine hydrochloride (Gujarat, India); Dimercaptosuccinic Acid (DMSA) and Calcium Disodium EDTA ($CaNa_2EDTA$) were from VEGIsci pharm; Trypan blue was purchased from Invitrogen, Company, USA; Penicillin, and Streptomycin were from MedChem Express, USA.

2.2 Experimental animals

Male Albino mice (18–20 g) were purchased from Central Animal Farm, Faculty of Basic Medical Sciences, University of Cross River State (UNICROSS), Okuku Campus, Nigeria. The experimental animals were housed in rodent facility at ambient condition with 12-hour day/night cycle and given feed and drinking water *ad libitum*. They were acclimatized for two weeks prior to experiment.

2.3 Pancreatic Islet cell isolation by perfusion and digestion

Pancreatic Islet cell was isolated by perfusion and digestion as described by Hornblad *et al.* (2011) and Carter *et al*., (2009). Animals were anaesthetised using ketamine (90 mg/kg). The pancreas tissue was exposed, and the ampulla was properly clamped. The pancreas was perfused with 3 mL of collagenase V in HBSS via the bile duct. The swollen pancreas was removed, placed in a glass vial, and allowed to digest at 37^0C for 30 minutes. The glass vial was rocked intermittently to disrupt the tissue into a homogeneous solution. After digestion, the content was transferred into a tube containing 25 mL of cold calcium chloride and HBSS solution. The mixture was centrifuged for 30 minutes at 1000 g. The resulting pellet was collected, re-suspended in the same solution, and centrifuged under the same conditions, yielding a solution of pancreatic Islet cells.

2.4 Islet purification and identification

Gradient separation of Islet was performed using the method described by Carter *et al*., (2009). Pancreatic Islet cells previously obtained were washed three times in a cold solution of $CaCl_2$ and HBSS, then filtered through a pre-wetted 70 µm cell strainer. The content was further transferred on top of a discontinuous gradient of 1.096, 1.069, and 1.039 g/mL. After centrifugation at 1800 g for 15 minutes, the Islets were isolated from the interfaces of 1.069 and 1.096 g/mL. The Islets were then transferred from one culture dish to another containing penicillin, streptomycin, FBS, and RPMI 1640 medium. For identification, an aliquot of the mixture was mixed with a drop of trypan blue, incubating for 5 minutes, and visualised under a microscope using a haemocytometer (Freshney, 2008).

2.5 Islet cell inhibition by lead acetate

Nine (9) mL of Islet cell culture containing penicillin, streptomycin, FBS and RPMI 1640 medium were introduced into four (4) separate petri dishes designated as Group A to D (n=3). Groups A to D received 1 mL each of lead acetate in 0.1 % (v/v) dimethyl sulfoxide (DMSO) at varying concentration of 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL, respectively. The cell media were incubated for 24 hours, and cell viability was assessed using trypan blue and haemocytometer counting.

2.6 Experimental protocol

The selected concentration of 0.2 mg/mL lead acetate, corresponding to its half-maximal inhibitory concentration Islet cells, balances its inhibition, viability and function. Nine (9) mL of Islet cell culture containing penicillin, streptomycin, FBS, and RPMI 1640 medium were divided into four (4) separate petri dishes designated as Set 1 to 4 (n=3) and treated as follows: Set 1 (Normal control): Received 1 mL of 0.1 % DMSO (vehicle); Set 2 (Negative control): Received 1 mL of lead acetate in 0.1 % DMSO; Set 3 (DMSA): Received 0.9 mL of lead acetate in 0.1 % DMSO, followed by 0.1 mL of 15 mg/mL DMSA after 1 hour; Set 4 ($CaNa_2EDTA$): Received 0.9 mL of lead acetate in 0.1 % DMSO, followed by 0.1 mL of 15 mg/mL $CaNa_2EDTA$ after 1 hour. All the cell media were incubated for 24, 504, and 1008 hours, with glucose (3 mg) constantly added once per week. At the end of the experiment, glucose uptake and cell viability were assessed using a glucometer and haemocytometer counting of trypan blue-stained cells, respectively.

2.7 Statistical analysis

The results are presented as mean ± standard deviation (SD). The data were analyzed using GraphPad Prism version 5.00 for one-way ANOVA (Analysis of variance) and the significant differences among the groups was compared by Duncan's multiple range tests at $P<0.05$ significance.

3.0 Results

The photomicrograph of Trypan Blue-stained Islet cells showing viable healthy cells and non-viable death cell was presented in Figure 1. The healthy cells remain unstained or faintly coloured, while the death cells attract Trypan Blue stain to become deep blue.

Figure 2 depicts the effect of varying concentrations of lead acetate on Islet cell viability. Different concentrations of lead acetate (0.1 - 0.4 mg/dL) were prepared in standard vehicle dimethyl sulfoxide (DMSO). The cell viability count revealed that 0.2 mg/dL of lead acetate had the highest significant value of healthy living cells (127,000±4.75 cells/mL) and the lowest dead cells (7,400±3.64 cells/mL) compared to other dose concentrations. Hence, 0.2 mg/dL of lead acetate was considered and used throughout the study.

Table 1 shows the mean cell growth and death rates of Islet cells exposed to 0.2 mg/dL of lead acetate and subsequently treated with Dimercaptosuccinic Acid (DMSA) and Calcium Disodium EDTA ($CaNa_2EDTA$). The cell counting values were monitored at 24, 504, and 1008 hours. After 24 hours, Set 2 (Negative control) showed a high value of healthy living cells (119,000±4.56 cells/mL) and lowest value of death cells (6,200±3.22 cells/mL) compared to those treated with DMSA and $CaNa_2EDTA$. However, the normal control had the highest (123,000±5.08 cells/mL) and lowest (1,800±3.30 cells/mL) healthy living and dead cells, respectively. Treatment with both DMSA and $CaNa_2EDTA$ reduced the cells by 16% and 23% after incubating for 504 and 1008 hours, respectively.

Table 2 represents the total mean glucose uptake by Islet cells exposed to lead acetate and subsequently treated with DMSA and $CaNa_2EDTA$. The set of experiment exposed to lead acetate alone increased glucose uptake to (2.70±0.18 mg/dL) after 24 hours compared to normal control (1.73±0.10 mg/dL), but rapidly reduced by 75% and 65% when evaluated at 504 and 1008 hours, respectively. However, when incubated with $CaNa_2EDTA$ showed a higher value of 1.91±0.17 mg/dL compared to normal control (1.73±0.10 mg/dL). Interestingly, the Islet cells increased their ability from 2% to 9%, when evaluated at 504 an 1008 hours, respectively, compared to normal control values. Then followed by DMSA treated-set, which increased their glucose uptake ability from 0.91±0.20 mg/dL to 1.08±0.10 mg/dL, when evaluated at 24 hours and 1008 hours respectively.

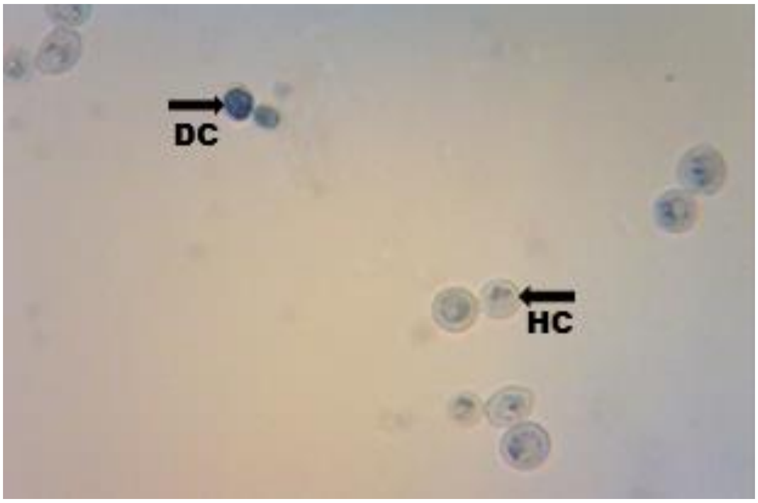

Figure 1. Photomicrograph of Trypan Blue-Stained Islet Cells Showing Healthy Cell (HC) and Death Cell (DC).

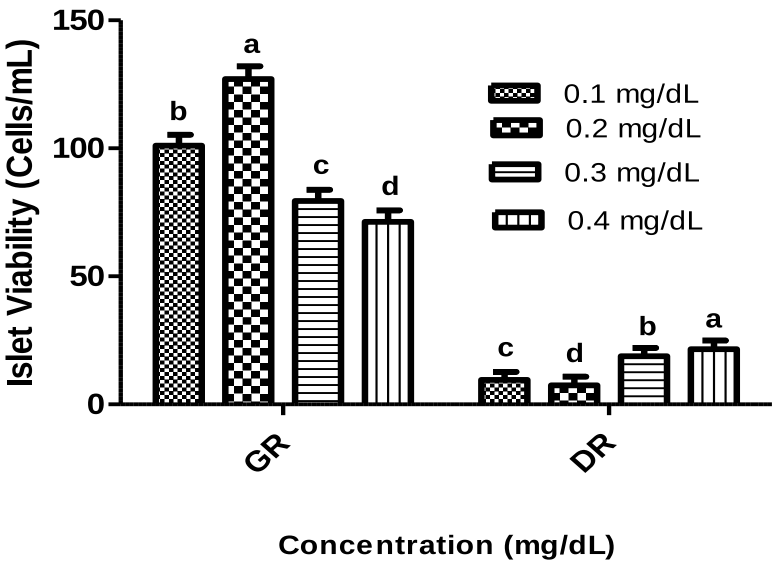

Figure 2. Effect of varying concentrations of lead acetate on Islet cell viability via growth rates (GR) and death rates (DR). Values are mean ± SD of three independent experiments. Values with different letters (a, b, c, d) are significantly different at $p<0.05$

Table 1: Periodic effect of DMSA and $CaNa_2EDTA$ on Islet cell exposed to lead acetate

	Islet cell viability	SET 1	SET 2	SET 3	SET 4
24 Hours Intervals	Growth rates (cells/mL)	123,000±5.08^{a}	119,000±4.56^{b}	62,000±4.15^{d}	68,000±4.08^{c}
	Death rates (cells/mL)	1,800±3.30^{d}	6,200±3.22^{c}	9,000±2.30^{a}	7,800±2.73^{b}
504 Hours Intervals	Growth rates (cells/mL)	89,000±4.55^{b}	117,000±4.44^{a}	52,000±3.54^{d}	56,800±3.68^{c}
	Death rates (cells/mL)	3,200±3.32^{d}	6,800±3.65^{c}	9,600±3.02^{a}	8,100±2.64^{b}
1008 Hours Intervals	Growth rates (cells/mL)	82,100±4.38^{b}	102,000±4.28^{a}	48,000±3.95^{d}	52,000±3.64^{c}
	Death rates (cells/mL)	4,000±3.16^{d}	10,400±3.54^{b}	11,600±3.26^{a}	7,900±2.86^{c}

Values are mean ± SD of three independent experiments. Values with different letters (a, b, c, d) across the row are significantly different at $p<0.05$. Dimercaptosuccinic Acid (DMSA) and Calcium Disodium EDTA ($CaNa_2EDTA$). SET 1 (Normal control); SET 2 (0.2 mg/dL Lead acetate); SET 3 (0.2 mg/dL Lead acetate + 15 mg/dL DMSA); SET 4 (0.2 mg/dL Lead acetate + 15 mg/dL $CaNa_2EDTA$).

Table 2: Periodic effect of DMSA and $CaNa_2EDTA$ on glucose uptake of Islet cell exposed to lead acetate

	SET 1	SET 2	SET 3	SET 4
24 Hours	1.73±0.10^{c}	2.70±0.18^{a}	0.91±0.20^{d}	1.91±0.17^{b}
504 Hours	1.94±0.10^{a}	0.67±0.15^{c}	1.01±0.10^{b}	1.98±0.12^{a}
1008 Hours	1.98±0.14^{a}	0.93±0.12^{c}	1.08±0.10^{b}	2.09±0.18^{a}

Values are mean ± SD (mg/dL) of three independent experiments. Values with different letters (a, b, c, d) across the row are significantly different at $p<0.05$. Dimercaptosuccinic Acid (DMSA) and Calcium Disodium EDTA ($CaNa_2EDTA$). SET 1 (Normal control); SET 2 (0.2 mg/dL Lead acetate); SET 3 (0.2 mg/dL Lead acetate + 15 mg/dL DMSA); SET 4 (0.2 mg/dL Lead acetate + 15 mg/dL $CaNa_2EDTA$).

4.0 Discussion

Since the year 2010, there has been a massive importation of chelation therapy as an emergency medical response to lead poisoning outbreak in Nigeria (Brown and Woolf, 2022; MSF, 2012). Chelation therapies, especially dimercaptosuccinic acid (DMSA) and calcium disodium EDTA ($CaNa_2EDTA$), have been distinguished for their excellent ability to mitigate lead toxicity. During our interactions with patients undergoing treatment, we observed that some non-diabetic patients exhibited signs and symptoms of diabetes, such as increased thirst and hunger, frequent urination, and fatigue (Okafor *et al,* 2013; Ugwu *et al,* 2013). Notably, diabetes is one of the leading cause of death worldwide, and a major health concern (Lund *et al.*, 2014). This observation prompted our research into the effects of lead poisoning on Islet cell function and the potential of chelation therapies to mitigate these effects.

Following the protocol of Carter *et al.* (2009), we successfully purified Islet cells from healthy mouse pancreatic tissue. Healthy versus death cells were assessed using Trypan Blue stain. From our study, the healthy cells remain unstained or faintly stained, while the death cells attract Trypan Blue stain to become deep blue (Hornblad *et al.*, 2011). Islet cells regulate insulin and hormone release in response to extracellular glucose concentration changes, defining their function. Insulin production and release from Islet beta cells play a vital role in regulating blood glucose, a significant feature of both type 1 and 2 diabetes (Ugwu *et al*, 2013; Wilhelm *et al*., 2006). To perform a detailed study, Islet cells were incubated with varying lead acetate concentrations (0.1-0.4 mg/dL). Our study showed that 0.2 mg/dL of lead acetate may cause significant changes in Islet cell growth and death within 24 hours, which was used as the standard concentration throughout the experiment. In this study, we assessed two critical aspects: Islet cell viability and functionality.

Our Islet cell viability study demonstrated that lead poisoning caused rapid cell proliferation, that can potentially lead to life-threatening complications like cancer (Balali-Mood *et al*., 2021). This particular effect was significantly reduced overtime. However, incubating lead-exposed Islet cells with DMSA and $CaNa_2EDTA$ mitigated cell proliferation by 47% and 43%, respectively, showing both drugs' ability to chelate lead poisoning, with $CaNa_2EDTA$ demonstrating higher potency. Furthermore, our study investigated the effect of these chelation therapies on lead-exposed Islet cell functionality. Islet cells-exposed to lead poisoning initially demonstrated effective glucose uptake after 24 hours but rapidly reduced by 75% as the experiment continued, indicating that cells viability does not necessarily ensure cell functionality (Balali-Mood *et al*., 2021). Incubating Islet cells exposed to lead poison with DMSA and $CaNa_2EDTA$ showed some improvement, with $CaNa_2EDTA$ enhancing its capacity to undertake glucose uptake.

5.0 Conclusion

Our research demonstrates the detrimental effects of lead poisoning on Islet cell viability and functionality, highlighting the potential for lead exposure to contribute to the development of diabetes. We also show that chelation therapy, particularly with $CaNa_2EDTA$, can mitigate these effects and improve Islet cell function. These findings have significant implications for the treatment of lead poisoning and the prevention of diabetes, emphasizing the need for prompt medical intervention in cases of lead exposure. Further research is warranted to explore the clinical applications of these findings and to develop targeted therapies for lead-induced diabetes.

Conflict of interest

The authors declare no conflict of interest

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