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RESEARCH ARTICLE

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Evaluation of Blood Lead Levels (Bll) of Albino Rat Offspring (Weanlings) Prenatally Exposed to Lead and Moringa Oleifera

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Abstract

This study evaluated the presence of lead in the offspring (weanlings) of prenatally exposed rats and the ameliorative effect of Moringa oleifera (MO). Independent group randomized design in a 3 X 4 factorial matrix was adopted. A total of 120 Wister Albino rat weanlings randomly selected from 490 offspring prenatally exposed to lead and MO and assigned into 12 treatment groups of 10 rats each were used. Multivariate Analysis variance at 0.05 level of significance was adopted. Rat weanlings prenatally exposed to higher lead doses had more lead in their blood compared to rat weanlings exposed to MO combined with lead, rat weanlings exposed to lead only and rat weanlings exposed to MO only. Lead was present in the blood of rat weanlings prenatally exposed to lead while non was recorded in blood of rat weanlings exposed to MO and the controls. Control of environmental lead is therefore imperative.

Keywords: Lead, Moringa oleifera, Blood Lead Level (BLL), Albino Rats, Offspring

1 | INTRODUCTION

Measurement of Blood Lead Level (BLL) is very critical in understanding the major health hazards associated with exposure to lead, Particularly in children; this need regular monitoring in order to avoid lead exposure as much as possible (H. Needleman, 2009). There are several research evidence showing behavioral deficits associated with high BLL mostly in children (Liu et al., 2011 Tatsuta et al., 2020; Abushady et al., 2017).

Lead is a hazardous chemical which occurs naturally and categorized as a heavy metal and also non-biodegradable. Lead is used extensively in production and recycling of batteries, paints, lead containing gasoline, and for several other purposes resulting

into extensive contamination of the environment and exposure to humans and animals, causing significant community health problems in various parts of the world (Al-Megrin et al., 2020). World Health Organization (WHO) report of 2016 indicate that exposure to lead through human activities accounted for an estimated death of 143,000 people who die every year, accounting for 0.6% of the worldwide disease burden. (WHO, 2016).

Lead is a toxin that accumulates in the body and damages many physiological systems (WHO, 2020). It has substantial consequences for the central nervous system, hematologic, reproductive, hepatic, and excretory systems (Obeng-Gyasi et al., 2018 Qi et al., 2019). The amount of lead collected in the body is accounted for by blood lead levels, although nothing is known about the safe blood lead level. However, other studies have found that a low lead level of 5 g/dL can contribute to decreased intellect in children, learning impairments, and behavioral issues (Mason et al., 2014). The impacts of lead toxicity are more severe in developing countries because they are predominantly at high risk of lead toxicity, and they bear the highest burden of this hazard, according to a WHO report ((WHO, 2020).

According to Yousef et al.,(2019), Lead can enter the body through a variety of methods, including inhalation, skin absorption, and ingestion of contaminated food or water. Several researches indicate that after absorption, lead is transported from the bloodstream and deposited in the soft tissues of living organisms (Al-Quraishy et al., 2016 Aladaileh et al., 2020). Lead is recognized to be hazardous, especially to developing neural systems. Lead can pass from a pregnant woman's placenta to the fetus or from breast milk to a baby, indicating that safeguarding infants from lead exposure is critical for lifetime health. There is evidence that even at low levels of lead in the blood, lead can impact IQ, attention span, and academic accomplishment (Aladaileh et al., 2020).

Because children are exposed to lead throughout their lives, they are more vulnerable to lead poisoning than adults. If their mothers have lead in their bodies, they might well be exposed to lead in the womb. Babies can consume lead when they breastfeed, eat other meals, or drink lead-contaminated water (Aziz et al., 2012 ATSDR, 2020). While playing on the floor or the ground, babies and children might swallow and breathe lead from dirt, dust, or sand. Children are more likely than adults to be exposed to lead as a result of these activities. Lead particles may be present in the soil or dust on the children's hands, toys, and other items. In other situations, children ingest nonfood substances like paint chips, which can contain extremely high levels of lead, especially in and around older buildings painted with lead-based paint. The paint on these structures frequently peels and combines with dust and filth. Children are more vulnerable to the health impacts of lead than adults, and there is no acceptable blood lead level in children (CDC, 2012 Mazumdar et al., 2011).

Lead affects children differently depending on how much lead they consume. A child that consumes excessive levels of lead may suffer anemia, renal damage, colic (severe "gut ache"), muscle weakness, and brain damage, all of which can be fatal (CDC, 2012). In rare situations, the amount of lead in a child's body can be reduced by administering particular medications that aid in the elimination of lead from the body. Smaller amounts of lead, such as dust containing lead from paint, may be swallowed by a kid, causing far less severe but nonetheless significant effects on blood, development, and behavior. Recovery is expected in this situation after the child is removed from the source of lead exposure, but there is no guarantee that the newborn will totally avoid the long-term repercussions of lead poisoning. Lead can harm a child's mental and physical development even at low levels of exposure. Fetuses exposed to lead in the womb as a result of their mothers' high lead levels may be born prematurely and with lower birth weights (ATSDR, 2020). Exposure during pregnancy, infancy, or early childhood may also

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impede mental development and result in poorer IQ later in life. Lead is highly hazardous to the nervous system, especially throughout the developing stages of life (Baranowska-Bosiacka et al., 2013 Metryka et al., 2020). Lead exposure throughout childhood has been linked to several abnormalities in the developing brain (Falkowska et al., 2015 Chibowska et al., 2016 Metryka et al., 2018), leading to a variety of cognitive impairments (Gilbert & Lasley, 2002 Verina et al., 2007; Al-Saleh et al., 2009). Prenatal lead exposure has been linked to many neurodevelopmental diseases, including autism spectrum disorder (ASD (Amin, 2012 Saghazadeh & Rezaei, 2017; El-Ansary et al., 2017; Smith et al., 2018; Chibowska et al., 2020), hyperactivity, emotional symptoms, and conduct disorder (Sioen et al., 2013), attention-deficit hyperactivity disorder (ADHD) (Nicolescu et al., 2010 Hong et al., 2015). There is evidence that these impacts may last longer than childhood (Needleman et al., 1990 ;Cecil et al., 2008 ;Wright et al., 2008). There appears to be no threshold level below which lead causes no harm to the growing human brain (Sanders et al., 2009 ; Wani et al., 2015). Though no specific symptoms may be observed in children with high blood lead levels, blood samples from such infants exposed to hazardous levels of lead may be taken to detect the blood lead levels. This can also be discovered in the bone using specific x-rays of the finger, knee, or elbow (Agrawal, 2012).

To assess if lead exposure has occurred, the level of total lead in the blood can be measured. This test determines whether or not a person has recently been exposed to lead. Lead can be assessed in teeth or bones using x-ray techniques, but these procedures are not commonly available and only show long-term lead exposures. Lead exposure can also be assessed by detecting erythrocyte protoporphyrin (EP) levels in blood samples. EP is a component of red blood cells that is known to rise when there is a high level of lead in the blood. However, the EP level is insufficiently sensitive to identify children with high blood lead levels of less than 25 micrograms per deciliter (g/dL). These tests typically necessitate the use of specialized analytical equipment that is not widely available (Ettinger et al., 2020 ;CDC, 2020). A variety of laboratory procedures are available to detect blood lead concentrations in newborns (Flanagan et al., 2008 ATSDR, 2020). The most frequent laboratory method is atomic absorption spectrometry. Anodic stripping voltammetry (ASV) and inductively coupled plasma mass spectrometry are two others (ICP-MS). Any method used to determine blood lead levels will be heavily influenced by its analytical capabilities, price, and technical requirements. These considerations influence the method selection choices.

Research efforts were able to establish that some chemical compounds could minimize the hazardous effects of lead due to the destructive effect of lead on the blood, brain, kidney, and other organs. Some of the research efforts include the role of exogenous hydrogen peroxide (H_2O_2) in inducing tolerance to lead exposure (Li et al., 2009), garlic and EDTA (Reckziegel et al., 2011), anti-oxidants like vitamin C and E (Hassan & Jassim, 2010 El-Masry et al., 2011), nutrients containing methionine, taurine, zinc, ascorbic acid and glycine (Fan et al., 2009), supplements containing iron, calcium and zinc (Davuljigari & Gottipolu, 2020), extracts of plant origin such as *Rubia cordifolia* (Maitra & Satardekar, 2017), *Moringa oleifera* (Mahdy et al., 2012), *Coriandrum sativum* (Sharma et al., 2011). However, there has been a recent surge in interest in the use of medicinal plants, which provide significant therapeutic compounds that can help reduce the harmful effects of lead and other environmental toxins (Maitra & Satardekar, 2017; Alves et al., 2006). Plant study has become more popular around the world, and a substantial body of information has been gathered that demonstrates the immense potential of medicinal herbs employed in diverse traditional systems. The protective properties of natural antioxidants have received more attention (Sánchez-Machado et al., 2006). Moringa oleifera is currently thought to be a source of dietary ingredients with biological and pharmacological activity that may have health advantages for humans.

The problem with chelating and other chemical agents now used to treat lead toxicity is that they cannot remove lead without damaging other cellular critical metals, and they can also cause toxicant redistribution and hepatic or renal failure, according to AL-Megrin et al., (2019). The use of medicinal plants as a therapeutic method, on the other hand, may

reduce lead toxicity. For example, Abdel Moneim, (2016), found evidence that the nutritional components polyphenols, flavonoids, and organic acids in Indigofera oblongifolia extract protected liver tissue from lead acetate in a research.

Moringa oleifera is a perennial Indian tree that belongs to the Moringaceae family and is widely cultivated around the world due to its biological qualities (Khalil et al., 2020). Because the flowering, stem, and leaves of the tree offer therapeutic effects, all parts of the tree are essential (Abdel-Daim et al., 2019). Previous research has shown that the flower can be used as a tonic and to cure rheumatism and inflammation; the stems and seeds have hepatoprotective and anti-hypertensive actions; and the leaves can be used to fight microbial infections and to regulate hyperglycemia (El-Khadragy et al., 2018 Oguntibeju et al., 2020). Because of the high quantity of vitamins (A and C), antioxidants (anthocyanidins, glucosinolates, flavonoids, and phytosterols), and proteins, the leaves are consumed raw, boiled, or air-dried to help with nutritional deficiencies (Kandeil et al., 2020). There are some signs that Moringa oleifera extract is safe. Human investigations employing up to a single dose of 50 g (William et al., 1993) or an 8g/day dose for 40 days revealed no harmful effects (Kumari, 2010).

Although there is a wealth of research evidence demonstrating the negative effects of prenatal and childhood lead exposure on behavior, such as poor neuro-developmental outcomes, particularly in cognitive functioning (Gomaa et al., 2002 WHO, 2010), shortening of attention span, and disruption of behavior (Wright et al., 2008), and that these effects may persist beyond childhood (Needleman et al., 1990 Wright et al., 2008 Cecil et al., 2008), there is a scarcity of evidence on the presence of lead in the blood of prenatally exposed children. The majority of investigations have focused on maternal blood lead levels (Bellinger et al., 2005 WHO, 2010 Ettinger et al., 2020).

Lead poisoning and toxicity treatment and management is a worldwide concern. There appear to be no universally recognized therapeutic standards for lead toxicity. According to a World Health Organization report, many countries establish their own treatment standards for levels of lead exposure that they deem safe (WHO, 2013). The Center for Disease Control and Prevention (CDC) in the United States of America, for example, has suggested follow-up testing for both pregnant women and their newborn babies if the blood lead level is greater than 5g/dl, and chelation therapy if the blood lead level is greater than 45g/dl (Ettinger et al., 2020). However, treatment of the lead poisoning and toxicity with chelating and other chemical agents has been reported to be inadequate (AL-Megrin et al., 2019), suggesting that the toxicity may be lessened by the use of medicinal plants as a therapeutic strategy (Abdel Moneim, 2016). There is evidence that extracts from different medicinal plants such as *Indigofera oblongifolia*, *Moringa Oleifera* etc. containing nutritional ingredients like polyphenols, flavonoids, and organic acids are known to protect tissues from lead toxicity (Rehman Ali et al., 2020). Although many researchers have reported the beneficial effects of *Moringa oleifera* in humans and animals, information regarding the utilization of the plant in lead remediation particularly at the prenatal stage of development is scanty.

This current study is therefore, aimed to evaluate the blood lead levels of Albino rat weanlings prenatally exposed to different doses of lead, relate it to the possible behavioral deficits associated with lead exposure and to investigate the remediation effect of *Moringa oleifera* leaf extract on the control of blood lead levels of prenatally exposed rat weanlings.

The following research questions were raised to provide answers to them.

1. What are the blood lead levels of Albino rat weanlings prenatally exposed to lead at different doses of 5, 8 and 10 micrograms per deciliter of lead?

(ii) What are the blood lead levels of Albino rat weanlings prenatally exposed to *Moringa oleifera* at different doses of 20 and 60 milligrams per kilogram body weight?

1. What are the blood lead levels of Albino rat weanlings prenatally exposed to combined doses of lead and Moringa oleifera at 5, 8,

and 10 micrograms per deciliter of lead and 20 and 60 milligrams per kilogram body weight of Moringa oleifera?

The following hypotheses were tested in determining the blood lead levels of prenatally exposed Albino rat weanlings to lead and the ameliorating effects of *Moringa oleifera*;

- Albino rat weanlings prenatally exposed to different doses of lead will have traces of the presence of lead in the blood
- Albino rat weanlings exposed to different doses of Moringa oleifera will have no traces of lead in the blood
- Albino rat weanlings exposed to combined doses of lead and Moringa oleifera at different doses will show less traces of lead in their blood.

2 | METHODOLOGY

2.1 | Design

The design for this study is a completely randomized design adopting a 3 x 4 factorial matrix. The independent variables are lead treatment which is at 4 levels; 5μg, 8μg, 10μg per kilogram body weight of rats, and no lead treatment (saline) and *Moringa oleifera* treatment which is at 3 levels; 20mg, 60mg per kilogram body weight of rats and no *Moringa oleifera* treatment (saline). The dependent variables are the blood lead levels of rat weanlings prenatally exposed to lead and *Moringa oleifera*.

2.2 | Subjects

Wister albino rats were used for this study. The rats fall into three main categories as follows

Category 1: - A total of 120 mature male Wister albino rats weighing between 220g – 250g for the purpose of mating.

Category 2: - A total of 120 mature female albino rats weighing between 180g – 200g randomly selected into the different experimental groups.

Category 3: - Offspring (Weanlings) of the Dams (female) albino rats in category 2 randomly selected from the different experimental groups of 5 micrograms, 8 micrograms, 10 micrograms of lead, 20 milligrams, 60 milligrams of *Moringa oleifera* and control groups (saline treatment).

Allocation of rats to the different cells was done by first acclimatizing the rats to the laboratory environment for two weeks, matching them according to gender and randomly allotting the rats to the different treatment groups represented in the cells below. It was ensured that the weight of rats in each cell was within the same range by random allotment of rats in the cells.

Table 1 Factorial Design Cells

	Moringa oleifera		
Lead	B1 20mg	B2 60mg	B3 0mg
A1 0µg	1 A1B1	2 A1B2	3 A1B3
A2 5µg	4 A2B1	5 A2B2	6 A2B3
A3 8µg	7 A3B1	8 A3B2	9 A3B3
A4 10µg	10 A4B1	11 A4B2	12 A4B3

Cell 1 - 20mg/kg body weight *Moringa oleifera*

Cell 2 - 60mg/kg body weight *Moringa oleifera*

Cell 3 - Control group (No Moringa and Lead), Saline treatment

Cell 4 - Combined 5μg/kg lead and 20mg/kg *Moringa oleifera*

Cell 5 - Combined 5$\mu\gamma$g/kg lead and 60mg/kg *Moringa oleifera*

Cell 6 - 5μg/kg lead only

Cell 7 - Combined 8μg/kg lead and 20mg/kg *Moringa oleifera*

Cell 8 - Combined 8μg/kg lead and 60mg/kg *Moringa oleifera*

Cell 9 - 8μg/kg lead only

Cell 10 - Combined 10μg/kg lead and 20mg/kg *Moringa oleifera*

Cell 11 - Combined 10μg/kg lead and 60mg/kg *Moringa oleifera*

Cell 12 10μg/kg lead only

2.3 | Housing of Rats

The subjects (Wister Albino rats) were raised and maintained in the experimental animal laboratory of the Faculty of Veterinary Medicine, University of Ibadan, in a regular day-night cycle (12: 12-hour light dark cycle) in RCI North Kent Plastic cages. The RCI plastic cages have dimension of 56cm x 40cm x 18cm. The rats were housed in twos – one male and one female until 3 days when conception was assumed to have taken place. The animals were fed with Mouse cubes (a standard laboratory rat feed) and water. All the observations were carried out between 9:00 am to 2:00 pm. The experimental protocols were approved by the UI/UCH Institutional Ethics Committee Number UI/UCH/EC/15/0040.

2.4 | Equipment / Materials

The following instruments were used for the study:

1. Rat cages: These were RCI North Kent plastic cages measuring 56cm x 40cm x 18cm for breeding and general housing of subjects in groups and RI North Kent plastic cages measuring 45cm x 27cm x 40cm for observation purposes.

2. Two weighing balances were used for the study. They were:

i. A Chatillon, New York. N.Y. 11415 weighing balance with capacity of 500 x 2g this was used for weighing rats, and

ii. A computerized Metler-Toledo weighing balance with a maximum capacity of 5kg and minimum capacity of 0.01mg. This was used for weighing lead, *Moringa oleifera* and other chemical substances that require very sensitive weights because of minute quantity involved.

3. A 250ml graduated measuring cylinder was employed for measuring saline used to dilute the substances.

4. Water bottles with stainless steel tunnels were used to provide drinking water for the subjects.

5. An oral cannula was used for oral administration of lead, *Moringa oleifera* and saline.

6. Disposable 5ml and 2ml syringes and needles were employed for the collection of lead, *Moringa oleifera* and saline during dilution process.

7. Standard animal feed (mouse cubes) was used for feeding the subjects.

8. Substances for the experiment were lead, *Moringa oleifera* and saline.

9. Atomic Absorption Spectrophotometer was used to determine blood lead levels of weanlings of the rats.

2.5 | Experimental Procedure

The experiment started with random assignment of female rats into individual cages for the purpose of mating. The male rats also were individually and randomly assigned into the individual cages containing the female rats on the day of commencement of the experiment. The male rats were only used for the purpose of mating the female rats for three days only within which it was expected that the female rats have mated. The female rats on the other hand were weighed and randomly assigned into the following treatment groups:

1. Control group with saline treatment

2. 5 micrograms per deciliter / kg body weight lead treatment group.

3. 8 micrograms per deciliter / kg body weight lead treatment group

4. 10 micrograms per deciliter / kg body weight lead treatment group.

5. 20 milligrams per kilogram body weight *Moringa oleifera* treatment group.

6. 60 milligrams per kilogram body weight *Moringa oleifera* treatment group.

7. Combined 5 microgram per kilogram body weight of lead and 20 milligrams per body weight of *Moringa oleifera*.

8. Combined 8 microgram per kilogram body weight of lead and 60 milligrams per kilogram body weight of *Moringa oleifera*.

9. Combined 10 microgram per kilogram body weight of lead and 60 milligrams per kilogram body weight of *Moringa oleifera*.

10. Combined 5 microgram per kilogram body weight of lead and 60 milligrams per kilogram body

weight of *Moringa oleifera*.

11. Combined 8 microgram body weight of lead and 20 milligram body weight of *Moringa oleifera.*

12. Combined 10 microgram body weight of lead and 20 milligrams per kilogram body weight of *Moringa oleifera.*

Each treatment group comprised ten dams.

Lead was prepared into fresh solutions containing 5μg/dl/kg body weight, 8μg/dl/kg body weight and 10μg/dl/kg body weight concentration by diluting a stock solution of lead into one million parts per million (PPM). A stock solution of *Moringa oleifera* isolate was also prepared into 20mg/kg and 60mg/kg concentration by the dilution into one thousands part per million (PPM). The dams were then given oral administration of lead with the help of the oral canula. They were allowed a period of 30 minutes before food and water was restored to them to ensure that the action of the drug has begun. On the third day of the experiment, the male rats were removed leaving only the females.

The procedure of treatment was repeated for 16 days of the gestation period. This was to ensure:

a. That the action of the drug covers three trimesters of the dam's gestational period which normally spans for duration of 21 + 1 day.

b. Dams will not be given drugs on the days of parturition. On this basis it will be claimed that treatment covers most days of gestation.

The dams were only weighed and observed from day 17 to parturition without treatment. At parturition, the litter, size and the mean birth weight was taken at the nearest experimental time. The offspring were allowed to breast feed for 21 days after which they were weaned. The next stage of the experiment continued with the offspring (weanlings) which were gathered into groups according to their experimental groupings. Blood samples of each weanling according to their experimental groups were collected using EDTA bottles. The blood samples were analyzed for the presence of lead to determine the blood lead levels of the rat weanlings using the Atomic Absorption Spectrophotometer.

3 | RESULTS

The findings of this research on the assessment of blood levels of Albino rat weanlings prenatally exposed to lead and Moringa oleifera are reported in accordance with the hypotheses proposed. The obtained data was subjected to Randomized Block Analysis of Variance (ANOVA), descriptive statistics of mean and standard deviation, and graphical representation.

Table 2: Summary of Factorial ANOVA table showing Comparison of the blood- lead levels of rat weanlings prenatally exposed to lead and *Moringa oleifera*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.099	11	.736	102.338	.000
Within Groups	.777	108	.007		
Total	8.876	119			

In Table 2, ANOVA analysis of the blood- lead levels of rat weanlings prenatally exposed to lead shows that there was a significant difference between rat weanlings prenatally exposed to Moringa Oleifera in combination with lead, rat weanlings exposed to lead only and rat weanlings exposed to Moringa Oleifera only ($F(11,108) = 102.34$, $p<.001$). Rat weanlings prenatally exposed to higher lead doses had more lead in their blood compared to rat weanlings exposed to Moringa Oleifera in combination with lead, rat weanlings exposed to lead only and rat weanlings exposed to Moringa Oleifera only.

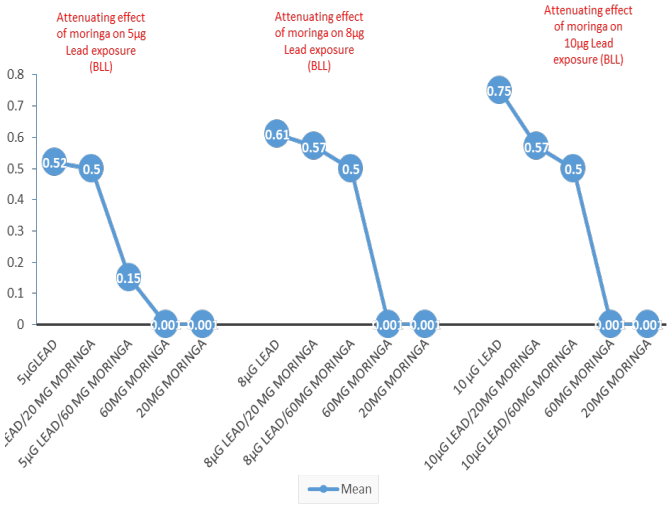
Table 3. Descriptive and scheffe *post hoc* comparison values of the blood lead levels of rat weanlings prenatally exposed to different doses of lead and *Moringa oleifera*

		1	2	3	4	5	6	7	8	9	10	11	12
1	10 µg lead	-											
	10µg lead/20mg	.18*	-										
2	moringa 10µg lead/60mg	.25*	.07										
3	moringa 20mg	.75*	.57*	.50*									
4	moringa 5µg lead	.23*	.05	-.02	-.52*								
5	5µg lead/20 mg moringa	.25*	.07	0.00	-.50*	.02							
6	5µg lead/60 mg moringa	.60*	.42*	.35*	-.15*	.37*	.35*						
7	60mg moringa	.75*	.57*	.50*	0.00	.52*	.50*	.15*					
8	8µg lead	.14*	-.04	-.11*	-.61*	-.09*	-.11*	-.46*	-.61*				
9	8µg lead/20 mg moringa	.18*	0.00	-.07	-.57*	-.05	-.07	-.42*	-.57*	.04			
10	8µg lead/60mg moringa	.25*	.07	0.00	-.50*	.02	0.00	-.35*	-.50*	.11*	.07		
11	Control	.75*	.57*	.50*	0.00	.52*	.50*	.15*	0.00	.61*	.57*	.50*	
12	Mean	0.75	0.57	0.50	0.00	0.52	0.50	0.15	0.00	0.61	0.57	0.50	0.00
	S.D	0.08	0.07	0.00	0.00	0.04	0.00	0.24	0.00	0.11	0.05	0.00	0.00

***mean comparison significantly different at 0.05 of significance.**

Table 4. Summary of mean scores of blood lead levels of rat weanlings prenatally exposed to different doses of lead and *Moringa oleifera*

	Mean±S.D
5µgLead	0.52 ± 0.04^{c}
8µg lead	0.61 ± 0.11^{d}
10 µg lead	0.75 ± 0.08^{d}
20mg moringa	0.00 ± 0.00^{a}
60mg moringa	0.00 ± 0.00^{a}
5µg lead/20 mg moringa	0.5 ± 0.01^{c}
8µg lead/20 mg moringa	0.57 ± 0.05^{cd}
10µg lead/20mg moringa	0.57 ± 0.07^{cd}
5µg lead/60 mg moringa	0.15 ± 0.24^{b}
8µg lead/60mg moringa	0.5 ± 0.00^{c}
10µg lead/60mg moringa	0.5 ± 0.00^{c}
Control	0.00 ± 0.00^{a}



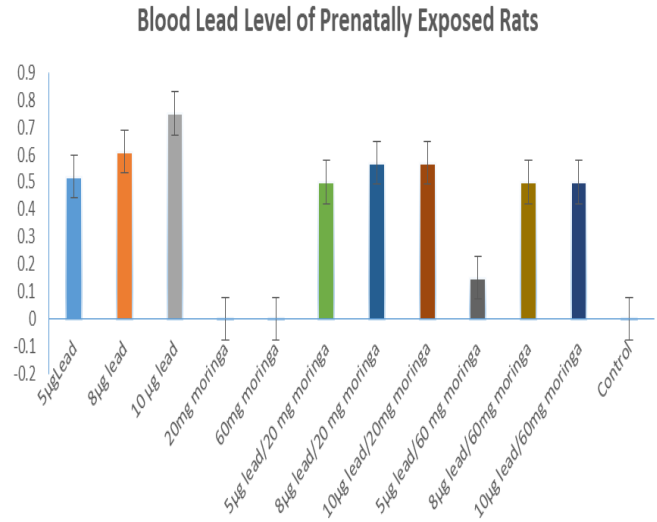


Fig1: Blood Lead Level of Prenatally Exposed Rats

Fig.1 shows that rat weanlings exposed to higher doses of lead (10μg and 8 μg) had more lead in their blood compared to rat weanlings exposed to Moringa oleifera in combination with lead, rat weanlings exposed to lead only and rat weanlings exposed to Moringa oleifera only. This confirmed that there was significant amount of lead in the blood of the prenatally exposed rat weanlings.

Analysis of the blood lead levels of rat weanlings prenatally exposed to different doses of lead and *Moringa oleifera* in Table 2 shows that there was a significant difference between blood lead levels of rat weanlings prenatally exposed to *Moringa oleifera* in combination with lead, lead only and *Moringa oleifera* only.

The first hypothesis which stated that Albino rat weanlings prenatally exposed to different doses of lead will have traces of the presence of lead in the blood compared to weanlings who were not exposed was confirmed. In Table 3 and 4, the pair wise comparison reveals that the rats in the control group (= 0.00) which were not exposed to lead significantly (p<.05) demonstrated no traces of lead in their blood compared to weanlings exposed to lead (10 μg lead = (= 0.75); 5μg lead = (= 0.52); 8μg lead= (= 0.61)).

The second hypothesis which stated that Albino rat weanlings exposed to different doses of *Moringa oleifera* will have no traces of lead in the blood was also supported. Pair wise comparison also demon-strated that the rat weanlings in the 20mg *Moringa oleifera* (= 0.00), 60mg moringa oleifera (= 0.00) experimental groups whose mothers were not ex-posed to lead significantly (p<.05) demonstrated no traces of lead in their blood compared to weanlings exposed to lead (10 μg lead = (= 0.75); 5μg lead = (= 0.52); 8μg lead= (= 0.61)).

The third hypothesis which stated that Albino rat weanlings exposed to combined doses of lead and *Moringa oleifera* at different doses will show less traces of lead in their blood was also supported. Rat weanlings prenatally exposed to higher doses of lead had more lead in their blood compared to rat weanlings exposed to *Moringa oleifera* in combination with lead, lower doses of lead only and *Moringa oleifera* only. The mean comparison significantly show that rat weanlings exposed to higher doses of lead had more lead in their blood compared to rat weanlings exposed to *Moringa oleifera* in combination with lead, lower doses of lead only and *Moringa oleifera* only.

4 | DISCUSSION

This study was primarily designed to investigate the blood lead levels (BLL) of rat offspring (weanlings) prenatally exposed to single and combined doses of lead and *Moringa oleifera*. Blood Lead Level (BLL) of the prenatally exposed rat weanlings was analysed by the researcher using Atomic Absorption Spectrophotometer to investigate the presence of lead in the blood of prenatally exposed rat weanlings. Different levels of lead were detected in the blood of the weanlings according to their levels of prenatal exposure to lead and *Moringa oleifera*. The result shows a significant difference between blood lead levels of rat weanlings prenatally exposed to lead in combination to *Moringa oleifera*, (rat weanlings exposed to lead only and rat weanlings exposed to *Moringa oleifera* only ($F(11, 108)= 102.34$, $p<.001$)). Rat weanlings prenatally exposed to higher doses of lead had more lead in their blood compared to rat weanlings exposed to *Moringa oleifera* in combination with lead, and rat weanlings exposed to Moringa oleifera only. The mean comparison significantly show that rat weanlings exposed to higher doses of lead had more lead in their blood compared to rat weanlings exposed to *Moringa oleifera* in combination with lead, and rat weanlings exposed to *Moringa oleifera* only (10μg lead (= 0.75); 10μg lead/60mg Moringa (= 0.5); 60mg Moringa(= 0.0).

According to some research, there is a possible physiologic mobilization of bone lead storage during pregnancy and lactation, making unborn fetuses more susceptible to increasing lead concentrations from pregnant women (Ettinger et al., 2020). According to Markowitz (2000), lead that has been deposited in the mother's bones for years is released into the blood during pregnancy due to the metabolic stress. Throughout pregnancy, lead easily transfers from maternal to baby circulation, and the infant's blood lead levels becomes nearly comparable to that of the mother (Markowitz, 2000). According to Bellinger, (2008), Lead moves readily through passive diffusion from mother to fetus indicating a possibility of the occurrence of prenatal lead exposure as seen from the findings of this research. There are research evidences that Lead can cross into the baby (WHO, 2010 Ettinger et al., 2020 Bellinger, 2008). Although it is unknown how early lead can reach a growing baby during pregnancy, some reports have shown lead in a developing fetus as early as the 13th week of pregnancy. (Ladele et al., 2019 Ettinger et al., 2020).

Agrawal, (2012), discovered that lead is strongly attached to red blood cells, boosting the transfer from maternal circulation to the fetus via the placenta, and that the placental transfer occurs as early as the twelfth week of gestation. Previous animal studies of brain lead content have revealed that the blood-brain barrier is more vulnerable to lead during pregnancy, becoming more effective during weaning and much more so following weaning (Rossouw et al., 1987 Toews et al., 1978). Even at very low levels of exposure, the developing human brain is particularly vulnerable to lead, and once in the infant, lead can permeate the immature blood–brain barrier and enter the developing brain (Lidsky & Schneider, 2006 Shell, 2016).

Lead can be passed to the unborn by young women who live in lead contaminated homes or who were lead poisoned as children, according to Vigeh et al., (2011). As a result, there is a substantial relationship between maternal and umbilical cord blood lead levels, demonstrating that lead is transferred from mother to fetus (Torabi et al., 2018). According to research, lead accumulates and is stored in bone for decades, and these bone lead deposits may pose a risk to women of reproductive age long after their lead exposure has ended (Agrawal, 2012). As a result,

lead can enter the fetus from the environment (exogenous lead) or through the bones (endogenous lead). In addition to prenatal lead transmission, lead levels in breast milk increase with maternal blood lead levels, increasing the likelihood of newborn blood lead (Weitzman & Kursmark, 2009). Furthermore, Cormick et al., (2019), observed that high calcium intake may reduce pregnancy induced increases in maternal blood lead concentrations by reducing maternal bone resorption or demineralization during pregnancy and the consequent flow of lead from the bone to the fetus. Ladele et al., (2019), discovered a high positive connection between maternal and umbilical cord blood lead levels in a study. This conclusion implies that maternal blood lead levels, as reported in this work, are a robust indicator of the presence of lead in the blood of prenatally exposed rats. Similar investigations found a direct link between maternal and umbilical cord blood lead levels.

The results of this research revealed that rat weanlings prenatally treated to Moringa oleifera alone or as a control had no trace of lead in their blood. However, rat weanlings treated to a combination dose of 60 mg/kg Moringa oleifera/10g lead showed less trace of lead presence than those subjected to a high dose of 10g/kg lead alone. The findings of this research is suggestive of the ameliorative effects of Moringa oleifera on lead poisoning effect. The submissions of Jiraungkoorskul & Jiraungkoorskul, (2016) that Moringa oleifera in its different forms, fruit, seed, leaf, flower, bark, and root, have been extensively employed in traditional medicine, including the decrease of heavy metal toxicity, confirm the conclusions of this study. Moringa oleifera has been shown to have a variety of chemical compounds with biological activities such as metal concentration depletion and antioxidant capabilities that have the ability to reduce oxidative stress caused by heavy metals. Sharayu & Asmita, (2017) showed in a similar investigation that the leaves and seeds of Moringa Oleifera exhibit anti-metal toxicity properties.

The ameliorative effect of Moringa oleifera in its many forms on lead toxicity is undeniable, as proven by several research findings. (Owolabi, et al., 2012 Mohamed et al., 2020 Omóbòwálé et al., 2020 Farid & Hegazy, 2020). This could be attributable to Moringa oleifera's chemical composition. Scientific evidence supports the medical use of Moringa oleifera as claimed by numerous cultures and groups. Moringa oleifera has been discovered to contain a wide range of vital nutrients, including vitamins, minerals, and amino acids (Kasolo et al., 2010 Fahey, 2016 Abd El-Hack et al., 2018). Moringa oleifera leaves are thought to contain significant levels of vitamin C, calcium, potassium, and protein. Moringa oleifera is a good source of natural antioxidants since it contains a variety of antioxidant components such as flavonoids, ascorbic acid, carotenoids, and phenolics. (Dillard & Bruce German, 2000 Nizioł-ŁUkaszewska et al., 2020).

Moringa oleifera's ameliorative effect on lead toxicity may be linked to the high calcium content of Moringa oleifera leaves. Some studies have examined reductions in blood lead concentrations in calcium supplemented women and found that calcium supplementation may be useful in lowering blood lead levels in pregnant women with elevated lead levels (Ettinger et al., 2009 Ladele, Fajolu, et al., 2019). Lead absorption is known to increase significantly with lack of calcium consumption particularly in pregnancy. Researches with humans and experimental animals have shown that diets low in calcium can enhance gastrointestinal lead absorption in humans and experimental animals (Ettinger et al., 2007 Dubey & Patel, 2018). On the contrary, diets that are rich in calcium reduce lead absorption and are known to provide additional protection against lead toxicity Ettinger et al., 2007).

5 | CONCLUSIONS

This study highlighted prenatal exposure of lead and its effect on the offspring. Blood Lead Levels of the offspring was particularly investigated with a strong evidence that prenatal exposure to lead increases the incidence of lead poisoning of the fetus. This was exhibited by the presence of lead in the blood of the offspring prenatally exposed to lead. Lead as an environmental toxin has the ability to permeate the placenta and is especially toxic to the growing foetus. The heavy metal is not filtered by the placenta from mother to child and is directly deposited in

growing foetal tissues. This study also concluded that *Moringa oleifera,* a medicinal plant has the ability to ameliorate the toxic effect of lead at all doses and on a dose dependent manner. It is recommended that given the toxic effect of lead on the fetus, pregnant women are advised to stay away from environments prone to lead inhalation or ingestion considering the implications brain development and behavior.

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CONFLICT OF INTEREST

The Authors hereby declare that there is no conflict of interest in this study.

AUTHORS CONTRIBUTION

J. I. Osuh conceptualized the study, designed the protocols, did some of the literature search and supervised the data collection for the whole study

A. M. Sunmola supervised the study and read the manuscript.

S. K. Balogun co supervised the work and read the manuscript.

A. A. Ishola did some of the literature search and analyzed the data

Finally, All the authors read through and approved the final copy of the manuscript.

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Conflict of Interest

The Authors unanimously declare that there is no conflict of interest in this study.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (National Research Council **(2011) GUIDE FOR LABORATORY ANIMALS FOR THE CARE AND USE OF ANIMALS** (8ed) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee" as referenced above.

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