THERAPEUTIC EFFECTS OF HYDRO-ETHANOLIC EXTRACT OF *Nigella sativa* (BLACK SEED) ON SOME HAEMATOLOGICAL PARAMETERS OF ALBINO RATS AFTER LEAD EXPOSURE

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**ABSTRACT**

**Background:** Lead poisoning is a great public health concern in Africa and Nigeria, especially regions where illegal mining activities occur such as widespread gold ore mining in Zamfara by artisan miners using rudimentary and unsafe processing techniques. Lead (Pb) which often have no biological function remain in the system causes havoc and distortion of normal physiologic functions. Currently, the toxic effects of lead poisoning are clinically treated using chelation therapy which have been associated with many side effects and setbacks. Since ancient times, medicinal plants have been used as a major source of treatment for numerous human diseases. Plants that possess hematinic and antioxidant properties in the plant kingdom are often used in such scenarios. One of such plants is *Nigella sativa* commonly known as black cumin. **Objectives:** In this study, therapeutic effects of hydro-ethanolic extracts of *Nigella sativa* was assessed on hematological parameters of lead-poisoned albino rats as a curative therapy for management of lead poisoning. **Method:** Thirty-five (35) adult albino wistar rats of both sexes were used for this study. Group I (Normal control) received 2ml/kg of distilled water, Group II (Negative control), Group III treated with 10 mg/kg of Meso-2,3 Dimercaptosuccinic acid (DMSA), IV treated with 200 mg/kg of *Nigella sativa* seed extract, V treated with 400 mg/kg of *Nigella sativa* seed extract, and VI treated with 800mg/kg of *Nigella sativa* seed extract for Group VII (Recovery) were allowed to recover without treatment. The study lasted for a duration of 21days. Blood samples were collected from the rats through cardiac puncture after anesthetizing the animals and analysed for haematological parameters which included RBC count, PCV, Hb concentration, platelet count, WBC profile and haematological indices using an automated digital blood analyser. **Results:** *Nigella sativa* seed extract significantly (P<0.05) reversed the adverse effect of Lead exposure on RBC count, PCV, Hb concentration, platelet count, WBC profile and haematological indices. **Conclusion:** *Nigella sativa* showed therapeutic effects on hematological parameters and indices of lead poisoned albino rats.

**Keywords:** Hematological parameters, Lead, *Nigella sativa*, Therapeutic effects.

**INTRODUCTION**

Lead poisoning is a great public health concern in Africa and Nigeria, especially regions where illegal mining activities occur such as the widespread gold ore mining in Zamfara by artisan miners using rudimentary and unsafe processing techniques. Lead poisoning is specifically pronounced in children (≤ 6 years) and contributes to 0.6% of the global burden of disease. The Institute of Health Metric and Evaluation (IHME) estimated that in 2016, lead exposure accounted for 63.2% of global burden of idiopathic developmental intellectual disability, 10.3% of the global burden of hypertensive heart diseases, 5.6% of global burden of the ischemic heart disease and 6.2% of global burden of stroke. Over...
200 children from Zamfara state in Nigeria have reportedly died from lead intoxication since March 2010 and an estimated 18,000 children and adults have been affected by wide spread lead contamination, resulting from informal extraction of gold from lead-bearing ore. According to the World Health Organization, high levels of lead exposure can cause anemia, brain, liver, kidney, nerve, and stomach damage, as well as permanent intellectual and developmental disabilities. Individuals with certain risk factors such as calcium deficiency, iron deficiency and disease of organs target by lead (such as brain or kidney) and possibly genetic susceptibility are more prone to lead toxicity. Anemia and socioeconomic factors are also important risk factors of lead poisoning. The symptoms arising from lead poisoning are often subtle and patients remain asymptomatic until significant reduction of renal function has occurred. Lead directly affects the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting various key enzymes (δ-aminolevulinic acid dehydratase (ALAD), aminolevulinic acid synthetase (ALAS), and ferrochelatase) involved in the heme synthesis pathway. Lead intoxication also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes through increased free radicals. The combined aftermath of these two processes leads to anemia which possess severe health burden in Africa and is one of the major public health problems worldwide. It is characterized by hemoglobin concentration less than 13g/dl in males and 12g/dl in females according to WHO. Anemia is disastrous and has been attributed to be an independent risk factor for cardiovascular disease outcome in patients with chronic kidney disease and severe heart failure. The toxic effects of lead poisoning are clinically treated by the use of chelation therapy which involves the formation of a metal ion complex in which the metal ion is associated with a charged or uncharged electron donor referred to as the ligand. Although newly synthesized chelating agents such as esters of DMSA (e.g monoisoamyl DMSA, monomethyl DMSA and monocyclohexyl DMSA) and combination therapy (use of two structurally different chelators) are currently being tried and employed in the treatment of lead poisoning, it is clear that most conventional chelators have been associated with many side effects such as nausea, stomach cramps, vomiting, chelation of essential ions (Iron, Magnesium) and setbacks which implies no safe and effective treatment available for lead poisoning. Since ancient times medicinal plants have been used as a major source of treatment for numerous human diseases. Despite modernization and advent of modern medicine and therapies, a WHO report reveals that about 80% of the world population still relays on traditional medicine for the treatment of various ailments. Plants that possess hematinic and antioxidant properties in the plant kingdom are often used using such scenarios. *Nigella Sativa* (Black Seed) is one of the most common herbal medicines that have been used several years as curative remedy for various disorders as reported by Hussain and Hussain, 2016 as curative remedy for various disorders. The seeds of *Nigella sativa* L. (Ranunculite) (NS) known as Black seed or Black cumin (“السوداء” or “حبة البركة”) have long been used in folk medicine in several part of the world. Hussain and Hussain reported that black seed contains over 100 healing components that work together to produce a synergetic effect. Phytochemical screening of *Nigella sativa* seed extract revealed many active components such as thymoquinone, alkaloids (nigellicines and nigelledines), saponins (Alpha-hederin), flavonoids, proteins, fatty acids, and several other elements that have positive effects in the treatment of patients with different diseases. Hematinics substances are essential to the proper formation of the components of blood. The high prevalence of lead poisoning and its severe health impacts call for the investigation of the condition so as to find effective measure of control and prevention. In this study, therapeutic effects of hydro-ethanolic extracts of *Nigella sativa* was assessed on hematological parameters of lead poisoned albino rats as curative therapy for treatment of lead poisoning.
MATERIALS AND METHOD
Collection and Identification of Plant Seeds
The seeds of *Nigella sativa* used in this study were purchased from the local market (Monday market) from an herbal vendor in Maiduguri Metropolitan Council, Borno state, Nigeria. The seeds were identified and authenticated by a plant taxonomist in the Department of Biological Sciences, Faculty of Science, University of Maiduguri.

Preparation and Extraction of Nigella *Sativa* Seeds
Black seeds were washed, dried at room temperature and grounded into fine powder. The grounded material (2000g) was then transferred into a stoppered container with 60% v/v ethanol and allowed to stand at room temperature for a period of 72 hours with frequent agitation until the soluble matter has dissolved.

The mixture was then strained and the damp solid material is pressed, the resulting suspension was filtered and concentrated using a rotatory evaporator with the aid of a vacuum pump. The concentrate was evaporated further to dryness in an oven at 40°C to obtain a powder and then stored in a desiccator. Percentage yield was determined.

Experimental Animals
A total normal of thirty-five (35) albino rats of both sexes weighing between 100-180g were used for this study. The rats were bred in the animal house of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Maiduguri. They were acclimatized under standard laboratory conditions and normal photoperiod (12h light: dark cycle) for 7 days. They were fed with commercial pellet diet and water *ad libitum* throughout the period of study. This study was approved by the ethical and research committee of the College of Medical Sciences, University of Maiduguri.

Lead intoxication of rats
The animals were administered sub-lethal dose of 60mg/kg body weight based on the reported oral LD$_{50}$ of lead acetate for Wistar rats$^{22,23}$ for a period of 36 days. Lead intoxication was established by estimation of packed cell volume ($\leq$25%) and Hemoglobin concentration ($\leq$7g/dl) in the rats.

Treatment of Lead Poisoned Rats
The lead poisoned albino rats were divided into groups and treated as follows. The *Nigella sativa* experimental groups received hydroethanolic extract of *Nigella sativa* seeds at doses of 200, 400, 800mg/kg b.wt. orally during the period of the experiment. The doses used were based on reports of previous studies$^{17,18,19}$

- **Group I (Normal Control)**
- **Group II (Negative Control)**
- **Group III:** received 10 mg/kg b.wt of standard drug (Meso-2, 3 dimercaptosuccinic acid).
- **Group IV:** received 200 mg/kg b.wt of *Nigella sativa* extract solution.
- **Group V:** received 400 mg/kg b.wt of *Nigella sativa* extract solution.
- **Group VI:** received 800 mg/kg b.wt of *Nigella sativa* extract solution.
- **Group VII:** (Recovery group)

At the end of 21 days of treatment, all the animals were anaesthetized with mild ether inhalation and sacrificed on the 22nd day (24hrs after last dosage). The blood samples for haematological analysis were collected through cardiac puncture after sedating the animals. The collected blood (2ml) were transferred into EDTA bottles, for the determination of the following haematological parameters; Red Blood Cell (RBC) count, Haematocrit (Packed cell volume), Haemoglobin concentration, White Blood Cell (WBC) count and Platelet counts, Granulocyte count, Monocyte and Lymphocyte count. Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) were calculated from the values obtained. The automated blood analyser Landwind, model LW D3600 was used for the determination of haematological parameters.
**Statistical Analysis**

The data obtained from the haematological parameters were expressed as Mean±SEM and subjected to one-way analysis of variance (ANOVA) after statistical analysis using GraphPad InStat version 3.0. Tukey Kramer's test was used for multiple comparison and values with P value <0.05 were considered significant.

**RESULTS**

The result obtained from the administration of graded doses of Hydro-ethanolic extracts of *Nigella sativa* seed on the haematological parameters are presented in the tables below;

**Table 1:** Effects of Hydro-ethanolic extract of *Nigella sativa* seeds on some haematological parameters of Lead Poisoned Albino rats.

<table>
<thead>
<tr>
<th>(Dose: mg/kg)</th>
<th>RBC count (x)</th>
<th>PCV (%)</th>
<th>Hb (g/dl)</th>
<th>Platelet count (x 10^5/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: Distilled water</td>
<td>6.81 ± 0.20</td>
<td>37.88 ± 1.20</td>
<td>12.12 ± 0.40</td>
<td>697.60 ± 39.18</td>
</tr>
<tr>
<td>Negative control: Pb acetate (60)</td>
<td>5.10 ± 0.26</td>
<td>24.63 ± 1.50</td>
<td>6.00 ± 0.22</td>
<td>1119.00 ± 23.95</td>
</tr>
<tr>
<td>DMSA (10)</td>
<td>6.80 ± 0.13</td>
<td>39.52 ± 1.30</td>
<td>12.00 ± 0.63</td>
<td>770.40 ± 53.27</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (200)</td>
<td>6.92 ± 0.31</td>
<td>41.78 ± 1.20</td>
<td>10.68 ± 0.33</td>
<td>887.20 ± 47.49</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (400)</td>
<td>7.04 ± 0.09</td>
<td>42.44 ± 0.95</td>
<td>12.00 ± 0.06</td>
<td>789.60 ± 57.15</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (800)</td>
<td>6.90 ± 0.10</td>
<td>39.64 ± 1.00</td>
<td>11.34 ± 0.06</td>
<td>846.80 ± 56.03</td>
</tr>
<tr>
<td>Recovery</td>
<td>6.68 ± 0.23</td>
<td>39.80 ± 0.31</td>
<td>8.52 ± 0.24</td>
<td>842.20 ± 11.09</td>
</tr>
</tbody>
</table>

*Note: The values are presented as Mean±SEM and n=5 for each group.

<p>| | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
</table>
| *a*,*b*,*c* represent statistically significant values between compared groups P<0.05.

**Table 2:** Effects of Hydro-ethanolic extract of *Nigella sativa* seeds and Lead acetate solution on Blood indices of Lead poisoned Albino rats.

<table>
<thead>
<tr>
<th>(Dose: mg/kg)</th>
<th>MCHC (%)</th>
<th>MCH (pg)</th>
<th>MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: Distilled water</td>
<td>32.04 ± 0.91</td>
<td>17.84 ± 0.50</td>
<td>55.64 ± 0.78</td>
</tr>
<tr>
<td>Negative control: Pb acetate (60)</td>
<td>24.52 ± 0.71</td>
<td>11.82 ± 0.48</td>
<td>48.52 ± 2.01</td>
</tr>
<tr>
<td>DMSA (10)</td>
<td>30.57 ± 2.17</td>
<td>17.83 ± 1.31</td>
<td>58.12 ± 1.20</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (200)</td>
<td>25.60 ± 0.79</td>
<td>15.56 ± 0.81</td>
<td>60.62 ± 1.58</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (400)</td>
<td>28.33 ± 0.65</td>
<td>17.05 ± 0.18</td>
<td>60.27 ± 0.86</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (800)</td>
<td>28.59 ± 0.94</td>
<td>16.42 ± 0.74</td>
<td>57.51 ± 1.51</td>
</tr>
<tr>
<td>Recovery</td>
<td>21.41 ± 0.63</td>
<td>12.79 ± 0.37</td>
<td>59.91 ± 2.32</td>
</tr>
</tbody>
</table>

*Note: The values are presented as Mean±SEM and n=5 for each group.

*ab* represent statistically significant values between compared groups P<0.05.
Table 3: Effects of Hydro-ethanolic extract of *Nigella sativa* seeds on White blood cell, Granulocytes, Lymphocytes and Monocyte count of Lead poisoned Albino rat

<table>
<thead>
<tr>
<th>(Dose: mg/kg)</th>
<th>WBC (x10^9/µl)</th>
<th>Granulocytes (%)</th>
<th>Lymphocytes (%)</th>
<th>Monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: Distilled water</td>
<td>1.54 ± 0.06</td>
<td>4.40 ± 0.49</td>
<td>88.44 ± 1.15</td>
<td>7.16 ± 0.84</td>
</tr>
<tr>
<td>Negative control: Pb acetate (60)</td>
<td>3.28 ± 0.24a</td>
<td>2.07 ± 0.14a</td>
<td>96.70 ± 1.10a</td>
<td>1.23 ± 0.23a</td>
</tr>
<tr>
<td>DMSA (10)</td>
<td>1.74 ± 0.26b</td>
<td>1.60 ± 0.13c</td>
<td>94.40 ± 1.16</td>
<td>4.00 ± 1.11</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (200)</td>
<td>2.48 ± 0.19a</td>
<td>2.40 ± 0.66a</td>
<td>91.54 ± 3.04</td>
<td>6.06 ± 2.53</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (400)</td>
<td>1.34 ± 0.12bcd</td>
<td>2.88 ± 0.56</td>
<td>90.10 ± 1.34</td>
<td>7.08 ± 0.89d</td>
</tr>
<tr>
<td><em>Nigella sativa</em> seed (800)</td>
<td>1.56 ± 0.07bcd</td>
<td>2.30 ± 0.35a</td>
<td>92.88 ± 0.51</td>
<td>4.82 ± 0.27</td>
</tr>
<tr>
<td>Recovery</td>
<td>2.42 ± 0.20b</td>
<td>1.28 ± 0.12a</td>
<td>93.36 ± 1.13</td>
<td>5.36 ± 1.16</td>
</tr>
</tbody>
</table>

*Note: The values are presented as Mean±SEM and n=5 for each group. a,b,c represent statistically significant values between compared groups P<0.05

DISCUSSION

The therapeutic effects of *Nigella sativa* seed extract on some haematological parameters in lead poisoned rats investigated in this study revealed that administration of 200 mg, 400mg, and 800mg/kg of *Nigella sativa* seed extract was able to reverse the adverse effect of Lead acetate on Red blood cell count, packed cell volume and haemoglobin concentration of the albino rats (Table 1). Although, the standard drug, Meso-2,3 Dimercaptosuccinic acid (DMSA) also reversed these parameters in the Lead poisoned albino rats, *Nigella sativa* seed extract especially at medium dose (400mg/kg. b.wt.) was more efficient. The recovery group without treatment with either DMSA or *Nigella sativa* seed extracts also showed an appreciable reversal of these parameters (RBC count, PCV and Hb concentration) but they were not restored to normal levels (Table 1), this is similar to the studies of Ekanem et al. and Nuhu et al. The increase in the RBC count as confirmed by an increased PCV and Hb concentration in lead poisoned rats treated with 200 mg, 400mg, and 800mg/kg of *Nigella sativa* seed extract could likely be due to the roles played by the flavonoids, folic acid, Fe, Zn and thymoquinone content of the extract which have been reported to have both stimulatory and hematinic effect on the bone marrow during blood cell formation while the chelating activity of DMSA (Standard drug) was responsible for the increase of these parameters in the DMSA treated rats. It is a conceivable fact that during tissue anoxia or hypoxia which is very common during most forms of anemia, there is an increased formation of erythropoietin (a glycoprotein) which stimulates an increased production of erythrocytes. This therefore, could likely be the cause of partial reversal of RBC count, PCV and Haemoglobin concentration towards normal seen in the recovery group which were left untreated.

The Red blood cell indices; Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) which demonstrate the state of erythrocytes in terms of shape, size and quantity of haemoglobin content distribution were all significantly reversed towards normal values...
in the lead poisoned albino rats treated with 200 mg, 400mg, and 800mg/kg of *Nigella sativa* seed extract and DMSA (Standard drug) (Table 2). This is not surprising as the determinants of this indices (RBC count, PCV and Hb Concentration) were all reversed to normal following treatment. However, the red cell indices in the recovery group without treatment failed to restore to normal because the RBC count, PCV and Hb concentration were not restored to normal during the recovery period of the study. This is in agreement with previous studies in which red cell indices were altered as a result of alteration of the determining factors (RBC count, PCV and Hb concentration) after treatment of animals with different plant extracts.\(^{29,30}\)

The red cell indices of lead poisoned albino rats treated with *Nigella sativa* seed extract and DMSA revealed a better restoration of their parameters when compared to recovery group without treatment. The findings in this study further revealed a significantly increased MCV value across all treated rats and may imply the presence of large number of reticulocyte in circulation. This perhaps might have accounted for the increased MCV values obtained in this study, although reticulocyte count was not estimated in this study, elevated reticulocyte count is an indicator of extensive presence of immature RBC in circulation to replace destroyed RBC.\(^{31}\) Reticulocytes are often known to be larger and present larger volumes (MCV) than matured red blood cells. This spontaneous increase in circulating reticulocyte count transport sufficient oxygen to meet cellular demands as result of substantial number of RBC destroyed by Lead poisoning. In view of the observed changes across group treated with graded doses of *Nigella sativa* seed extract, medium dose (400mg) seemed to exert maximal beneficial recovery effect on the blood indices than the DMSA treated and recovery groups. The improvement observed in the values of MCH across all groups treated with either graded doses of *Nigella sativa* seed extract or DMSA (Standard drug) could be attributed to the recovery seen in haemoglobin synthesis of the Lead poisoned rats when treated. Although there was increased MCH values across all the treated and non-treated groups (recovery), restoration of the values to normal were only observed at higher doses of NS seed extract (400mg and 800mg) and DMSA. This suggests a possible effective dose dependent treatment of Lead poisoning in rats. The MCHC values obtained from the rats treated with graded doses of *Nigella sativa* seed extract revealed a gradual and progressive recovery towards normal as the doses increased, DMSA treated animals showed a maximal recovery while MCHC values of the untreated rats failed to recover during the period of study. This could be attributed probably to the period of treatment observed in this study because; extracts from other plants have shown to be more effective in prolonged treatment time.

The Leucocytic profile in this study revealed a gradual restoration of total white blood cell count towards normal in Lead poisoned albino rats as a result of treatment with DMSA and graded doses of *Nigella sativa* seed extract (Table 3). Although an effective restoration was only observed in rats treated with DMSA and higher doses of the extract (400mg and 800mg), The Total leucocyte count (White blood cell count) of the non-treated rats (recovery group) were still at low levels below normal. The findings in this study also showed the percentage lymphocytes and monocytes counts in the treated rats were being gradually reversed to normal while the granulocytes value remained unchanged. In view of this, it is likely that the extract of *Nigella sativa* seed extract exerts an anti-inflammatory function since lymphocytes have been reported to play role during tissue inflammation. This is consistent with the study that suggests anti-inflammatory activities of *Nigella sativa* seed extract\(^{32}\) while the partial reversal in lymphocyte and monocyte count in experimental group treated with DMSA could be attributed to the chelation activity of DMSA.\(^{13}\) The non-significant change in values of granulocytes across all the groups suggests the presence of some amount of tissue damage and inflammation,
particularly as neutrophils which are a major component of granulocytes and the first responders during cell damage and inflammation are often destroyed during their activities. Lead poisoned albino rats treated with graded doses of *Nigella sativa* seed extract that had their Platelet count increased, was reversed towards normal (Table 1) especially at medium and high doses (400 mg/kg and 800 mg/kg). This suggests that *Nigella sativa* seed extract reversed the tissue damages caused by Lead acetate administered, as an increased platelet count have been reported to occur during Lead intoxication since platelets play important role in tissue repair and inflammation during Lead intoxication. Platelet counts were also reversed towards normal (Table 1) in rats treated with DMSA (Standard drug), which is attributed to its chelation activity on Lead in blood and soft tissue thereby reducing the level of Lead intoxication and consequently reducing the gross tissue damage by Lead acetate administered. The non-treated group also showed some degree of reversal of the platelet count towards normal.

This work has established that graded doses of *Nigella sativa* seed extract (200 mg/kg, 400 mg/kg and 800mg/kg) and DMSA (standard drug) showed almost equal effectiveness on the reversal of the increased platelet count of the lead poisoned rats, and therefore provided a greater therapeutic effect when compared to the recovery group without treatment of the Lead poisoned rats.

**CONCLUSION**

Administration of 200 mg/kg, 400 mg/kg and 800mg/kg of Hydro-Ethanolic extract of *Nigella sativa* seed offered therapeutic effects on haematological parameters and indices after lead exposure in albino rats.

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