Effects of Single and Combined Exposure of Albino Rats to Cadmium, Arsenic and Iron on some Haematological Parameters and Possible Abatement using Vitamin C and Bitter Leaf (Vernonia amygdalina)

1Sese Owei Ekaye, 2Martin Ehiabhi Akhigbe, 3Prosper Opute, 4Prekeyi Tawari-Fufeyin

1-4Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Keywords: Cadmium, Arsenic, Iron, Vitamin C, Bitter Leaf, Haematology

Acknowledgments: The authors are grateful to Dr. Eze, and Dr. Omorogie of the Anatomy department, University of Benin, Nigeria for their technical assistance.

Corresponding Author: 3Prosper Opute, ashibudike.opute@uniben.edu, +2348033644134
ABSTRACT

This study evaluated the effects of chronic toxicity of single, binary and trinary combination of cadmium, arsenic and iron on Rattus norvegicus. The use of synthetic vitamin C and bitter leaf (Vernonia amygdalina) as a compound abatement was assessed. 10-weeks old R. norvegicus were exposed concurrently to 200mg of CdSO$_4$, 200mg of Na$_2$HA$_3$O$_4$, 200mg of FeSO$_4$ per kilogram of feed as single and combined doses for 12 weeks. Vitamin C and bitter leaf were administered in equal proportions per week throughout the duration of the experiment. Haematological parameters were used as specific endpoints. The mean PCV values showed significant difference ($p < 0.05$) between control, treatment and abatement groups. The values for WBC were generally higher in control and abatement groups than the various treatment groups without abatement. WBC differentials of neutrophil, lymphocyte, monocyte, eosinophil showed significant difference at $p < 0.05$ for control, treatment and abatement groups. Combined exposure of R. norvegicus to cadmium, arsenic and iron showed more severe effects than single exposure suggesting metal interaction and antagonistic effects. However, abatement groups in both single and combined exposure showed a reduction in effects when compared to treatment groups without abatements. This study therefore revealed the antagonistic effects of the three metals and the potential of vitamin C and bitter leaf as abatement for heavy metal poisoning preferably in combination and on a long term basis.
INTRODUCTION

Exposure to heavy metals has continued to increase in some parts of the world particularly in less developed countries. Heavy metals are natural components of the earth’s crust that have relatively high density and cannot be degraded or destroyed (Mehtap and Ethem, 2006). As trace metals, some heavy metals are essential to maintain the metabolism of the human body, however, the presence in the environment of large quantities of toxic metals such as Cadmium, Arsenic, Mercury Lead, Zinc and others poses risks to humans (TOXINET, 2007). This puts the scientific community under pressure to develop new methods to detect and eliminate toxic contaminants from food, air and waste waters in efficient and economically viable ways (Carlos, 2007).

Cadmium is a byproduct of the mining and smelting of lead and Zinc. It is used in Nickel-cadmium batteries, PVC plastics and paint pigments. When inhaled or ingested and absorbed through the gastrointestinal system, target organs are the liver, kidneys lungs, brain, bones and the placenta (ASTDR, 2001). Arsenic is however released into the environment by smelting processes of Copper, Zinc and Lead, as well as manufacturing of chemicals and glasses. Other sources are paints, rat poisoning, fungicides and wood preservatives. It is most common cause of acute heavy metal poisoning in adults. Target organs are the blood, kidneys, Central nervous system, digestive and skin systems (Roberts, 1999; ATSDR, 2001). Iron is a heavy metal of concern, particularly because ingesting dietary iron supplements may acutely poison young children (Roberts, 1999). Iron deficiency affects over 600 million people throughout the world, particularly in developing countries (Latunde-Dada, 1990). The toxic effect is largely due to ingestion and rapid absorption in the gastrointestinal tract. Sources of iron are drinking water; foodstuff such as meats, vegetables, legumes, molasses, dried fruits etc, iron pipes and cookware while target organs include the liver, cardiovascular system and kidneys (Roberts, 1999).

Vernonia amygdalina popularly known as bitter leaf is a shrub of 2-5m tall with petiolate green leaves of about 6 mm diameters which are characteristically bitter (Ojiako and Nwayo, 2006). The plants have been proven in human medicine to possess antimalaria and antihelminthic properties (Abosi and Raseroka, 2003), as well as antitumourigenic and antiparasitic efficacy (Izevbigie et al., 2004; Huffman, 2003). It has also proven to be a successful supplement in weaning foods (Eleyinmi, 2005). Iwalokun et al, (2006) reported that V. amygdalina elicits hepatoprotectivity through antioxidant activity on acetamino-phen induced hepatic damage in mice. The leaves reported to be consumed by goat in some parts of Nigeria (Areghore et al., 1998) while extracts of V. amygdalina showed protection against thrombosis in mice (Awe and Makinde, 1998).
MATERIALS AND METHODS

ANIMALS AND HUSBANDRY
A total of 75 locally bred Wister rats of initial average body weight of 165g were obtained from the animal unit, Department of Animal and Environmental Biology, University of Benin. All animals were kept under the same standard laboratory conditions. They were housed in a wooden cage of 15 compartments and fed with growers mash poultry feed from the Bendel Flour and Feed Mill, Ewu. The animals had unlimited access to drinking water. Rats were used in this study after a 2 week acclimation period. No consideration was given to sexual selection.

CHEMICALS
All chemicals and reagents were of high purity. Cadmium sulphate, sodium arsenate, iron sulphate, nitric acid and perchloric acid used for intoxication, digestion and for atomic absorption spectrometry analysis were from BDH Chemical Company, Poole, England.

EXPERIMENTAL DESIGN
The experiment was conducted for 12 weeks. The 10–week–old rats, after the acclimatization period, were randomly divided into fifteen experimental groups of two each. The compartments were labelled A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, with Group O serving as control. Prior to metal exposure, the animals were starved for a period of 24 hours after which their weights were taken. Before the commencement of exposure, the toxicants were weighed using electronic weighing balance (Scout Pro SPU202, England). During the experimental period, the various chemicals were measured for the single, binary and trinary mixtures as follows: 200mg CdSO₄/kg of feed/ body weight, 200mg Na₂HAsO₄/kg of feed/ body weight and 200mg FeSO₄/kg of feed/ body weight.
The metals were administered orally and daily to the various groups by incorporating it in their feed. Before feeding, thorough mixing of the toxicants and feed was ensured. The various groups, toxicants and amounts administered are shown in Table 1.

**Table 1: Toxicant, feed and abatement schedule**

<table>
<thead>
<tr>
<th>Group</th>
<th>Contaminant(s)</th>
<th>Type of Feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>200mg CdSO₄</td>
<td>Feed only</td>
</tr>
<tr>
<td>B</td>
<td>200mg Na₂HASO₄</td>
<td>Feed only</td>
</tr>
<tr>
<td>C</td>
<td>200mg FeSO₄</td>
<td>Feed only</td>
</tr>
<tr>
<td>D</td>
<td>200mg CdSO₄</td>
<td>Feed + Abatement</td>
</tr>
<tr>
<td>E</td>
<td>200mgNa₂HAsO₄</td>
<td>Feed + Abatement</td>
</tr>
<tr>
<td>F</td>
<td>200mgFeSO₄</td>
<td>Feed + Abatement</td>
</tr>
<tr>
<td>G</td>
<td>200mg CdSO₄ + 200mg Na₂HAsO₄</td>
<td>Feed only</td>
</tr>
<tr>
<td>H</td>
<td>200mg CdSO₄ + 200mg FeSO₄</td>
<td>Feed only</td>
</tr>
<tr>
<td>I</td>
<td>200mg Na₂HASO₄ + 200mg FeSO₄</td>
<td>Feed only</td>
</tr>
<tr>
<td>J</td>
<td>200mgCdSO₄ +200mg Na₂HAsO₄</td>
<td>Feed + Abatement</td>
</tr>
<tr>
<td>K</td>
<td>200mgCdSO₄ + 200mgFeSO₄</td>
<td>Feed + Abatement</td>
</tr>
<tr>
<td>L</td>
<td>200mgNa₂HAsO₄ +200mg FeSO₄</td>
<td>Feed + Abatement</td>
</tr>
<tr>
<td>M</td>
<td>200mg CdSO₄ + 200mg Na₂HAsO₄ + 200mg FeSO₄</td>
<td>Feed only</td>
</tr>
<tr>
<td>N</td>
<td>200mgCdSO₄ + 200mgNa₂HAsO₄ +200mg FeSO₄</td>
<td>Feed + Abatement</td>
</tr>
<tr>
<td>O</td>
<td>NIL</td>
<td>Feed only</td>
</tr>
</tbody>
</table>
Abatement

The abatement feed consists of synthetic vitamin C tablets complexed with dried and powdered leaves of the plant *Vernonia amygdalina* (bitter leaf). The vitamin C tablets of 500 mg, produced by MAY and BAKER NIG. PLC was grinded into powdery form using laboratory mortar and pestle. The sun-dried leaf of bitter leaf plant was blended into powder. These were administered in equal portions of 2000mg of vitamin C and bitter leaf per week to the test animals. This was done throughout the duration of the experiment along with the diet. With the onset of exposure, feed were given in a ration of about 10g daily while water was administered *ad libitum*.

Collection of Blood Samples

Blood samples were collected immediately from the dissected wister rats using 2 ml disposable sterile syringes from the abdominal heart aorta and transferred into sterile EDTA bottles. The samples were kept in ice and immediately transferred to the haemotological laboratory for full blood count analysis.

Haematological Analysis

Small amounts of each blood sample were utilized for haematology values such as PCV, WBC and blood count differentials which include neutrophile, lymphocytes, monocyte, eosinophil and basophile. Haematological analysis was done as described by Cheesbrough (2000). After blood collection using plain capillary tube and centrifugation for 5 minute at 5000 rev/mm the Packed Cell Volume (PCV) was read using a Hawskley micro-haematocrit reader (model 850179, Great Britain). The White Blood Cells (WBC) counts and differentials were determined using Olympus Biological Microscopes (Models CHA ad CHB).
RESULTS

Hematological changes were evaluated for packed cell volume (PCV) white blood cell (WBC), blood differentials such as neutrophil, lymphocyte, monocyte, eosinophil and basophil.

![Graph](image)
Figure 1: Haematological parameters for control, single metals and abated groups
Figure 2: Haematological parameters for control, binary combination of metals and abated groups
Figure 3: Haematological parameters for control, trinary combination of metals and abated groups

**Packed Cell Volume (PCV)**

The value for PCV ranged from 30 ± 5.43% to 56 ± 1.82%. There was a general decrease in PCV values compared to control. Mean PCV was 48 ± 9.08%. The mean PCV values for control, treatment and abated groups showed significant differences with exposure groups ($F_{values} = 68.295$ df. 14) at p<0.05. There was slight increase in PCV value with abatement except in single dose of arsenic and iron and the trinary mixture where PCV values for abatement was lower than the treatment groups.

**White Blood Cell (WBC)**

WBC values for the various groups ranged from 3700 ± 158.11 cells/mm$^3$ to 15300 ± 370.14 cells/mm$^3$ in group N (trinary mixture) and group L with a binary combination of arsenic and iron. The mean WBC was 10065 ± 3689.12 cells/mm$^3$. The values for WBC were generally higher than control of 6800 cells/mm$^3$ except in groups F (48000 cells/mm$^3$) K (5300 cells/mm$^3$) and N (3700 cells/mm$^3$). WBC showed significant differences at p<0.005; ($F = 1181.479$).

**Neutrophil**

The range of values for neutrophil was 32 – 52% and the mean was 44 ± 7.14%. There was significant difference between the poisoned groups and control ($F= 29.874$) at p<0.05.

**Lymphocyte**

The values ranged from 40 – 48%. The mean value was 44 ± 4.06%. Significant difference occurred with toxic metals ($F= 6.460$) at p<0.05.
Monocyte

The values ranged from 4 – 13% with a mean of 8%. The values were generally higher than control (4%) except in group L (4%) with a binary combination of arsenic and iron. Significant difference occurred between control and the poisoned groups as well as the abatement groups (F= 7.142) at p<0.05.

Eosinophil

The value ranged from 2 – 9% in groups F and K respectively. The mean was 6% with a significant difference between control, poisoned and abated groups at p<0.05.

Figures 1, 2 and 3 illustrates the comparative changes in haematological parameters with single, binary, trinary metals for control, poisoned and abated groups.

DISCUSSION

The results from this study demonstrated that chronic doses of Cadmium, Arsenic and Iron when administered concurrently, through dietary intake in single, binary and trinary combination had adverse effects on the experimental animals.

HAEMATOLOGICAL PARAMETERS

Pack Cell Volume

The use of haematological parameters as an index of physiological and pathological status in humans and animals is well documented (Ogwumike, 2002). The most frequently investigated include haemoglobin, pack cell volume, white blood cell count and platelet count (Adeneye and Benebo, 2007). Accordingly, pack cell volume, total and differential white blood cell counts were evaluated. The results from this study clearly indicated that single, binary and trinary mixtures of the toxic metals significantly changed the blood parameters evaluated. The fundamental role of stem cells or progenitor cells in haematopoiesis is well documented (Weissman, 2000). The haematopoietic stem cells are the progenitor cells for erythrocytes, platelets and the various subsets of leucocytes, which develop in the bone marrow throughout life. In the rat marrow population, erythroid cells constitute 39%, myelopoietic 34%, lymphopoietic 24% and reticulum cells 3%. In wistar rats, the mean normal value range of PCV and total leucocytes count are documented to be 40.5 - 53.1% and 7,063 – 8,760 cell/mm3, respectively (Adeneye and Benebo, 2006). Several literature have also shown that oral ingestion of medicinal compounds or drugs can alter the normal range of these measured haematological parameters (Abatan and Arowolo, 1989). In this study, single, binary and trinary mixtures induced decrement in PCV (haematocrit) in the different groups when compared to the control. The difference in changes between the binary and trinary combinations of metals tended to be small in magnitude. The comparative changes between treatment and abated groups were shown to be inconsistent and statistically not significant. The reduced PCV value was suggestive of anaemia due to metal toxicity. Single doses of Cadmium and Arsenic have demonstrated haemolytic effect and are known to reduce pack cell volume (Andrew and Morgan, 1997; ATSDR, 1998). ATSDR (1999), similarly reported that Arsenic alone significantly decreased haematocrit with a concentration of approximately 2.5mg As/kg/day. Arsenic is reported to have toxic effects on the erythropoietic cells of the bone marrow and increases haemolysis.
Cadmium may inhibit heme synthesis indirectly by decreasing the absorption of iron from the gastrointestinal tract (ATSDR, 1999). These could be potentially responsible for the decrease in pack cell volume associated with the single doses. The magnitude of change between binary and trinary mixtures was small and showed inconsistent variations between the poisoned and abated groups. For example, in group F, the PCV value was significantly low, relative to the control (Fvalue = 68.295, df = 14 at p< 0.05). The factors responsible could include the interactions of Vitamin C, dietary iron, heavy metals and active components of *V. amygdalina* (Nicolas *et al.*., 2003).

The observed reduction in PCV suggested a decrease in the number of red cells and was indicative of both aplastic and particularly haemolytic anaemia (Cheesbrough, 2000). Apart from other several clinical factors in this study, these conditions could have resulted as a result of destructive effects of the toxic heavy metals used as poison.

**White Blood Cells**

The mean values of white blood cells were generally higher than that of the control. This condition is referred to as leucocytosis. The significant increase in WBC in the various groups with the exception of group F (given iron and abatement) and K (cadmium, iron and abatement) can be attributed to inflammatory responses and tissue necrosis due to the heavy metals (Cheesbrough, 2000). The observed leucopaenia (reduction in WBC) in group F was due to heavy metal effects on the haemopoietic system (Andrew, 2000). Agranulocytosis has been reported with heavy metal (Goodman and Gilman, 1980), but it can also be attributed to the toxic metals, hypersplenism and other factors such as infections and aplastic anaemia (Cheesbrough, 2000). Selective improvements observed in groups F, K and N in WBC of abated groups relative to poisoned groups showed likely effects of the abatement used. This is consistent with the findings of Ojiako and Nwajo, (2006) who reported that *V. amygdalina* was hepatoprotective in rat by inhibiting and even reversing carbon tetrachloride – induced hepatotoxicity.

**Neutrophil**

The changes in neutrophils when control and poisoned groups were compared, was small in magnitude and inconsistent for abated groups. The overall implication was a significant change in neutrophil indicative of the destructive effects of the heavy metals. The reductions were similarly lowest in groups F and K. A reduction in circulating neutrophils (neutropenia) occurs with bone marrow failure and toxic drugs treatments (ATSDR, 2000)

**Lymphocyte**

There was significant change in lymphocytes when compared to the control (Fvalue = 6.460 at p < 0.05). The percentage lymphocyte composition increased but showed inconsistent variations across treatment and abated groups. The increased number of circulating lymphocytes (lymphocytosis) was likely due to tissue damage and inflammation from the toxic metals as seen in their histopathology (Cheesbrough, 2000; Adeneye and Benebo, 2007).
**Monocyte**

The circulating monocytes increased with poisoned and abated groups when compared with the control. The trend suggested that the absolute increase in monocytes (monocytosis) and significant difference (F = 7.142, at p < 0.05) between control, poisoned and abated groups were due to toxic metals, their interaction and the effects of the used abatements. The circulating eosinophils increased. The data available for basophils were too scanty to permit for any form of inference.

ATSDR (1999) reported that anaemia was a common feature of cadmium intoxication in animals exposed orally or parenterally. This was attributed, to impaired intestinal absorption of iron and may be ameliorated by dietary supplementation with iron or ascorbic acid (Fox *et al.*, 1971). For Arsenic, Jolliffee (1991) observed that once absorbed, it is bound to haemoglobin, leucocytes and plasma protein before being distributed to tissues. This results in haemolysis with consequentes of anaemia. Furthermore, bone marrow depression/failure and haemolysis may result with Arsenic intoxication. Rezuke *et al.* (1991) explained that peripheral haematologic such as leucopenia, anaemia and thrombocytopenia are associated with Arsenic interaction. Cadmium is also known to have caused destruction of erythrocytes with no indication of either effect on haemoglobin synthesis or haemolytic anaemia reported in rabbits (ATSDR, 1999).
CONCLUSION

The findings obtained from this study provided insight on chronic toxic effects of single, binary and trinary mixtures of cadmium, arsenic and iron in terms of blood parameters. Oral exposure through feed for an intermediate duration resulted in significant haematological alterations which included general decrease in haematocrit (PCV), increase in white blood cells and marked variations in white blood cells differentials.

REFERENCES


Carlos Universidad Ray Juan (2007). New wastewater Treatment System Removes Heavy Metals. *Science Daily*


