An investigation on the Effects of *Napoleona imperialis* Methanol seed extract on Haematological parameters and Cardiac function enzymes in Albino Rats.

ABSTRACT

This study was aimed at determining the effect of methanol seed extract of *N. imperialis*on Haematological parameters and some cardiac function biomarker enzymes. The LD_{50} and antinutrient contents were also determined using standard methods. The experiment comprised of 25 albino rats divided in to 5 groups of 5 rats each in which group 1 was the control, Group 2 received 100 mg/kg, Group 3 received 200 mg/kg, Group 4 recieved 400 mg/kg andGroup 5 recieved 600 mg/kg of the methanol extract of *N. imperialis* seeds. The rats were administered the extract orally for 28 days. On day 29, after an overnight fast, blood samples were collected from ocular vein for analysis of haematological parameters and cardiac function biomarker enzymes. Results showed that the LD_{50} was 3605.55 mg/kg a significant decrease in PCV, WBC in the group given 600 mg/kg of the extract, RBC and platelets also decreased significantly in the test groups compared to the control. There was however no significant changes in the cardiac function enzymes; CK and LDH in all the experimental groups compared to the control. This research revealed that the methanol seed extract of *N. imperialis* posed some haemolytic effects on the blood manifested by significant decrease in haematological indices but no significant changes in the cardiac function enzymes.

Keywords: Antinutrients,Blood, Heart, *Napoleona imperialis,*Toxicity,

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1.0 INTRODUCTION

1.1 Background to the study

Blood is a vital and distinct circulatory tissue.Maintaining homeostasis is the major goal of this system, w hich consists of cells suspended in a fluid intercellular substance termed plasma (Isaac *et al.,* 2013).Tracki ng feed toxicity can be aided by haematological components, especially when feed additives affect the blo od and general health of farm animals.According to Oyawoye and Ogunkunle (2004), these components i nclude leucocytes, or white blood cells, mean corpuscular volume, mean corpuscular haemoglobin, and m ean corpuscular haemoglobin concentration.Erythrocytes, or red blood cells, are the cells that carry haemo globin.According to Johnson and Morris (1996) and Chineke et al. (2006), during respiration, this haemoglobin combines with the oxygen transported in the blood to generate oxyhaemoglobin.T he movement of carbon dioxide and oxygen throughout the body is facilitated by red blood cels, as stated by Isaac et al. (2013).According to Ugwuene (2011) and Soetan et al. (2013), a lower re d blood cell count therefore indicates a lower amount of oxygen that would be transported to the tissues as well as a lower amount of carbon dioxide that would be removed from the lungs.

The main jobs of white blood cells and their derivatives include producing or at least distributing antibodies in response to an immune response, fighting infections, and protecting the body from foreign invaders through phagocytocis.Accordingly, animals with low white blood cell counts are more susceptible to infection by diseases, whereas animals with high counts can produce antibodies during phagocytosis, which increases their resistance to illnesses (Soetan*et al*., 2013). Additionally, high counts increase an animal's ability to adapt to the local environment and conditions that are conducive to disease (Kabir*et al.,* 2011; Okunlola*et al*., 2012; Iwuji and Herbert, 2012 and Isaac *et al.,* 2013).Blood clotting may be aided by platelets.

Low platelet concentration raises the possibility of a protracted blood clotting process, which could lead to significant blood loss in the event of an accident (Okunlola *et al*., 2012).The proportion (%) of red blood cells in blood is known as the erythrocyte volume fraction (EVF), often known as the haematocrit (Ht or Hct).Packed Cell Volume is implicated in the transport of ingested nutrients and oxygen, as stated by Isaac et al. (2013).Better transportation is demonstrated by an increased packed cell volume, which leads to a rise in primary and secondary polycythemia.(Herbert and Iwuji, 2012)All vertebrates, with the exception of fish family members and invertebrate tissues, include haemoglobin, an iron-containing metalloprotein that transports oxygen in red blood cells (Maton*et al.*, 1993), excluding the tissues of invertebrates and the fish family, channichthyldae.The physiological role of haemoglobin is to carry carbon dioxide out of an animal's body and deliver oxygen to its tissues for the oxidation of food consumed, which releases energy for other bodily processes (Ugwuene, 2011; Omiyale*et al.*, 2012; Soetan*et al.,* 2013; Isaac *et al.,* 2013).According to Peters et al. (2011), previous studies have shown that hemoglobin-packed cell volume and mean corpuscular haemoglobin are significant indicators for evaluating circulatory erythrocytes.In addition to being useful markers of the ability of the bone marrow to create red blood cells, akin to what occurs in mammals, these markers are significant in the diagnosis of anaemia (Awodiet al., 2005; Chinekeet al., 2006).Furthermore, a high Packed Cell Volume (PCV) measurement may indicate either a decrease in the volume of circulating plasma or an increase in red blood cells (RBCs), according to a 2006 study by Chinekeet al.The values of mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration show the blood level conditions.A low level suggests anaemia (Aster, 2004).The most common type of blood cell, red blood cells, or erythrocytes, are the main source of oxygen (O_2) that is carried to the body's tissues by blood flow through the circulatory system in vertebrate species.As they squeeze through the body's capillaries, they take in oxygen in their lungs or gills and expel it.Haemoglobin, an iron-containing biomolecule that can bind oxygen and give blood its red colour, is abundant in the cytoplasm of these cells.Ford (2013)

Because of the complications and death that come with acute myocardial infarction (AMI), it is a major contributor to the burden of cardiovascular disease (Roger, 2007).The long-term progression of atherosclerotic lesions results in acute myocardial infarction.Reactive oxygen species (ROS) may be important in the pathophysiology of myocardial infarction, according to

the evidence (Leoper*etal*., 1991).ROS are generated during the reperfusion period after ischemia (Espat and Helton, 2000; Zweier *et al.,* 1987).According to Salvemini and Cuzzocrea (2003),RO S have the ability to react with unsaturated lipids and start the self-
perpetuating chain reactions that lead to lipid peroxidation in membranes.
According to Misra et al. (2009), there are two different forms of heart damage associated with A MI: ischemia injury and reperfusion injury, both of which cause heart cells' mitochondria to malf unction.It has been discovered that reduced myocardial perfusion is linked to diminished antioxi dative defence upon reperfusion (Kaminski et al., 2008).According to Fujii et al. (2002), oxidativ e stress can also result in the development of major side effects such left ventricular remodelling following AMI.While left ventricular function during AMI can be predicted by the redox status o f arterial blood (Ohsawa *et al*., 2004).Nearly every bodily tissue contains the enzyme lactate deh ydrogenase (LDH), which is essential to cellular respiration.

Even though LDH is widely distributed in tissue cells, tissue damage caused by illness or injury causes tissues to release more LDH into the bloodstream, which can be used as a screening tool f or tissue damage.A different enzyme that is also a more accurate measure of cardiac or muscle in jury is creatine kinase (CK).In the early stages of suspected ischemia myocardial damage, assays for serum AST, LDH, and CK are frequently carried out (Fontes*et al.*, 1999).According to Kazm iet al. (2009) and Mayret al. (2013), CK is a valid marker for predicting the size of the infarct an d left ventricular function during the acute phase as well as the later cardiac events following A MI.

When a person exercises or has a myocardial infarction, their serum can have elevated overall C K activity as well as elevated CK-
MB activity.When using laboratory techniques to confirm myocardial infarction, CK activity are essential.A myocardial infarction markedly increases serum CK activity.If the activity of CK-
MB accounts for 6-
25% of the total CK activity in serum, there is a significant risk of myocardial infarction.Serum CK activity is markedly and quickly elevated during myocardial infarction.About five hours after the infarction starts, activity starts to increase.After 10–
12 hours, the serum's CK activity reaches its peak (Fontes *et al.,* 1999).

Pyruvate and NADH+ are interconverted to L-lactate and NAD+ by the enzyme L-
lactate dehydrogenase (L-
LDH).The interconversion of Dlactate and ferricytochrome c to pyruvate and ferrocytochrome c is catalysed by H-lactate dehydrogenase (H-
LDH).In humans, lactate dehydrogenase (LDH) is a crucial enzyme.It manifests in several bodily areas, each of which has a distinct subunit configuration.One essential enzyme for anaerobic res piration is LDH.

So much efforts are been done through research to improve the quality of blood cells and heart tissues considering their metabolic roles in the system. Also, efforts are on the way to investigate

the nutritional potentials of *N. imperialis* seeds as an alternative or complimentary feed product for animals and humans although currently, the seeds are discarded once the pulp is eaten thereby drawing attention for further studies on the seeds. In this research, we investigated the toxicological effects of *Napoleona imperialis* on the haematological parameters and cardiac function marker enzymes.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

N. imperialis fresh fruits were collected from a habitat in Aku, Enugu State, Nigeria's Igbo-Etiti Local Government Area.Mr. Alfred Ozioko of the Bioresources and Development Centre, f ormerly the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, ide ntified and verified the plant materials.For reference, a specimen was placed in the Department's herbarium..

2.1.2 Animals

Adult wistar albino rats and mice were utilised in this study.They were acquired from the Univer sity of Nigeria, Nsukka's Animal House, which is part of the Faculty of Vertinary Medicine.They received a regular diet and access to clean water during their seven-day acclimation period in typical surroundings.

2.1.3 Chemicals and Reagents

Analytical-grade chemicals and reagents from Sigma Aldrich (U.S.A.), British Drug House (BDH), Burgoy ne (India), Harkin and Williams (England), Qualikems (India), Fluka (Germany), May and Baker (England) were all used in this study.All of the experiment's reagents were purchased kits made by Teco (TC) and RANDOX (U.S.A.).**2.2 Methods**

2.2.1 Experimental Designs of all Studies

Twenty five (25)adult albino rats were divided into five groups of five rats each and treated as follows;

Group 1: Normal control (1 ml/kg of normal saline), Group 2: 100 mg/kg of methanol extract of *N. imperialis* seeds, Group 3: 200 mg/kg of methanol extract of *N. imperialis* seeds, Group 4: 400 mg/kg of methanol extract of *N. imperialis* seeds, Group 5: 600 mg/kg of methanol extract of *N. imperialis* seeds. The rats were administered the extract orally for 28 days. On day 29, after

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an overnight fast, blood samples were collected from ocular vein for analysis of haematological parameters and cardiac function marker enzymes.

2.2.2 Preparation of the Plant Extract

The ripe and mature fruit's seeds were taken out of the pods and cleaned with fresh water.After b eing allowed to air dry at room temperature, the seeds were covered, then ground into a coarse p owder.300g of the ground powder was steeped in 1.5 litres of methanol for 72 hours at room tem perature, stirring now and then.The suspension was concentrated in a water bath set at 50˚C after being filtered through a mesh and Whatman No. 4 filter paper.The extract was stored in the refrig erator for later use.

2.2.3 Acute Toxicity Test

The Lorke (1983) approach was utilised to ascertain the median lethal dose (LD50).There are tw o stages to this method: phases 1 and 2, respectively.Nine rats were employed in the first phase.T hree groups of three rats each were formed from the nine rats.The extract was given to each anim al group at varying levels (10, 100, and 1000 mg/kg).For a full day, the rats were kept under obse rvation to look for signs of death.In phase two,three rats were used, they were distributed into three groups of 1 rat each. The rats were administered higher doses (1600, 2900 and 5000 mg/kg) respectively of the extract and then observed for 24 hours for behavior as well as mortality. At the end, the LD_{50} was calculated using the formula;

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where, D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality.

2.2.4 Determination of Phytates

Phytates were measured using Ali's (2009) colorimetric absorption technique at 420 nm.The solu tion that was used included 1.2% HCl and 10% Na2SO4, 0.6% HCl with 5% Na2SO4, and 3 mill ilitres of nitric acid.

2.2.5 Determination of Oxalates

This was carried out in compliance with ElOlemyet al.'s (1994) methodology.In summary, 1g of granulated zinc was added after 2g of the extract had been dissolved in 4 ml of water and 3 ml of strong hydrochloric acid.After the combination was heated in a boiling water bath for one minut

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e and left to stand at room temperature for two minutes, the supernatant solution was poured into a test tube that held 0.25 millilitres of a 1% phenylhydrazine hydrochloride solution.After the liq uid quickly cooled after boiling, it was put into a glass cylinder and an equivalent volume of stro ng hydrochloric acid was added.After thoroughly mixing 0.25 ml of a 5% potassium hexacyanof errate (III) solution, it was let to stand for 30 minutes.In a 10 mm cell, the absorbance was measu red at 520 nm. The results were compared to a standard solution made in the same way, which co nsisted of 1 ml of oxalic acid standard solution diluted with 3 ml of deionized water. The standar d solution was made by dissolving a calculated amount, in mg, equivalent to the limit specified f or oxalic acid or oxalic acid dihydrate in 1000 ml of deionized water.

2.2.6 Determination of Cyanogenic glycosides (HCN):

Using Onwuka and Olopade's alkaline picrate technique, the cyanogenic glycoside was identified (2005).Weighing the 2.0 g ground sample, it was dissolved in 50 cm3 of distilled water.After an overnight stay, the cyanide extraction was filtered.

Preparation of cyanide standard curve:

KCN solutions with cyanide contents ranging from 0.1 to 1.0 mg/mL were made.4 mL of an alka line picrate solution (1 g of picrate and 5 g of Na_2CO_3 in 200 cm3 of distilled water) was added t o 1 mL of the sample filtrate and standard cyanide solution in test tubes, and the mixture was the n incubated in a water bath for 15 minutes.Following colour development, the absorbance was m easured at 490 nm in comparison to a blank that contained 4 cm3 of alkaline picrate solution and 1 mL of distilled water alone.The cyanide standard curve was used to extrapolate the cyanide con tent.

Calculation:

$$\text{Cyanogenic glycoside (mg/100 g)} = \frac{\text{C (mg)} \times 10}{\text{Weight of sample}}$$

Where,C (mg) = Concentration of cyanide content read off the graph

2.6.7 Determination of Packed Cell Volume (PCV):

Ochei and Kolhatkar (2008) provided a standard method for determining the packed cell volume. The idea is that greater molecular weight red blood cells and other blood components settle to the top, where the supernatant plasma may be separated.In short, capillary action was used to draw

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blood samples from the rats into heparinized PCV hematocrit tubes.After the blood was collected , one end of the tube was sealed with plasticine and spun for five minutes at 3000 rpm using a hematocrit centrifuge.A PCV hematocrit reader was used to read the test result.

2.6.8 Haemoglobin Estimation (Hb)

The method developed by Miale (1972) was used to determine the Hb.Red blood cells can be hydrolyzed by Drapkin's solution, releasing haemoglobin for counting.In a nutshell.Twentyµl of well combined anticoagulated whole blood was added to a 4 millilitre Drabkins solution in a test tube.After inverting the tube, it was incubated for five minutes at 25ºC.At 540 nm, the solution's absorbance was measured in relation to a reagent blank.Haemoglobin concentration was determined by multiplying absorbance by a factor of 36.8.

$$Hb = A_{540} \times 36.8$$

2.6.9 Determination of Total White Blood Cell (WBC) Count:

The White Blood Cell Count was calculated by applying the Dacie and Lewis (1991) technique. White blood cells remain unaltered after being hydrolyzed by Turk's solution (2% glacial acetic acid), which allows them to be counted.To put it briefly, Turk's solution (2% glacial acetic acid) was used to dilute the blood sample (1:20).Using a Pasteur pipette, the diluted material was placed into a Neubaer counting chamber.By counting the necessary number of squares on the counting chamber using a (×1000) magnification under a microscope, the total WBC was determined.

2.6.10 Determination of Red Blood Cell Count

The standard haematological technique outlined by Ochei and Kolhatkar (2008) was used for this .Red blood cells are easily counted in the Neubaeur counting chamber because they are big and noticeable under a microscope.Briefly put, 10% Na2CO3 solution was used to dilute well-mixed anticoagulated blood 1:20.After that, the mixture was put into a modified Neubauer counting chamber.To calculate the overall number of red cells, appropriate squares were tallied and combined together.

2.6.11 Determination of Platelets Count:

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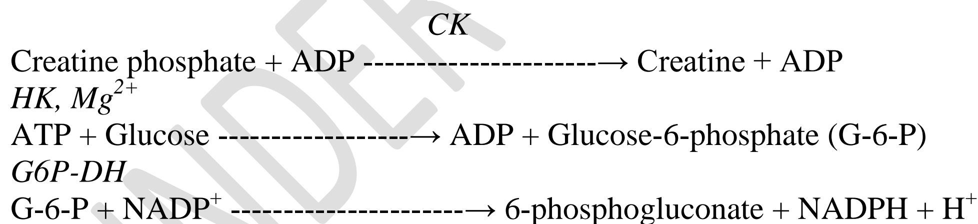
The methodology outlined by Umarani and Shashidhar (2016) was followed in this instance.Leishman stain readily stains platelets, making them visible enough to count.In summary, Leishman stain was applied to thin smears that had been airdried from all of the samples.These PBS (platelets blood stains) were viewed with an x100 oil immersion lens under a light microscope.Platelets were counted concurrently with RBCs in a monolayer zone of the smear until 1000 RBCs were counted.Thus acquired, the number of platelets per 1000 RBC was multiplied by 15000.

2.6.12 Assay of Creatine kinase Activity

The RANDOX handbook for CK estimation was followed in order to perform a spectrophotometric assessment of the activity of creatine kinase (CK) using the IFCC (2002) method.The IFCC approach has been modified to create this CK process.1 and 2.In order to produce creatine and adenosine triphosphate (ATP), CK reversibly catalyses the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP).Using the ATP created, glucose is converted to glucose6phosphate and ADP.Hexokinase (HK), the catalyst for this reaction, needs magnesium ions to function at its best.The coenzyme nicotinamide adenine dinucleotide (NADP) is reduced concurrently with the oxidation of glucose-6-phosphate by the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), producing NADPH and 6-phosphogluconate.The amount of CK present in the sample directly correlates with the rate at which absorbance increases at 340/660 nm as a result of NADPH production.



Room temperature was used for the test.In summary, the sample (0.1 ml and 0.4 ml) macro and semimicro, respectively, and the macro and semimicro, 2.5 ml and 1.0 ml, respectively, of the enzymes/coenzymes/substrate were mixed and incubated for 3 minutes. The first absorbance, A1, was measured at 340 nm, and the final absorbance, A2, was measured at 340 nm, three minutes later.This is how the creatine kinase activity was determined:CK activity (unit/L) = (A2 - A1) × 4127

2.6.13Assay of Lactate Dehydrogenase Activity

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This was carried out using the RANDOX manual's instructions for LDH determination, which ar e detailed in the (IFCC, 2002) technique.The following reaction scheme forms the basis of the pr ocedure.

LDH

L-lactate + NAD^+ --------------------------------→ Pyruvate + NADH + H^+

The increase in absorbance at 340 nm is a direct indicator of the LDH catalytic activity, which is correlated with the rate of NADH production.The IFCC's standard techniques for evaluating the catalytic activity of enzymes at 37ºC are followed in order to optimise test concentrations.Reage nt A (100 µL) and the samples (4 µL) were pipetted into the cuvette.Reagent B (20 µL) was adde d to the mixture after it had been incubated for 30 seconds at 37ºC.After giving the mixture a full 120 seconds of incubation, the lactate dehydrogenase was estimated as follows:LDH (U/L) = $(A_2 - A_1)/\text{min.} \times 6230\ M^{-1}\ cm^{-1}$

2.7 Data analysis

The data obtained were analyzed using one way ANOVA in SPSS version 23.0 for the mean and standard deviations followed by post Hoc test for the level of significance between groups was determined using Duncan test. The significant level was set at $p<0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.1.1 Qualitative and Quantitative Anti-nutrient Composition of Methanol Extract of *Napoleonaimperialis* Seeds.

The qualitative anti-nutrients composition of the seed extract include hydrogen cyanide, phytates, tannins and oxalates. Cyanide (HCN) had the highest concentration followed by Saponins. Others were found in low concentration.Quantitave determination of anti-nutrients showed that the amount of Cyanide (HCN) was 18.74±3.57 g/100g, phytates, 1.56±0.31, Tannins, 1.35±0.61 and oxalates 0.85±0.19 g/100g.

Table 1: Qualitative and quantitative anti-nutrients composition of methanol extract *Napoleonaimperialis* seed extract

Anti-nutrient	Qualitative composition	Quantitative composition (g/100mg)
HCN (mg/kg)	+++	18.74±3.57

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Phytates	+	1.56 ±0.31
Tannins	+	1.35±0.61
Oxalates	+	0.85±0.19

Results are expressed in mean ± SD (n = 3), + = Present in low amount, ++ = Present in moderate amount, +++ = Present in high amount

3.1.2 Median Lethal Dose (LD_{50}) of Methanol Extract of *N. imperialis* Seeds

No mortality was observed in the mice treated with different doses of the extract in phase one.

Table 2: Phase 1 acute toxicity studies of methanol extract *Napoleonaimperialis* seed extract

S/NO.	Dose (mg/kg b.w)	Mortality
1	10	0/3
2	100	0/3
3	1000	0/3

The phase two showed one death at the highest dose of 5000 mg/kg b.w. (table 3) hence the LD50 of the extract was calculated to be 3605.55 mg/kg

Table 3: Phase 2 acute toxicity studies of methanol extract *Napoleonaimperialis* seed extract

S/NO.	Dose (mg/kg b.w)	Mortality
1	1900	0/1
2	2600	0/1
3	5000	1/1

3.1.3 Effect of methanol extract of *N. imperialis* seeds on haematological parameters

Table 4 shows non-significant ($p > 0.05$) decrease of PCV in the test groups (2- 4) compared to PCV of the control rats in group 1 except group 5 rats that were administered 600mg/kg b.w of the extract which was significantly ($p < 0.05$) lower compared to the control.There was no significant ($p > 0.05$) decrease in the Hb of the treated groups (2, 3and 5) compared to the

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control group while group 4 rats showed slight increase in value of Hb but this was also found to be non-significant ($p > 0.05$). For WBC, a non-significant ($p > 0.05$) decrease in the test groups compared to the control was observed, except group 5 rats that were administered 600mg/kg b.w extract which shows significant ($p < 0.05$) decrease, as shown in table 4 above.RBC of the rats was found to be significantly ($p < 0.05$) lower in all the test groups compared to the RBC of the control. There was also a significant ($p < 0.05$) decrease in platelets count in the test groups compared to the control.

Table 4: Effect of methanol extract of *Napoleonaimperialis* seeds on haematological profile in rats

Treatment Group	**Haematological Indices**				
	PCV (%)	**Hb Conc. (g/dl)**	**WBC Count (10^{12}/L)**	**RBC Count (10^6/mm^3)**	**Platelet Count (10^6/mm^3)**
Group 1	48.40±5.46^a	17.80±1.92^b	7492.20±384.90^c	282.6±37.45^d	192.00±78.61^k
Group 2	46.20±5.89^a	14.00±2.24^b	6180.00±258.84^c	169.8±13.37^e	94.40±5.18^l
Group 3	46.60±5.18^a	15.40±3.21^b	5420.00±496.99^c	162.2±8.47^f	90.60±11.15^m
Group 4	42.20±8.14^a	16.80±5.89^b	5080.00±881.48^c	195.6±75.04^g	75.40±14.69^n
Group 5	29.80±15.2^d	18.80±6.79^b	3780.00±192.95^e	194.0±96.54^h	61.60±17.16^o

Results are expressed in Means ± SD (n = 5). Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$, Group 1 = Normal Control, Group 2 = 100 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds, Group 3 = 200 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds, Group 4 = 400 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds, Group 5 = 600 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds

3.1.4 Effect of Methanol Extract of *N. imperialis* seeds on Cardiac function markers

As shown in Table 5, a non-significant ($p > 0.05$) increase in the activities of CK was observed in all the test groups compared to control.LDH in all the treated groups compared to the control was observed to increase non-significantly ($p > 0.05$) when compared to the control as shown in table 5.

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Table 5: Effect of Methanol Extract of *Napoleonaimperialis* seeds on Cardiac markers of Rats

Treatment	**Enzyme Activity**	
Group	**Creatine Kinase (IU/L)**	**LDH (IU/L)**
Group 1	37.28±10.38[a]	3.01±1.35[b]
Group 2	46.28±6.89[a]	13.78±6.16[b]
Group 3	42.45±2.22[a]	6.99±3.13[b]
Group 4	43.12±4.62[a]	5.38±2.4[b]
Group 5	47.42±3.64[a]	4.62±2.06[b]

Results are expressed in Means ± SD (n = 5). Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$, Group 1 = Normal Control, Group 2 = 100 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds, Group 3 = 200 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds, Group 4 = 400 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds, Group 5 = 600 mg/kg b.w. of methanol extract of *Napoleonaimperialis* seeds

3.2 Discussion

This study found that the N. imperialis methanol seed extract included phytates, oxalates, tannins , and hydrogen cyanide.The concentration of cyanide (HCN) was highest, followed by saponins. Some were discovered in trace amounts.The results of the quantitative analysis of anti-nutrients revealed that the amounts of phytates (1.56±0.31), oxalates (0.85±0.19 g/100g), tannins (1.35±0.61), and cyanide (HCN) were as follows.Given that CN is known to disrupt the respiratory chain that provides energy, resulting in a significant and lethal energy crisis, the presence of HCN and other anti-nutrient chemicals in the seed extract may have contributed to the death of the rat given 5000 mg/kg in the phase 2 LD50 (acute toxicity) test.According to Auwal et al. (2012), oxalates and phytates have been shown to prevent the body from absorbing certain minerals, rendering them inaccessible for use by the body.This suggests that the seed extract may not be suitable for ingestion by humans or animals at that dosage (5000 mg/kg b.w.).The haematological parameters' results revealed a marked decline in the quantity of red blood cells.This is consistent with the findings previously presented by Ukpabi et al. (2003), which suggested that large doses of the extract could c

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ause hypochromic macrocytic anaemia in the rats.According to Ukpabi et al. (2003), N. imperialis seeds may lyse erythrocytes 100% of the time.According to Smith et al. (1974), the release of immature erythrocytes in reaction to circulating red blood cell breakdown typically results in an increase in mean corpuscular volume (MCV) and a decrease in the proportion of Hb per erythrocyte. For the WBC, the values decreased significantly at 600 mg/kg b.w which might be an indication that high dose of the extract gradually diminished the ability of the rats to fight the intruder (the extract) because it showed significant ($p< 0.05$) decrease in WBC (3780±192) compared to the control (7492.20±384.90). According to Afolayan and Yakubu (2009), this might be the result of decreased leucopoiesis in the bone marrow and could have an impact on the immune system and the phagocytic activities of the animal blood cells.The repair of minute fractures in capillaries and other small vessels may be hampered by the notable decrease in platelets that has been seen (Guyton and Hall, 2016).Because platelets are essential for preventing blood loss and healing vascular damage, chronic highdose treatment of the extract may cause extensive bleeding because of coagulation deficiencies.

Hypertension is linked to creatine kinase, also referred to as creatine phosphokinase.According to Hropot et al. (2003), rats given nitric oxide inhibitors had considerably higher systolic blood pressure due to the systolic enzymes' (LDH and CK) activity.Rat systolic blood pressure was not adversely affected by the plant extract at the levels given, as evidenced by the lack of a significant increase in CK values in the treatment groups when compared to the control group.

Practically all bodily tissue's cells contain the enzyme lactate dehydrogenase (LDH).It catalyses the simultaneous interconversion of NADH and NAD+ with the interconversion of pyruvate and lactate.(Gropper et al.1900a)

LDH can enter the circulation through cellular damage in tissues that contain it.Certain disorders can be diagnosed more easily when the various isoenzymes of LDH in the blood are analysed (Groff and Gropper, 2000b).According to Iemitsu et al. (2003), spontaneously hypertensive rats had significantly higher levels of LDH mRNA expression on the glycolytic pathway in the heart when compared to control rats.There is evidence linking the release of lactate dehydrogenase (LDH) to injury to heart tissue.An elevated level of LDH may indicate myocardial injury.Table 5 above indicates that there was no significant ($p>0.05$) difference in the LDH values between the treated

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groups and the control group when the plant extract was used. This suggests that the doses of the seed extract that were used did not have any negative effects on heart function.

4.0 Conclusion

This study evaluated the effects of *N. imperialis* methanol seed extract on haematological parameters and cardiac function enzymes. Some antinutritional content of the seed extract was also determined. The results revealed the presence of hydrogen cyanide in high amounts, phytates, tannins and oxalates. The LD50 was calculated to be 3605.55 mg/kg, group 5 rats that were administered 600mg/kg b.w of the extract showed significant ($p < 0.05$) decrease in PCV, WBC, RBC and platelets count in the test groups compared to the control. CK and LDH however showed no significant changes due to *N. imperialis* methanol seed extract administration, indicating that the methanol seed extract may exert haemolytic activity on blood cells but no toxic effect on cardiac tissues.

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