EFFECTS OF *VERNONIA AMYGDALINA* ON HEMATOLOGY PARAMETERS IN WISTAR RATS SUBJECTED TO 1-NITROPYRENE EXPOSURE

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IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES DEGREE IN MEDICAL BIOCHEMISTRY

FEBRUARY, 2025

ERTIFICATION

We the undersigned hereby certify that Mary Ese AKHIMIEN (BMS2008998) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

_____ _____

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(Head of Department)

_____ _____

External Examiner **Date**

DEDICATION

To the Hands that Shaped Me

With deepest gratitude and love, I dedicate this project to those who have profoundly impacted my life and academic journey. To my loving parents, Mr. and Mrs. AKHIMIEN, your unwavering support and guidance have been a constant source of strength. To the Department of Medical Biochemistry and the National Association of Medical Biochemistry Students (NAMBS), Uniben Chapter, I appreciate the knowledge, mentorship, and camaraderie that have shaped me into a better biochemist. May this project be a testament to the love, hard work, and dedication that have brought me thus far.

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ABSTRACT

1-Nitropyrene (1-NP), a widespread environmental pollutant, is known to induce oxidative stress and hematological alterations. This study investigated the protective role of *Vernonia amygdalina* (VA) against 1-NP-induced hematological toxicity in female wistar rat. Our results showed that 1-NP significantly altered hematological parameters, including red blood cell count, hemoglobin, hematocrit, white blood cell count, and platelet count. Co-administration of *V. amygdalina* extract significantly attenuated these 1-NP-induced changes, particularly at a dose of 100 mg/kg. These findings suggest that *V. amygdalina* possesses potential protective effects against 1-NP-induced hematological damage, possibly due to its antioxidant and anti-inflammatory properties. This study provides new insights into the therapeutic potential of *V. amygdalina* in mitigating the adverse effects of environmental pollutants.

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INTRODUCTION

1.1 BACKGROUND OF STUDY

A common medicinal plant in many traditional medical systems, bitter leaf (Vernonia amygdalina L.), is commonly used in African and Asian traditional medicine. As a result of it's numerous medicinal applications, this plant has been shown to have antibacterial, anticancer, antidiabetic, and anti-inflammatory qualities (Ogidi, 2019). To maximize the optimum potential of medicinal plants, it is essential to understand how their phytochemical content and antioxidant activity vary depending on the solvent used during extraction (Wenli *et al*., 2023). Due to their strong antioxidant properties, phenolics and flavonoids are the major bioactive chemicals that bring about these health benefits (Ma'aruf *et al*., 2024 and Egharevba *et al*., 2020)

In medical or biological field, oxidative stress is a physical, mental or emotional factor that results to bodily or mental tension. In this study, we will be working on the effects of 1-Nitropyrene; an environmental pollutant responsible for oxidative stress. There are different kinds of stress and these could be from our regular routine stress to sudden negative change stress and even to traumatic stress, and these are usually accompanied with physical and mental health risks (Jaap *et al* ,2011)

The pharmacological study of Vernonia amygdalina (V. amygdalina) have been reported by Abosi *et al* (2003) to demonstrate antihelmintic and antimalarial properties, antitumorigenic properties by Izevbigie, *et al* (2004), analgesic and antipyretic activities by Tijjani *et al* (2017), hypoglycemic and hypolipidaemic effect in experimental animals. The biologically active phytoconstituents from V. amygdalina are alkaloids, flavonoids, terpenes, saponins, coumarins, xanthones, phenolic acids, lignans, steroids,

anthraquinones as reported by Tona *et al* (2004), edotides (Izevbigie *et al*,2003), and sesquiterpene lactone. Despite the vast traditional use of V. amygdalina, it is still thought-about as amongst the beneath utilized crops of economic significance.

Considering the employment of the ironweed amygdalina leaves by the overall public, this research thus seeks to verify whether or not the leaves of the plant once employed in the management of diabetes will reverse the effects of oxidative stress caused by the environmental pollutant 1- Nitropyrene as seen on it's influence on the hematological parameters of the blood.

1-Nitropyrene (1-NP) is a known environmental pollutant that has been shown to induce oxidative stress, DNA damage, and hematological alterations in experimental animals. Exposure to 1-NP has been linked to an increased risk of hematological disorders, including anemia, leukemia, and lymphoma. Vernonia amygdalina, a plant commonly used in traditional medicine, has been shown to possess antioxidant, anti-inflammatory, and antimutagenic properties. However, its potential to mitigate 1-NP-induced hematological alterations has not been fully explored. This study aims to investigate the protective effects of Vernonia amygdalina on hematology parameters in Wistar rats subjected to 1-NP toxicity. The findings of this study may provide valuable insights into the potential use of Vernonia amygdalina as a therapeutic agent for the prevention and treatment of hematological disorders associated with 1-NP exposure.

1.2 Aim of the Study

The aim of this study was to investigate the protective effects of Vernonia amygdalina on hematology parameters in Wistar rats subjected to 1-nitropyrene (1-NP) toxicity.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Vernonia amygdalina*

Since ancient times, plants have been used to treat various ailments but due to lack of proper documentation, the knowledge was not properly transferred (Ajila *et al*., 2012). *Vernonia amygdalina*, commonly known as bitter leaf, is a shrub or small tree belonging to the Asteraceae family (Iwu, 1993). It is native to tropical Africa and is widely cultivated for its medicinal and nutritional properties (Oboh, 2005). However, recently various pharmacological researches have been reported for different types of medicinal plants (Omede *et al*., 2018). Phytochemicals such as flavonoids, alkaloids, saponins, phenolics amongst others are responsible for the medicinal properties of this plant species (Omede *et al*., 2018). The plant *Vernonia amygdalina* (Asteraceae) has a common name 'bitter leaf' due to the fact that it is bitter to taste. However, the leaf is delicious in soup. The plant is about three feet or more tall and grows plentifully in moist places. The leaf medicinal and nutritional properties cannot be underestimated (Omede *et al*., 2018). Anti-bacteria, anti- malaria, anti-cancer and more recently antioxidant activities of some of parts of the plant has been reported in literature (Omede *et al*., 2018). The leaf is applied in the treatment of scurvy, rheumatism, pile, indigestion, blood sugar control amongst others (Ajila *et al*., 2012).

2.1.1 Taxonomy

The taxonomy of the *Vernonia amygdalina* popularly known as bitter leaf is as follows:

Kngdom: Plantae

Phylum: Angiosperms

Class: Eudicots

Order: Asterales

Family: Asteraceae

Genus: Vernonia

Species: *Vernonia amygdalina*
(plants of the world online,2020)

Figure 2.1: *Vernonia amygdalina*(Asteraceae)

V. amygdalina is a shrub or small tree belonging to the Asteraceae family (also known as the daisy or sunflower family) (Ijeh *et al*., 2011). It typically grows to a height of 2-5 meters (6.6-16.4 ft), though it can sometimes reach up to 7 meters. The bark is light grey or brown, rough, and longitudinally flaking, with brittle branches. The leaves are

elliptical or oblong-lanceolate, measuring 10-15 cm in length and 4-5 cm in width, with a characteristic bitter taste (Akah *et al*., 1992). They are medium to dark green, sometimes with visible red veining (Sileshi *et al* 2024). The plant produces small, creamy-white, thistle-like flower heads in dense clusters.

2.2 Botanical Characteristics of *Vernonia amygdalina*

Habit: *V. amygdalina* is a perennial plant that can grow up to 5 meters tall. It has an erect or scrambling growth habit

Leaves: The leaves are simple, elliptical to lanceolate, with serrated margins. They are typically 5-20 cm long and 2-8 cm wide. The leaves have a characteristic bitter taste, hence the common name "bitter leaf".

Flowers: The flowers are small, white, and arranged in terminal or axillary panicles. They are hermaphroditic (possessing both male and female reproductive organs).

Fruit: The fruit is a small, dry achene (a type of simple dry fruit).

2.2.1 Traditional Uses of *Vernonia amygdalina*

Medicinal Uses: V. amygdalina has a long history of traditional medicinal use in various African communities. It is used to treat a wide range of ailments, including:

Malaria: Extracts of V. amygdalina have demonstrated antimalarial activity in vitro and in vivo (Odugbemi *et al*., 2007).

Diabetes: Studies have shown that V. amygdalina can help lower blood glucose levels (Akah *et al*., 2004).

Gastrointestinal Disorders: It is used to treat stomach aches, diarrhea, and other gastrointestinal problems (Gill, 1992).

Wound Healing: The leaves are sometimes applied topically to promote wound healing (Du-Bois *et al*., 2024).

Nutritional Uses: The leaves of *V. amygdalina* are consumed as a vegetable in various African dishes. They are a good source of vitamins, minerals, and antioxidants (Iwara *et al*., 2013). The bitter taste is often reduced by boiling or washing the leaves before consumption.

Table 1.1: Ethnobotanical Uses of *Vernonia amygdalina* Extracts

Extract Type	Reported Medicinal/pharmacological benefits	References
Aqueous Extract	Antidiabetic effects(lowering blood glucose) Antiinflammatory properties Hepatoprotective effects(protects the liver)	Adedapo,*et al*(2014) Adeniyi,*et al*(2006) Akah,*et al*(1992) Atangwho,*et al*(2007)
Ethanolic/methanolic extract	Anticancer activity(prevents the growth of cancer cells) Antimalarial activity(prevents the growth of malaria parasite) Antimicrobial activity(prevents the growth of bacteria and fungi)	William,*et al*(2017) Iwalokun,*et al*(2008) Oboh,*et al* (2010)
Chloroform extract	Antioxidant and hepatoprotective	Ojiako,*et al* (2006)

	effects	
Hydroxy-ethoxy-vernolide A(HEVA)	Antioxidant action Exhibits antidiabetic effects Anticancer	Iwalokun,*et al*(2006) Akah,*et al*(1992)

2.3 Physiological Properties of *Vernonia amygdalina*

2.3.1 Antioxidant Properties

V. amygdalina is a rich source of various bioactive compounds, including flavonoids, phenolic acids, and saponins, which contribute significantly to its antioxidant potential (Oboh, 2005). These compounds act as scavengers of free radicals, protecting cells against oxidative stress and damage (Farombi, 2003). Studies have demonstrated the strong antioxidant activity of V. amygdalina extracts in various in vitro assays, including DPPH radical scavenging, ABTS radical scavenging, and ferric reducing antioxidant power (FRAP) assays (Iwalokun *et al*., 2006). This antioxidant activity may play a crucial role in the plant's traditional use for treating various diseases associated with oxidative stress.

2.3.2 Anti-inflammatory Properties

Inflammation is a complex biological process involved in various diseases. V. amygdalina has demonstrated promising anti-inflammatory effects in several studies. Extracts from the plant have been shown to inhibit the production of pro-inflammatory mediators, such as nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α), in vitro (Adedapo *et al*., 2014). Additionally, studies have reported the effectiveness of V. amygdalina extracts in reducing inflammation in vivo models of inflammation. These

findings suggest that V. amygdalina may have therapeutic potential for managing inflammatory conditions.

2.3.3 Antimutagenic Properties

Mutagens are agents that can cause DNA damage and increase the risk of mutations, which can lead to various diseases, including cancer. V. amygdalina has exhibited antimutagenic activity in several studies, suggesting its potential to protect against DNA damage. Extracts from the plant have been shown to inhibit the mutagenicity induced by various mutagens in vitro assays (Toyin *et al*., 2008). This antimutagenic effect may be attributed to the plant's rich content of antioxidants and other bioactive compounds that can prevent DNA damage.

2.4 Chemical Constituents

FLAVONOIDS have been shown to provide antibacterial, antiinflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity. Flavonoid also has immense antioxidant and anti-inflammatory activity because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Atinga *et al*., 2018).

TANNINS have been used in the treatment of wounds especially those emanating from varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision.

The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Atinga *et al*., 2018) that will aid in chemo taxonomical classification system and further phylogenetic studies in Asteraceae

family.The phytochemical screening of *V.amygdalina* leaf extract recorded the presence of compounds carbohydrates, flavonoids, saponins and alkaloids present in the extract and responsible for antimicrobial activities as reported in similar studies (ibikunle *et al*., 2012). A study conducted by Evbuomwan *et al*. 2018 reported the presence of fatty acids and terpenes responsible for bactericidal activity. Ibikunle *et al*. (2012) also reported that phenolics, flavonoids, saponins and phorbol esters as antimicrobial compounds in *V. amygdalina*.

2.5 Pharmacology of *Vernonia amygdalina*

People all over the world, including modern medicine professionals, have used bitter leaf as traditional medicine. Common illnesses are treated with a variety of plant parts, including the leaves, roots, seeds, shoots, and stems (Ugbogu *et al*., 2021). Nowadays, phytochemicals from plants are used in herbal medicine; hence, it is essential to know about and explain the compounds present in medicinal plants in order to ensure their successful utilization and preservation. To date, not many investigations have been conducted to evaluate the pharmacological activity of the isolated chemicals from VA using a variety of in vitro and/or in vivo techniques. Few studies have reported the anti-inflammatory (Nguyen *et al*., 2021), antioxidant (Erasto *et al*., 2007), antibacterial, antifungal (Erasto *et al*., 2006), anti-cancer (Luo *et al*., 2011), anti-diabetic, and anti-helminthic (IfedibaluChukwu *et al*., 2020) activities of isolated compounds from VA. Vernolide and Vernodalol have antioxidant (Erasto *et al*., 2007;Djeujo *et al*., 2023), antibacterial (Erasto *et al*., 2006; Habtamu and Melaku, 2018), and antifungal (Erasto *et al*., 2006) properties.

Vernodalol'sin silico pharmacokinetics and toxicity profile, as reported by Djeujo *et al*. (2023), indicate that the compound could be a good drug candidate due to its appropriate pharmacokinetic characteristics. The toxicity and pharmacokinetics study on luteolin; another extract of *Vernonia amygdalina* indicates that the compound is safe and has adequate pharmacokinetic good qualities (Djeujo *et al*., 2023).

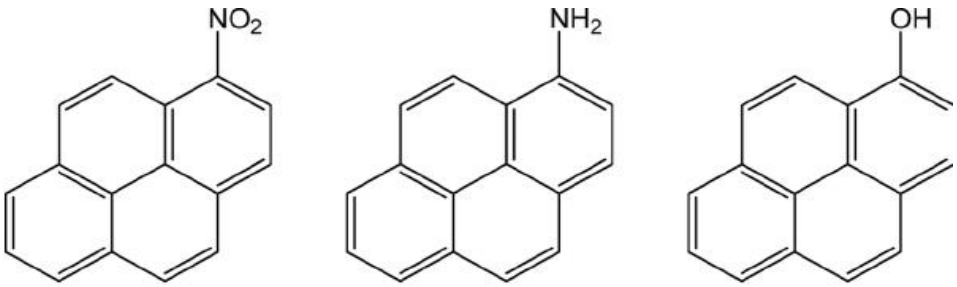
2.6 1-Nitropyrene

1-Nitropyrene has a structure based on pyrene, which consists of four fused benzene rings. The "1-" indicates that the nitro group is attached to the carbon atom at position 1 of the pyrene molecule. 1-Nitropyrene (1-NP), is a nitro-PAH and has been proposed as a marker for Diesel exhaust. Its presence in diesel exhaust is far more than any other particulate-bound nitro-PAHs and, more importantly, its formation is not significantly driven by atmospheric photochemical reactions (Bamford *et al*, 2003; Toriba *et al*, 2007). 1-NP has been reported to cause genetic mutations and chromosomal aberrations (Kim *et al*; Anderson *et al*) and the IARC has classified 1-NP as a Group 2A chemical (IARC,2014). Exposure to 1-NP increases oxidative stress, inflammation, and endothelial dysfunction, leading to increased risk of cardiovascular disease. While it is widely agreed that no single analyte can perfectly represent the complex and variable composition of diesel particulate matter in all scenarios, the employment of 1-NP as a metric could potentially provide an accurate assessment of diesel exhaust exposure.

Furthermore, such an approach could also provide a more accurate estimate of the carcinogenic nature of diesel exhaust (Scheepers,*et al*,1995). Assessing the urinary concentrations of 1-NP metabolites, including 6-hydroxy-1-nitropyrene (6-OHNP), 1-

aminopyrene (1-AP), and N-acetyl-1-aminopyrene (1-NAAP), is a necessary approach for determining environmental exposure to 1-NP and diesel exhaust particles.

Figure 2.2: The structural formulas of 1-nitropyrene (A), 1-aminopyrene (B), and 1-hydroxypyrene (C).



1-Nitropyrene (1-NP), a type of nitrated polycyclic aromatic hydrocarbon (nitro-PAH), is predominantly produced in the exhaust emissions from diesel engines and is commonly found within ambient particulate matter (PM). (Jongeneelen,*et al*,1988) This compound is distinguished by its substantial cytotoxic properties and has been placed as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC). Consequently, 1-Nitropyrene is regarded as a contributor to the adverse health effects associated with air pollution and functions as a representative organic pollutant for evaluating environmental contaminants in human respiratory systems (Jackson *et al* 1985). Extensive research, including animal experiments and in vitro cell assays, has indicated the carcinogenesis mechanism of 1-Nitropyrene, which involves the induction of DNA adduct formation, (Bauer *et al* 2022) the triggering of oxidative stress, (Jian *et al* 2024) and the disruption of cellular signaling pathways (Nana *et al* 2009). These combined effects contribute to the promotion of genetic mutations and cellular transformation. However, the full extent of the impact caused by 1-Nitropyrene exposure, particularly the perturbation of the

lipidome in human airways, is still limited and remains to be investigated using albino wistar rats in this study.

2.6.1 Environmental Sources of 1-Nitropyrene

1-Nitropyrene is primarily formed through the incomplete combustion of organic materials, particularly in the presence of nitrogen oxides (NOx). Major sources include:

Diesel exhaust: This is a significant source of 1-nitropyrene in urban air. The combustion of diesel fuel in engines produces PAHs, some of which react with NOx in the exhaust to form nitro-PAHs like 1-nitropyrene (Schuetzle, 1983).

Coal combustion: Burning coal for power generation and industrial processes also releases 1-nitropyrene into the atmosphere (IARC, 2012).

Other combustion sources: These include emissions from other types of internal combustion engines, wood burning, and even tobacco smoke.

Atmospheric reactions: 1-Nitropyrene can also be formed in the atmosphere through the reaction of pyrene with nitrogen dioxide (NO2) and other oxidizing agents (Atkinson, 1990).

2.7 Toxicity of 1-Nitropyrene

2.7.1 Effects of 1-Nitropyrene on Hematological Parameters

1-Nitropyrene (1-NP) is a significant environmental pollutant, classified as a probable human carcinogen. It's found in diesel exhaust, urban air, and other combustion sources (IARC, 1989). Exposure to 1-NP has been linked to various adverse health effects, including potential impacts on the blood and its components (hematological parameters).

2.7.2 Evidence from Animal Studies

Inhalation Study (NTP, 1996): A 13-week inhalation study on rats conducted by the NTP investigated the toxicity of 1-Nitropyrene. While the primary focus might have been on other endpoints like carcinogenicity, the study did observe slight variations in certain hematological parameters. Unfortunately, the publicly available summaries of this report don't provide extensive details on the specific changes observed in blood cell counts, hemoglobin levels, or other hematological measures. This highlights the need for this extensive study being conducted.

Metabolism and Protein Binding (van Bekkum *et al*., 1998): This study explored the biological fate of 1-Nitropyrene in rats after it was administered intragastrically (directly into the stomach). A key finding was that metabolites of 1-Nitropyrene could associate with blood proteins, including hemoglobin. Hemoglobin is crucial for oxygen transport in the blood, and its interaction with 1-NP metabolites raised concerns about potential disruptions in oxygen delivery and other hematological functions. This interaction could potentially lead to:

- Changes in hemoglobin structure or function: Affecting its ability to bind and transport oxygen.
- Oxidative stress: Leading to damage of red blood cells and other blood components.
- Altered red blood cell production or survival: Potentially affecting red blood cell counts and leading to anemia or other blood disorders.

2.7.3 Health and Environmental Concerns

The effects of 1-Nitropyrene is of great concern because it is a potent mutagen and is classified as a probable human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC, 2014). It can contribute to respiratory problems, DNA damage, and potentially cancer.

The available research, though limited in specific hematological details, suggests that 1-Nitropyrene exposure can influence blood-related parameters. The interaction of 1-NP metabolites with hemoglobin is a particularly concerning finding, as it indicates a potential mechanism for disrupting essential blood functions.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

1-Nitropyrene (1-NP) (≥ 96%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade and manufactured by Sigma-Aldrich (St. Louis, MO, USA) and British Drug Houses (Dorset, Poole, UK).

3.1.2 Plant Materials

The fresh leaves of *V. amygdalina* was obtained from Benin City, Nigeria. The plant was identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

3.1.3 Preparation of the Plants Extracts

Precisely 2 kg of each of the pulverized plants was steeped in distilled water for three days. The contents were stirred many times a day and filtered with Whatman filter paper at the end of the third day. The filtrate was evaporated to dryness with a freeze-dryer. The extracts were weighed, placed in an airtight container, and refrigerated at 4^{o}C until use.

3.1.4 Experimental Animals

Thirty-five female (35) Wistar albino rats weighing 150-200 g were obtained and housed in well-aerated suspended cages in the Animal House, Department of Anatomy at room temperature under a 12 h light/dark cycle. The animals were allowed access to standard laboratory rat chow and water ad libitum and after they were acclimated for 7 days, they were randomly distributed into 7 groups of 5 animals each.

3.1.5 Experimental Design for Toxicity study

The animals were grouped and treated as below:

- Group A: Corn oil 2ml/kg (Control)
- Group B: 1-NP 250mg/kg body weight
- Group C: VA 100mg/kg body weight
- Group D: 1-NP (250 mg/kg) + VA (50 mg/kg) body weight
- Group E: 1-NP (250 mg/kg) + VA (100 mg/kg) body weight

Treatments were by oral gavage for a period of 7 days. The dose of 1NP chosen was based on pilot study of different graded doses, and was dissolved in corn oil. After the duration of the treatment the animals were anesthetized after an overnight fast and blood samples were collected.

3.1.6 Blood Sample Collection

Blood samples were collected directly from the heart with a syringe and separated into tubes containing EDTA as anticoagulant. The samples were immediately centrifuged at 5000 g in 4 degrees for 5 min, to separate plasma and red blood cells. The plasma was stored at -80°C until further analysis.

3.1.7 Hematological assay

Packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelets, reticulocyte counts and differential white blood cell counts was analyzed using the Beckman Coulter JT series Hematology Analyzer (Jones *et al.,* 1996).

Principle and procedure

The Beckman Coulter method of sizing and counting particles uses measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte.

A suspension of blood cells passes through a small orifice simultaneously with an electric current. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone, each particle displaces its volume of electrolyte. Beckman Coulter measures the displaced volume as a voltage pulse, the height of each pulse being proportional to the volume of the particle. The quantity of suspension drawn through the aperture is for an exact reproducible volume. Beckman Coulter counts and sizes individual particles at a rate of several thousand per second. This method is independent of particle shape, colour, and density. The MAXM is a quantitative, automated, differential cell counter for *in vitro* diagnostic use. The MAXM measures these parameters in whole blood.

3.1.8 Statistical Analysis

The experimental results were expressed as mean ± standard error of the mean (SEM) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range tests using Graph Pad Prism Version 7. p values ≤ 0.05 were regarded as significant.

CHAPTER FOUR

RESULTS

4.1 Effects of *Vernonia amygdalina* on Hematology parameters in Wistar rats subjected to 1-Nitropyrene toxicity

	Control	1-NP alone	VA alone	1-NP + VA-1	1-NP + VA-2
WBC (u)	10.15 ± 1.07	5.12 ± 0.72*	10.45 ± 0.91	6.68 ± 1.06[a]	7.82 ± 2.17[a]
LYM%	79.19±5.19	58.44±5.48*	78.90 ± 4.00	62.85 ± 0.84[a]	66.21 ± 4.26[a]
GRAN%	8.11±0.19	2.24±1.00*	8.82 ± 0.11	5.43±1.13[a]	6.55±1.11[a]
HCT%	47.13±3.30	27.34±3.12*	47.09±3.05	23.12±3.12[a]	34.19±2.69[a]
RDW(%)	16.30±4.09	5.34±0.58*	15.59±1.13	10.45±1.70[a]	13.66±2.66[a]
RBC (ul)	7.91±1.88	3.89±.0.88*	7.00 ± 2.01	4.11±0.12[a]	5.65±0.67[a]

Values are expressed as mean ±standard deviation; n=5*Significant as compared with control; $p < 0.05$. 1-NP, 1-nitropyrene. VA, *Vernonia amygdalina*. VA-1, VA-2 denote 50 and 100 mg/kg of *Vernonia amygdalina* respectively. * shows significant difference from control ($p<0.05$). a shows significant difference ($p<0.05$) from 1-NP group.

CHAPTER FIVE

DISCUSSION, RECOMMENDATION AND CONCLUSION

5.1 Discussion

The study aimed to evaluate the potential protective effects of *Vernonia amygdalina* against 1-Nitropyrene-induced hematological alterations. The results show that 1-Nitropyrene significantly altered hematology parameters in Wistar rats. Specifically, 1-Nitropyrene decreased white blood cell (WBC) count, lymphocyte percentage (LYM%), granulocyte percentage (GRAN%), hematocrit (HCT%), and red blood cell (RBC) count. These findings are consistent with previous studies demonstrating the hematotoxic effects of 1-Nitropyrene. These results also show that *Vernonia amygdalina*, administered alone, did not significantly alter hematology parameters in Wistar rats. This suggests that *Vernonia amygdalina*, at the doses used in this study, is non-toxic and does not have any adverse effects on hematological parameters. Overall our findings demonstrate that *Vernonia amygdalina*, administered at doses of 50 and 100 mg/kg, significantly protected against 1-Nitropyrene-induced hematological alterations. Specifically, *Vernonia amygdalina* increased WBC count, LYM%, GRAN%, HCT%, and RBC count in 1-Nitropyrene-treated rats. These findings suggest that *Vernonia amygdalina* has potential protective effects against 1-Nitropyrene-induced hematotoxicity.

Furthermore, our results showed that both doses of *Vernonia amygdalina* (50 and 100 mg/kg) provided significant protection against 1-Nitropyrene-induced hematological alterations. However, the higher dose (100 mg/kg) appeared to provide more pronounced protection, as evidenced by the greater increases in WBC count, LYM%, GRAN%, HCT%, and RBC count. These findings suggest that the protective effects of *Vernonia*

amygdalina against 1-Nitropyrene-induced hematotoxicity may be dose-dependent. Though the exact mechanisms by which *Vernonia amygdalina* exerts its protective effects against 1-Nitropyrene-induced hematotoxicity are not fully understood. However, several possible mechanisms can be proposed based on the available literature.

Vernonia amygdalina may act by:

Antioxidant activity: *Vernonia amygdalina* has been reported to possess antioxidant properties, which may help to neutralize the oxidative stress induced by 1-Nitropyrene.

- Anti-inflammatory activity: *Vernonia amygdalina* may also exert anti-inflammatory effects, which could help to mitigate the inflammatory responses induced by 1-Nitropyrene.

- Immunomodulatory activity: *Vernonia amygdalina* may have immunomodulatory effects, which could help to regulate the immune system and prevent the immunosuppressive effects of 1-Nitropyrene.

5.2 Recommendations

Based on the findings of this study, the following recommendations/suggestions were made:

1. Further studies should be conducted to fully elucidate the mechanisms of the protective effects of *Vernonia amygdalina* against 1-Nitropyrene-induced hematotoxicity.

2. The potential protective effects of *Vernonia amygdalina* against other toxic substances should be investigated.

3. The safety and efficacy of *Vernonia amygdalina* as a therapeutic agent should be evaluated in clinical trials.

5.3 Conclusion

In conclusion, the results of this study demonstrate that *Vernonia amygdalina* has potential protective effects against 1-Nitropyrene-induced hematological alterations in Wistar rats. The exact mechanisms of these protective effects are not fully understood and require further investigation. However, the findings of this study suggest that *Vernonia amygdalina* may be a useful adjunct in the prevention or treatment of hematotoxicity induced by 1-Nitropyrene or other toxic substances.

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