Original Research Article

IN VIVO STUDY OF BITTER LEAF (*Vernonia amygdalina*) PLANT ON THE REMEDIATION OF THE CYTOLOGICAL AND PHYSIOLOGICAL DAMAGES ON WISTER RAT (ALBINO RAT) CAUSED BY *Streptococcus pyogenes*

ABSTRACT

Aim: To investigate the effectiveness of *V. amygdalina* leaf extract on the remediation of the cytological and physiological damages on albino rats caused by *streptococcus pyogenes.*

Study design: **Randomized Controlled Trial (RCT) design with multiple treatment groups**. Research was done at the department of Science Laboratory Technology, University of Calabar, Cross River state, between March 2023 and June 2023.

Methodology: Bitter extract was obtained through Soxlet extraction, and screened for the presence of secondary metabolites using standard phytochemical screening procedures. A pure culture of *S. pyogenes* was gotten from Microbiology Laboratory University of Calabar. Thirty five albino rats (100-175g), obtained from the Pharmacology department, University of Calabar were placed in 7 groups (A-G). Rats in groups A-F each was injected with the bacterium, observed for five days. Group A-E rats were subsequently treated with 0g/200 ml, 20g/200 ml, 40g/200 ml, 60g/200 ml, and 100g/200ml crude extract of bitter leaf respectively. While group F was treated with standard antibiotics. 0.5ml Cefuroxime Axetil tablets usp was injected into the rats. Group G rats served as negative control.

Results: Results showed the presence of alkaloids, acidic compounds, tannins, flavonoids and Saponins. Haematocrit increased slightly in group A-E (18.3-46.3%) treated with 0g/200ml, 20g/200ml, 40g/200 ml, 60g/200 ml, and 100g/200ml concentrations of the extract, respectively. But more in group F (50.2%) that received antibiotics treatment, when compared with the control. Similar trend was observed for haemoglobin (Hb) values (15.3g/dl) and red blood cell (RBC) counts (3.0 $x10^{12}$/l) that increased with increasing concentration of 100g/200ml extract treatment and antibiotics treatment (Hb = 15.7g/dl; RBC = 3.3 $x10^{12}$/l) respectively. The white blood cell counts decreased with antibiotic treatment.

Conclusion: Study established that *in vivo* administration of bitter leaf extract on albino rats was best at 60g/200 ml, and 100g/200ml in ameliorating haematotoxicity caused by *S. pyogenes.*

Keywords: Cytological, In Vivo, Physiological, *Vernonia Amygdalina*, Remediation, *Streptococcus pyogenes,* Wister rat

1. INTRODUCTION

The bitter leaf plant (*Vernonia amygdalina)*, which is prevalent in tropical Africa, is considered to be the largest family of flowering plants. With over 23,600 species and approximately 1,620 genera, this plant is characterized by its bitter taste [1]. The most common genus within this family is Vernonia, which consists of around 1,000 species of forbs, herbs, and shrubs. Among these species, *V. amygdalina* stands out as the most prominent and is part of the pan tropical tribes of the *Asteraceae* family [2]. While the plant thrives in various tropical African countries like Nigeria, Zimbabwe, and South Africa, it is also cultivated in certain regions of West Africa [3]. The bitter leaves of this plant are widely utilized in soup preparation and as a flavoring agent in African delicacies [4]. The bitter taste of these leaves can be attributed to their

anti-nutritional components, including alkaloids, saponins, glycosides, and tannins [5]. In Nigeria, the plant is known by different local names such as 'Ewuro' in Yoruba, 'Onugbu' in Igbo, 'Oriwo' in Bini, 'Ityuna' in Tiv, 'Chusar doki' or 'fatefate' in Hausa, and 'Etidot' in Ibibio [3].

The roots and leaves of *V. amygdalina* are commonly used in ethno medicine to treat various ailments such as fevers, hiccups, kidney problems, and stomach discomfort. Additionally, it is employed in the treatment of conditions like diarrhea, dysentery, hepatitis, and cough. Moreover, it functions as a laxative and fertility inducer [6]. To eliminate the bitter taste, the leaves of *V. amygdalina* are washed and boiled before being used as soup condiments [7]. They are particularly utilized in the preparation of the well-known Nigerian bitter leaf soup, 'Etidot,' and as a spice in the Cameroonian dish called 'Ndole' [8]. In certain parts of Africa, such as Nigeria, the plant is transformed into a tonic and consumed for medicinal purposes [3]. *V. amygdalina* also finds traditional use in the treatment of diseases like malaria, infertility, diabetes, gastrointestinal problems, and sexually transmitted diseases [9].
Furthermore, the leaves of this plant are commonly employed as a treatment against nematodes in humans and chimpanzees, as well as other intestinal worms [10]. Extracts of the plant have also been utilized in Nigerian herbal homes as a tonic, for tick control, and in the treatment of high blood pressure [11]. The reported efficacy of *V. amygdalina* is attributed to the complex secondary plant compounds that possess pharmacological activity [12]. Numerous studies have revealed the pharmacological and medical uses of the bitter leaf plant, including its traditional use in the treatment of venereal diseases, gastrointestinal problems, and malaria [11, 13, 14]. It is worth noting that the ethno-medical use of *V. amygdalina* is not limited to humans alone; it is also widely employed as an additive to horse feed in Northern Nigeria to provide a strengthening or fattening tonic known as 'Chusan Dokin' [7].
The treatment of parasite-related disease in wild chimpanzees in Tanzania has involved the use of *V. amygdalina*, according to a study [14]. Additionally, the potential therapeutic properties of extracts from the bitter leaf plant (*V. amygdalina*), including anthelmintic, antimalarial, antitumourigenic, bacteriostatic, and bactericidal effects on certain bacteria, have been extensively investigated [15, 16]. Furthermore, a study [17] has reported on the in vivo examination of the hypoglycaemic and hypolipidaemic effects of these leaf extracts. Traditional caregivers also recommend the use of aqueous extracts from the bitter leaf plant for treating various ailments, such as emesis, nausea, diabetes, loss of appetite, dysentery, gastrointestinal tract problems caused by pathogenic bacteria, as well as sexually transmitted diseases and diabetes mellitus [18].

1.1 Phytochemical composition of bitter leaf (*V. amygdalina*)

The presence of phytochemicals in fruits and leaves has been shown to prevent the development of chronic diseases, including cancer, diabetes, and heart disease. Several researchers [18, 19, 20] have reported the existence of phytochemicals such as saponins, flavonoids, alkaloids, and hydrocyanic acids in the extracts of *V. amygdalina* roots and barks. The phytochemicals found in *V. amygdalina* contain bioactive compounds with antiviral properties that have both prophylactic and therapeutic effects against cancer cells [21, 22]. In a study [23], the concentration (mg/100g) of specific phytochemicals in various plants (*Ocimum gratissimum* and *V. amygdalina*) was evaluated, and it was observed that *V. amygdalina* contained higher levels of bioactive compounds than Ocimum gratissimum, with the exception of phytate and cyanogenic glycosides (Table 1).

Table 1. Phytochemical components of ethanoic extracts of bitter leaf (*V. amygdalina)* (mg/100g)*

Phytochemicals	Concentration (mg/100g)
Oxalate	3.84
Phytate	3.95
Tannins	9.62
Saponins	5.97
Flavonoids	4.89
Cyanogenic glycoside	1.11
Alkaloids	2.16
Anthraquinone	0.14
Steroid	0.38
Phenol	3.24

***Source: [23]**

1.2 Nutritional composition of *Vernonia amygdalina*

The nutritional composition of *V. amygdalina* has been extensively researched. Proximate analysis of *V. amygdalina* has revealed the presence of protein, carbohydrate, moisture, ash, fiber, and fat, as reported by [18]. The moisture content was found to be 10.55%, which is higher than the value reported by [24] (10.02%). The crude fiber content of *V. amygdalina* is 8.78%, falling within the range for Nigerian vegetables. The ash content, which indicates the presence of

mineral elements, is 4.28%, lower than the values reported by [24] for bitter leaf (9.56%) and scent leaf (13.01%). The mineral elements present in *V. amygdalina* were found to follow the trend: K > Na > Ca > Mg > Fe > Zn > Cu > Mn [25]. Potassium was the most abundant mineral element detected, while manganese was the least detected. Inorganic mineral elements like potassium and calcium are known to play important roles in maintaining normal glucose tolerance and regulating insulin release from the beta cells of the islets of Langerhans, which helps control glucose levels in the human body [26]. Other researchers [26, 27] have also reported different proportions of proximate contents of bitter leaf (Table 2).

Table 2: Nutritional analysis (mg/100g dry matter) of bitter leaf (*V. amygdalina*)*

Nutrient	Value (mg/100g)
Crude protein	23.10g
Ash	17.13g
Cellulose	12.31g
Edible portion	100g
Fats	0.4g
Protein	5.2g
Water	82.0g
Energy	218g
Carbohydrates	10.0g
Dietary fibre	1.5g
Calcium	145mg
Phosphorus	6.7mg
Iron	5.0mg
Zinc	85.0mg
Manganese	710.0mg
Ascorbic acid	5.1mg

*Source: [26, 27]

1.3 Pharmacological and Medicinal properties of *V. amygdalina* leaves

Traditional medical practitioners have utilized the leaves of *V. amygdalina* in the treatment of malaria for many years. The significance of traditional medicine in rural communities, where there is a lack of efficient public healthcare systems, has been recognized [28]. In a study [29], the leaf extract of *V. amygdalina* was found to effectively treat Wister rats diseased with rodent malaria (*Plasmodium berghei*). The extract also exhibited analgesic activity and demonstrated clear and significant anti-plasmodial effects in mice. Toxicity levels in rats were within control values, indicating the safety of the extract. These findings support the claims made by traditional healers regarding the efficacy of *V. amygdalina* in pain and malaria treatment.

In Africa, traditional health workers often recommend the use of aqueous extracts of *V. amygdalina* for various ailments such as emesis, nausea, diabetes, loss of appetite, dysentery, gastrointestinal tract problems, sexually transmitted diseases, and diabetes mellitus [18]. Some of these assertions have been experimentally verified and documented, while others still require validation. Phytochemical compounds extracted from *V. amygdalina*, including saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides, and sesquiterpenes, have been found to elicit various biological effects in humans, including cancer chemoprevention [9].

Researchers [18, 30] have demonstrated that administration of an aqueous extract of *V. amygdalina* leaves can decrease plasma total cholesterol, low-density lipoprotein, and very low-density lipoprotein in animals with hyperlipidemia [31]. These findings indicate that the aqueous extract of *V. amygdalina* leaves may be beneficial in controlling blood lipids and preventing and treating coronary heart disease. Previous studies have shown that extracts of *V. amygdalina* leaves exhibit strong antimicrobial activity against clinical isolates [20, 23]. The antimicrobial properties of these leaves make them potential candidates for drug development due to their inhibitory effects on bacterial growth [20]. However, there is some contradictory evidence regarding the effects of *V. amygdalina* extracts on E. coli strains, which may be attributed to the genetic diversity of the pathogen and the resulting variation in resistance mechanisms [14, 22].

S. pyogenes typically colonizes the pharynx, anus, and genital mucosa. Infections caused by *S. pyogenes* are highly contagious and can be transmitted through various means, including airborne droplets, hand contact with nasal discharge or contaminated objects/surfaces, skin contact with contaminated lesions, or contaminated food sources. The frequency of *S. pyogenes* infections varies globally depending on the clinical manifestations of the infections [32]. This bacterium can cause a range of infections, such as streptococcal pharyngitis, impetigo, cellulitis, acute rheumatic fever, and scarlet fever [33]. While pharyngitis is mostly viral, GAS is responsible for a significant percentage of cases in both children and adults, with higher rates observed in children. Risk factors such as heart disease, diabetes, malignancy, trauma, surgical incision, and respiratory virus infections increase the likelihood of *S. pyogenes* infection [34]. In children, a prior

chickenpox infection is associated with a portion of *S. pyogenes* infections. Pregnancy is also a high-risk state for invasive GAS infection. Maternal *S. pyogenes* infection is more likely to occur in late pregnancy and postpartum, with a significantly elevated risk compared to the non-pregnant population [34].

1.4 Treatment and prevention of streptococcal infections

The prompt administration of antibiotics can prevent the rapid spread of streptococcal infections and their progression to the bloodstream and internal organs. As a result, cellulitis is often treated without the need for bacterial identification through culture. In such cases, doctors prescribe antibiotics that are effective against both streptococci and staphylococci, such as dicloxacillin or cephalexin [35]. Severe streptococcal infections, including necrotizing fasciitis, endocarditis, and severe cellulitis, require intravenous penicillin, sometimes in combination with other antibiotics. Patients with necrotizing fasciitis receive treatment in an intensive care unit, and surgical removal of dead, infected tissue is necessary [35]. Penicillin remains the preferred drug for treating all streptococcal infections, although most cases of impetigo can be treated with topical bacitracin, mupirocin, or retapamulin. Oral or parenteral administration of penicillin is only necessary in severe cases or during epidemics [36].

For the treatment of pharyngitis caused by group A strep, the preferred antibiotic options are penicillin or amoxicillin. It has never been documented that any strain of group A strep is resistant to penicillin. However, resistance to azithromycin and clarithromycin is prevalent in certain communities. For patients with an allergy to penicillin, recommended treatment plans include narrow-spectrum cephalosporins (such as cephalexin and cefadroxil), clindamycin, azithromycin, and clarithromycin [33]. Guidelines for antibiotic regimens suitable for group A streptococcal pharyngitis have also by research [37], which involve the use of Benzathine penicillin G, Amoxicillin, Penicillin V for patients without a penicillin allergy. For patients with the allergy, Cephalexin, Cefadroxil, Clindamycin, Azithromycin, and Clarithromycin are recommended.

These bacteria are known to spread through direct contact with the nasal and throat secretions of an infected individual, as well as through infected skin lesions. Given this information, it can be inferred that the transmission of all types of group A streptococcal infections can be minimized by practicing good hygiene habits [38], such as regular handwashing, especially after coughing and sneezing, before and after handling food, and prior to eating. Individuals with sore throats should seek medical attention to undergo tests for strep throat. If diagnosed, they should refrain from going to work, school, or daycare for at least 24 hours after starting antibiotic treatment. All wounds should be kept clean and monitored for potential signs of infection, including increased redness, swelling, and pain at the site of the wound [47].

Improved living conditions and the application of topical treatments to impetiginous lesions can help prevent the spread of the infection to susceptible individuals. Measures to prevent secondary cases should be taken for healthcare workers and family members who have prolonged and close contact with patients suffering from necrotizing fasciitis/streptococcal toxic shock syndrome, particularly those who are immunocompromised, have varicella, have recently undergone surgery, or have recently given birth [36]. Children are more susceptible to infection due to their lower awareness of hygiene compared to adults. Additionally, they tend to spend more time in crowded environments like schools and engage in close physical contact. Considering the epidemiology of *S. pyogenes* in relation to the antibacterial properties of *V. amygdalina*, it is evident that there is a lack of information in Nigeria regarding the antibacterial effectiveness of bitter leaf extracts as a means to counteract the cell and tissue damage caused by *S. pyogenes* toxins in Wister rats.

Therefore, the objective of this study was to examine the antibacterial potential of bitter leaf extracts in alleviating the cytological and physiological damage caused by *S. pyogenes* in albino rats. Considering the epidemiology of *S. pyogenes* in relation to antibacterial activities of *V. amygdalina,* it is apparently there is a dearth of information in Nigeria on the antibacterial potential of bitter leaf extracts as anti-toxin against cell and tissue damage caused by toxins of *S. pyogenes* on Wister rats. Therefore, the aim of this study was to investigate the antibacterial potential of bitter leaf extracts on the remediation of the cytological and physiological damage on albino rat caused by *S. pyogenes.*

2. MATERIAL AND METHODS

2.1 Collection of plant material and preparation of bitter leaf extract by Soxlet extraction method

Fresh bitter leaf, *V. amygdalina* were purchased from Goldie market, a local market in Calabar, Southern Nigeria. The bitter leaf extract was gotten through Soxlet extraction method. The leaves were washed, shade dried and powdered mechanically. Powdered

bitter leaf was weighed (100g) and subjected to Soxlet extraction using ethanol, chloroform and under 48hours solvent was recovered using Rotary vacuum evaporator followed by phytochemical screening. The five concentrations of extracts, 0g/200 ml, 20g/200 ml, 40g/200 ml, 60g/200 ml, and 100g/200ml used in this research were prepared by extracting 0g, 20g, 40g, 60g and 100g each of processed leaves in 200ml distilled water, according to the method described by [40]. The extract was stored in a refrigerator at 4^{o}C until used for further experiment.

2.2 Phytochemical analysis of bitter leaf extract

The aqueous extract of the leaves was screened for the presence or absence of various secondary metabolites using standard phytochemical screening procedures as described by [23].The extract was tested for resins, calcium, alkaloids, flavenoids, reducing sugars, saponins, glycosides, carbohydrates, steroids, acidic compounds, fats and oils.

2.3 Preparation of organism

A pure culture of *S. pyogenes* was gotten from Microbiology Laboratory University of Calabar. Buffer peptone water was sterilized using a pressure pot and gas cylinder at 121^{o}C for 15 minutes at 15 pound per square inch. It was allowed to cool and then inoculating wire loop was used aseptically to inoculate the organism into the peptone water and then incubated for 4 hours.

2.4 Experimental design

The study adopted a Randomized Controlled Trial (RCT) design with multiple treatment groups**.** Thirty five (35) Albino rats of Wister strain (100-175g) were obtained from the Pharmacology department, University of Calabar, Nigeria and were allowed acclimatization period of one week in a well-ventilated room with a temperature and relative humidity of 29±2^{o}C and 70% respectively. Rats were maintained on standard animal pellets and water.
At the end of the acclimatization period, they were numbered randomly placed in seven groups (Grp A-G) each group containing 5 albino rats. Rats in groups A-F each was injected with the bacterium. Group G rats served as negative control and were neither injected with the bacterium nor given any treatment. Groups A-E rats were subsequently treated with 0g/200 ml, 20g/200 ml, 40g/200 ml, 60g/200 ml, and 100g/200ml crude extract of bitter leaf respectively. While group F was treated with standard antibiotics. 1ml of saline water was used to dissolve the Cefuroxime Axetil tablets usp and 0.5ml injected into the rat. The following treatments were administered:

Grp A - Injected with bacterium + 0g/200ml of extract

Grp B - Injected with bacterium + 20g/200ml of extract

Grp C - Injected with bacterium + 40g/200ml of extract

Grp D - Injected with bacterium + 60ml/200ml of extract

Grp E - Injected with bacterium + 100ml/200ml of extract

Grp F - Injected with bacterium + standard antibiotics

Grp G – No bacterium injected, treatment was given (control)

2.5 Mode of organism inoculation and extract treatment

The *S. pyogenes* stock was inoculated into the mice through the skin with a sterile syringe. The route of administration of extract treatment was given orally (mouth) and treatment was given morning and evening consecutively.

2.6 Cytological/physiological procedure and extract dilution

Group A-F albino rats were inoculated with 0.5ml stock culture of the organism and observed for 5 days for some reactions. This was followed by concomitant inoculation of varied concentrations (0g/200 ml, 20g/200 ml, 40g/200 ml, 60g/200 ml, and 100g/200ml) of crude extract of bitter leaf into groups of rats previously inoculated with the bacterium. Treatment was continued for 5 days. Each day, the behavior of the rats were observed and recorded at 24hrs. Treatment with standard antibiotics was administered at the stated drug dose for rats in group F already inoculated with the bacterium for 5 days. To determine the pathological and physiological state of rats, blood samples were collected aseptically for full blood count analysis.

2.7 Full blood count (FBC) analysis

A full blood count (FBC), is a widely used clinical procedure that serves as the initial step in most medical investigations. Not only does it test for blood disorders and abnormalities, but it also provides insights into potential diseases in other organs [41], given that blood circulates throughout the entire body. The purpose of an FBC, as the name suggests, is to determine the count of blood cells in a blood sample. These cell counts are then used to estimate the levels of different types of blood cells within the body's circulatory system. The three main types of blood cells found in blood are red blood cells, white blood cells, and platelets. An FBC assesses the number, size, proportions, and hemoglobin levels of these cells. Hemoglobin, in particular, is the component responsible for carrying oxygen in red blood cells [42].

The full blood count determination involved the collection of whole blood from the animal's heart through cardiac puncture using a sterile syringe and needle. The collected blood samples were then placed in sample tubes treated with Ethylene di-amine tetra acetate (EDTA). The packed cell volume or hematocrit was determined using the method outlined by [43]. White blood cell count (WBC) was determined following the method described by [54], while hemoglobin (Hb) was determined based on the principle stated by [44]. Furthermore, the leucocyte differential counts were conducted as per the guidelines provided by [44].

3. RESULTS AND DISCUSSION

3.1 Results of phytochemical analysis of bitter leaf extract

The results of phytochemical analysis of bitter leaf extract are presented in Table 3. The results showed the presence of acidic compounds, alkaloids, flavonoids, tannins, saponins and fats and oils in varying concentrations, and total absence of steroids, terpenoids, glycoside, and resins. Acidic compounds were present in very high concentrations, alkaloids and flavonoids in moderately high concentrations, while other constituents were present in small concentrations.

Table 3: Phytochemical constituents of bitter leaf extract

Phytochemical constituents	Concentration
Acidic compounds	+++
Alkaloids	++
Flavonoids	++
Tannins	+
Saponins	+
Fats and oils	+
Steroids	-
Terpenoids	-
Glycosides	-
Resins	-

Key:

- = Negative
+ = present in small concentrations
++ = present in moderately high concentrations
+++ = present in very high concentrations

3.2 Haematological profile of albino rat treated with crude extract of bitter leaf

Table 4 below shows the results of assessment of the effect of crude extract of bitter leaf on some haematological parameters of Wister rat after treatment with 100g body weight of the extract. The results showed that the haematocrit (PVC) increased slightly in rats group A-E (18.3-46.3%) treated with 0g/200ml, 20g/200ml, 40g/200 ml, 60g/200 ml, and 100g/200ml concentrations of the extract respectively. But more in group F (50.2%) that received antibiotics treatment when compared with the control which was higher (58.4%). Similar trend was observed for haemoglobin (Hb) values (15.3g/dl) and red blood cell (RBC) counts (3.0 $x10^{12}$/l) that increased with increasing concentration of 100g/200ml extract treatment and antibiotics treatment (Hb = 15.7g/dl; RBC = 3.3 $x10^{12}$/l) respectively. The white blood cell (WBC) counts increased in group A-E treated with different concentrations of bitter leaf extract, and comparatively higher in group F which was treated with standard antibiotics. Total bilirubin also increased slightly in the groups treated with varied concentrations of crude extract of bitter leaf. While basophils, eosinophils and monocytes were not detected in all the experimental rats including the control.

Table 4: Haematological profile of albino rat treated with crude extract of bitter leaf

	Treatment						
Variables	**GRP.A**	**GRP.B**	**GRP.C**	**GRP.D**	**GRP.E**	**GRP.F**	**GRP.G**

	(0g/200ml)	(20g/200ml)	(40g/200ml)	(60g/200ml)	(100g/200ml)	(Antibiotics)	(Control)
PCV (%)	18.3	38.5	42.4	44.4	46.3	50.2	58.4
Hb (g/dl)	8.6	13.1	14.1	14.6	15.3	15.7	12.0
MCH (pg)	25.1	25.5	26.1	26.3	26.4	26.4	27.6
RBC ($x10^{12}$/l)	2.0	2.1	2.1	2.3	3.0	3.3	3.5
MCV (µl)	52.3	54.6	55.4	55.5	58.5	60.0	60.1
MCHC (%)	18.0	20.5	21.8	25.4	26.5	30.4	32.4
WBC ($x10^{o}$/l)	5.1	7.3	10.4	11.2	15.4	16.8	18.6
Total bilirubin (%)	20.0	20.1	20.0	20.4	21.3	21.4	21.5
Basophils (%)	-	-	-	-	-	-	-
Eosinophils (%)	1.1	1.9	2.0	2.2	2.5	2.5	5.5
Lymphocytes (%)	92	64	48	76	52	80	82
Monocytes (%)	-	-	-	-	-	-	-

PVC = packed cell volume; Hb –haemoglobin; RBC = red blood cell; MCV= mean cell volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; WBC = white blood cell

3.3 Discussion

Reduction in haematological parameters has been documented as an indication of an anaemic state in animals. Additionally, an increase in white blood cells signifies the immune system's response to disease-causing toxicants [45]. This elucidates the reason for the alteration in immune cells observed in this study. Blood serves as a means of evaluating the clinical and nutritional health status of animals in feeding trials, with the most commonly employed haematological variables being packed cell volume (PCV), red blood cell (RBC) count, haemoglobin (Hb) level, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) [46]. The findings of this study demonstrate that the bitter leaf extract has the potential to modify the distribution and occurrence of neutrophils and lymphocytes, indicating its ability to act as an immunostimulant. However, the leucocyte differential count did not reveal the presence of monocytes, eosinophils, and basophils, regardless of treatment with the extract. It has been established that neutrophils are the most abundant circulating granulocytes, and they migrate to tissues in response to infection when stimulated by a chemostatic factor [47].

The significant increase in packed cell volume (46.3%) and haemoglobin values (15.3g/dl) observed in albino rats, particularly after administering the bitter leaf extract at a concentration of 100g/200ml, suggests that the extract contains bioactive constituents or phytoconstituents with notable neutraceutical potentials capable of enhancing haematopoietic activities and regenerating damaged liver tissue. This study is in accordance with the findings of [48], who reported that methanolic extracts of *V. amygdalina* have the ability to protect the liver and kidneys of mice and albino rats from damage. Acute inflammation caused by pathogenic microorganisms often leads to haemolysis, which manifests as decreased haemoglobin and haematocrit (packed cell volume) levels [49].

Other haematological parameters measured in this study, such as mean corpuscular haemoglobin (MCH), red blood cell count (RBC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC), were only slightly affected by the experiment. The values did not show significant alterations in the albino rat groups treated with different concentrations of the bitter leaf extract compared to the control group that received no treatment and the group treated with antibiotics. The minor differences in MCH, MCV, and MCHC suggest that there were no significant variations in corpuscular sizes, even when the collected blood samples had different haemoglobin contents. These parameters are relevant for diagnosing anaemia and assessing the bone marrow's capacity to produce red blood cells [46]. However, the white blood cell (WBC) counts, although slightly different for the experimental albino rats, were enhanced by the bitter leaf extract and antibiotic treatments, approaching the values of the control rat group. This result indicates that the bitter leaf plant possesses immunostimulating properties, which is consistent with the earlier report by [50] that bitter leaf extract exhibits immunostimulant activity.

Phytochemical analysis of the bitter leaf extract in this investigation disclosed the presence of acidic compounds, alkaloids, flavonoids, tannins, saponins, and fats and oils in varying concentrations. The findings of this research align with those of [51], who reported the presence of phytochemical constituents such as saponin, flavonoids, alkaloids, and tannins in different quantities in the ethanol and methanol extracts of *V. amygdalina*. The identification of these compounds in the plant extract confirms previous reports that aqueous and ethanol leaf extracts of *V. amygdalina* possess antibacterial properties against bacterial isolates such as *S. aureus and E. coli* [30,52], as well as *S. aureus, P. aeruginosa*, and E. *coli* [20,23]. Earlier studies have indicated that specific organic compounds in bitter leaf can mitigate the toxic impact of bacterial exotoxins in vivo. The outcomes of this investigation correlate with the findings of [52], who also observed that a methanol leaf extract (60g/200ml) of *V. amygdalina* exhibited activity against various microorganisms (including *S. pyogenes*) and certain fungi, but did not demonstrate significant activity against *Candida albicans*.

The antimicrobial inhibitory activity observed in these organisms, as well as the wound healing potential in experimental albino rats, can be attributed to the presence of secondary plant metabolites such as alkaloids, saponins, and flavonoids in bitter leaf. Furthermore,

the extraction and isolation of compounds such as saponins, alkaloids, flavonoids, phenolic acids, lignans, xanthones, and anthraquinones from *V. amygdalina* have been reported to induce various biological effects in humans, including cancer chemoprevention, as reported by [9]. The chemopreventive properties of *V. amygdalina* are believed to be linked to its ability to scavenge free radicals, induce detoxification, inhibit stress response proteins, and interfere with the DNA binding activities of certain transcription factors [9].

Conclusion

There has been a great deal of interest in recent times in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases. Of the various classes of phytochemicals, interest has focused on the anti-inflammatory and antioxidant properties of polyphenols found in various botanical agents such as *V. amygdalina* commonly called the bitter leaf plant. The plant extracts have gained wide acceptance in traditional medicine and is currently gaining scientific research attention as the main alternative sources of prophylactic and chemopreventive drug discovery and development. This study was able to establish that, the *in vivo* administration of bitter leaf extract on experimental albino rat ameliorated haematotoxicity caused by *S. pyogenes*. It also revealed the neutraceutical potentials of the plant extract. Therefore, intake of bitter leaf should be encouraged among patients with microbial infections and also as medicinal and nutritional supplement in foods. Study established that *in vivo* administration of bitter leaf extract on albino rats was best at 60g/200 ml, and 100g/200ml in ameliorating haematotoxicity caused by *S. pyogenes.*

ACRONYMS

PVC = packed cell volume
Hb –haemoglobin
RBC = red blood cell
MCV= mean cell volume
MCH = mean cell haemoglobin
MCHC = mean cell haemoglobin concentration
WBC = white blood cell
GAS = Group A Streptococcal

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