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Research Article

The Effects of Ethanol Extract from The Seeds, Leaves and Barks of *Picralima Nitida On. Leucocytic Indices in Albino Rats*

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Abstract

Picralima nitida is widely used in West African traditional medicine for treating various blood related disorders including anaemia and diabetes. Despite substantial scientific evidence validating the ethno-medical use of different parts of this plant. This study was designed to investigate the haematinic values, and the safety profile of ethanol extracts of leaf, seed and bark of *P. nitida* in PHZ induced anaemic Wistar rats, by evaluating the effects on the leucocytic indices (total white blood cell (WBC) count, differential leucocyte count (lymphocyte, monocyte and granulocyte) A total of one hundred and ninety apparently healthy Wistar rats, weighing between 150-180 grams were used. The animals were randomly divided into 9 experimental groups: A-I, representing normal, negative and positive controls, as well as 200 and 400 mg/kg doses of leaf, seed and stem bark extracts of *P. nitida* respectively. Blood samples were evaluated at days 7, 14, and 21 following the oral administration of the different treatments post induction.

The different extracts showed varied abilities in restoring the abnormal increases in WBC and demonstrated significant recovery from PHZ induced reductions in RBC, and PCV. All the extracts significantly ameliorated the negative impacts of PHZ on the leucocytic indices of the Wistar rats, albeit not at the same strength.

Keywords: seeds, leaves, barks, *picralima nitida*, leucocytic indices, albino rats.

INTRODUCTION

For millennia, people have used naturally occurring minerals, plants, and animals as remedies to treat various illnesses. It is a dynamic activity that has been documented in early practitioners' writings as well as folklore. Decoctions, poultices, ointments, and solutions of plants, animal parts, and minerals were common ingredients in recipes for treating illnesses [1]. The trend shifted towards the production of pure drugs from plant and animal precursors after the discovery of pure drugs like quinine, atropine, and reserpine from plants. While many of these remedies have vanished over time, some are still in use today and are used to treat diseases by traditional medicine practitioners of all cultures.

The pharmacological potential of plants is still abundant despite the fact that this tendency was reversed with the introduction of exclusively synthetic medications. Of the 250 000 species of higher plants on the globe, only roughly 94 species have been or are now being used for drug manufacture [2]. Only a small portion of the 250 000 species of higher plants have been used as medicinal agents, even in the field of traditional medicine [3]. Apocynaceae is the family of plants that includes Picralima nitida.

The Picralima nitida shrub or tree can grow up to 35 meters tall and has glabrous white latex throughout. Its bole can reach a diameter of 60 cm. The bark of the tree is thick and brittle, ranging from pale to dark greyish black or brown, smooth to slightly rough or finely striped. The leaves are opposite, simple, and whole. There are no stipules, and the petiole is 1-2 cm long. The blade is elliptical to oblong, measuring (5–)10–26 cm × 2–13 cm, with a cuneate base and an abruptly acuminate apex. It is pinnately veined, with 14–23 pairs of lateral veins. The terminal or occasionally axillary

inflorescence is a compound, umbel-like cyme that is 6–10 cm long and has 10–35 flowers; the peduncle is 2–35 mm long and has three main branches; the bracts are extremely tiny [4].

The seeds, stem bark, fruit, and leaves of the Picralima nitida plant are among the parts of the plant that are used to make medicinal medicines [5]. The seeds have antipyretic and aphrodisiac properties, and they are used to treat pneumonia, malaria, and other respiratory tract ailments. Fever, dysmenorrhea, and gastrointestinal issues are all treated with the fruit. The leaf sap is administered to the ears to treat otitis, and the leaves are used as a vermifuge. Bark preparations are used as febrifuges, anthelmintics, laxatives/purgatives, and for the treatment of hernias and venereal disorders. Its root extract is used to treat gastrointestinal issues, malaria, pneumonia, and vermifuge, aphrodisiac, and febrifuge conditions.

To our knowledge, there is no published information on the toxicity profile of P. negida, despite the fact that characteristics like diuretic, carminative, and anthelmintic capabilities have been described. Therefore, the purpose of this study is to assess the toxicity effects, both acute and sub-acute, of the ethanol extract of P. Nitida's seeds, bark, and leaves. Many phytochemical components found in plants contribute to their poisonous and therapeutic qualities. When red blood cells are not producing enough oxygen to meet body requirements, anemia occurs. This condition can be caused by a variety of factors, including age, sex, altitude, smoking, and pregnancy status [6].

The majority of what is known about the uses of plants is the outcome of many years of human research and selection of the most successful, lustrous, and desirable plants that are available in the immediate environment at any particular time [7]. Eighty percent of people in underdeveloped nations still rely on native medicinal plants to meet their basic health needs, according to the World Health Organization [8]. In addition, the advantages of phytopharmacy are widely acknowledged, and medicinal plants already play a significant role in both plant research and medicine. Using herbal remedies at home is the primary line of treatment for 60% of children with high fevers in many African nations, including Ghana, Mali, Nigeria, and Zambia [9].

The present worldwide paradigm for obtaining pharmaceuticals from plant sources makes the use of plants in medicine even more significant. As a result, attention has been drawn to the medical benefit of herbal therapies for their safety, efficacy, and affordability [10]. In my community of Umunoha, Mbaitoli, picralima nitida is a plant that has long been utilized for its alleged hematinic and hypoglycemic qualities.It is equally used to treat a wide range of illnesses in different regions of West Africa. These conventional assertions are not supported by any scientific evidence, despite their frequent use.

This study aims to investigate the effects of Picralima nitida on. leucocutic indices (total white blood cell (WBC) count, differential leucocyte count (lymphocyte, monocyte and granulocyte) in phenylhydralazine and alloxan-induced anaemic and diabetic Wistar rats respectively.

MATERIALS AND METHODS

Plant material

Picrilima nitida leaves, seed and stem-bark were used for the study

Animals

One hundred and ninety (190) male albino Wistar rats (aged 3 – 4 months) bred in the Laboratory Animal House of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike were used for the study. The animals weighed between 150 – 180 g and were housed at ambient temperature (25°C) with lightening period of 12 h daily. Clean drinking water and standard commercial feed (Vital®, Nigeria) were provided *ad libitum*. Ethical standards governing the use of life animals for experiments were strictly observed. The ethical clearance with approval number MOUAU/CVM/REC/2020002 for this study was issued by the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike Ethical Committee.

Plant collection, identification and extraction

The leaves, seed and stem-bark of *Picralima nitida* were collected from Umunoha village in Mbaitoli local government area of Imo state and identified by a taxonomist (Prof. F.M. Mbagwu) in the Department of Botany, Imo State University, Owerri.

The plant samples were dried on laboratory bench and pulverized into coarse powder using hammer mill (Muliner®). Five hundred gram (500 g) each of the plant material were macerated in 2 litres of 80% methanol for 48 hours with intermittent shaking at 3 hours intervals and filtered with Whatman No. 1 filter paper. The filtrates were concentrated *in vacuo* using rotary evaporator and finally dried in hot air oven (40°C). The extracts were stored in the refrigerator (4°C) throughout the period of the experiment. Percentage yield was calculated using the formula:

$$\% \text{ yield} = a - b / a \times 100$$

Where, a = weight of original material (i.e. coarse powder) used for the extraction and b = weight of the dried extract.

Acute toxicity study:
This was done using Lorke method (Lorke, 1983). A total of 105 adult rats were used for the study. The study was carried out in two phases. In the first phase, four groups; A-D of 5 rat each were given (orally) distilled water, 10 mg/kg, 100 mg/kg and 1000mg/kg of the leaf extracts of *P. nitida*, respectively. Rats in group A served as the normal control. In the second phase, three (3) groups; E-G with 5 rats in each group, were given (orally), 1600 mg/kg, 2900 mg/kg and 5000 mg/kg (higher doses) of the leaf extract of *P. nitida*, respectively. The same procedure was repeated with the seed and stem-bark extracts of *P. nitida.*
The rats were observed for signs of toxicity and mortality over a period of 24 - 72 hours, post administration. They were allowed for another 7 - 14 days to observe any delayed toxicity.
The median lethal dose (LD_{50}) of the three extracts (leaf, seed and bark) were then calculated using the formula adopted by (Khan *et al.*, 2013).
$LD_{50} = \sqrt{}$ ~~(least dose with mortality × Highest dose without mortality)~~

Evaluation of the antianaemic effects of methanol extract of *Picralima nitida* leaf, seed and bark

Induction of anaemia
Briefly, anaemia was induced in the rats by intraperitoneal administration of 2.5% phenyl hydrazine hydrochloride (Fisher Scientific Company, New Jersey, USA) at a dose of 30 mg/kg body weight. The anaemia was maintained by the administration of 15 mg/kg body weight of 2.5% phenyl hydrazine hydrochloride at interval of 3 days, for the duration of the experiment.

Dose response effects of the extract against phenylhydrazine induced anaemia
Seventy-two (72) male albino Wistar rats were assigned to 9 groups (A – I; n = 8). Group A was a normal rat (non anaemic) while groups B – I was anaemic. The rats were treated as shown in Table 1 once daily for 21 consecutive days. Blood sample were collected from the retro-orbital plexus into EDTA and sodium citrate bottles on days 7, 14 and 21 of treatment. The blood in EDTA bottles were used for complete blood cell count while the blood in sodium citrate bottle were used for coagulation parameters analysis.

Table 1: Experimental design of antianaemic study

Group	Treatment	Number of animals
A	Normal control (Distilled water, 5 ml/kg)	8
B	Negative control (Untreated)	8
C	Positive control (Bunto, 5 ml/kg)	8
D	*P. nitida* leaf 200 mg/kg	8
E	*P. nitida* leaf 400 mg/kg	8
F	*P. nitida* seed 200 mg/kg	8
G	*P. nitida* seed 400 mg/kg	8
H	*P. nitida* stem-bark 200 mg/kg	8
I	*P. nitida* stem-bark 400 mg/kg	8

Haematological analysis
leucocutic indices tests was conducted with automated haematology analysers. leucocutic indices (total white blood cell (WBC) count, differential leucocyte count (lymphocyte, monocyte and granulocyte));

Induction of experimental diabetes
Alloxan was used to induce experimental diabetes mellitus in this study. Following 18 h fast, the blood sugar level (FBS) of the animals was determined using auto-analyzer (Accu-Check Active® glucose kit). Diabetes was then induced in the rats by a single intraperitoneal administration of alloxan monohydrate (160 mg/kg). The FBS of the rats were checked every other day, and on the sixth day rats with FBS ≥ 126 mg/dl were considered diabetic.

Effect of graded doses of the extracts on FBS of alloxan induced diabetic rats
Forty eight (48) alloxan-induced diabetic male Wistar rats (aged 8 – 10 weeks) were used for this study. The rats were randomly assigned into 8 groups of 6 rats per group and treated as shown in Table 2.

Table 2: Experimental design of antidiabetic study.

Group	Treatment
A	Negative control (Distilled water, 5 ml/kg)
B	Positive control (glibenclamide, 2 mg/kg)
C	*P. nitida* leaf 200 mg/kg
D	*P. nitida* leaf 400 mg/kg
E	*P. nitida* seed 200 mg/kg
F	*P. nitida* seed 400 mg/kg
G	*P. nitida* stem-bark 200 mg/kg
H	*P. nitida* stem-bark 400 mg/kg

All treatment were administered with oral gavage. The FBS of all the animals were measured at 0, 1, 3, 6 and 24 h post drug or extract administration using an auto analyzer (Accu Check Active®) glucose kit. The blood samples were collected from the tail vein after a tail snip.

Effects of repeated dosing of extracts on alloxan-induced diabetic rats

Forty-eight (48) diabetic male albino rats were randomly assigned into 8 groups of 6 rats per group and were treated as shown in Table 2 above. All animals were treated once daily for 21 consecutive days via oral gavage. The FBS levels of the rats were measured on days 0, 7, 14 and 21. On day 22 (24 h after the last treatment), blood samples were collected from the retro-orbital plexus through the median canthus of the eye, using heparinized capillary tubes. Blood collected from each animal was placed in plain sample bottles. The blood samples were placed in a slanting position for 30 minutes to allow for serum separation, after which they were centrifuged at 2000 r.p.m. for 10 minutes (Brar *et al*., 2000). The serum was harvested and used for biochemical analyses (lipid profile, liver and kidney function tests). The rats were afterwards sacrificed by cervical dislocation and vital organs like liver, kidney,and spleen were harvested into sample bottles containing 10% formal saline for histopathology.

Statistical Analysis

Data obtained were presented as mean (± S.E.M.) in tables and chart. They were analyzed using one-way analysis of variance (ANOVA) (SPSS software). The variant means were separated by Least Significant Difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$.

RESULTS

Table 4.5: Effects of methanol extracts of *P. nitida* leaf, seed and bark on the total WBC count on phenyl hydrazine induced haemolytic anaemia in wistar rats.

Treatment / Group	A	B	C	D	E	F	G	H	I	F value	P value
Day 7	11.08± 0.71*	17.30± 1.12#	15.88 ± 1.80	20.73± 0.97#	18.73± 2.91#	18.58± 2.05#	16.13 ± 2.10	20.25± 2.05#	16.80± 1.87#	2.43	0.04
Day 14	10.40± 0.60*	13.18± 0.85#	11.13 ± 0.76	12.25 ± 0.15	8.40 ± 0.70*	13.43± 0.90#	10.80 ± 0.40	10.13± 0.97*	10.95 ± 0.45	3.34	0.016
Day 21	8.50± 1.42*	16.45± 2.47#	14.55± 2.71#	9.28± 2.48*	9.98± 1.20*	8.73± 1.40*	8.18± 0.39*	8.05± 1.59*	13.15± 1.71	2.88	0.018

*$p < 0.05$ when compared with the negative control; # $p<0.05$ when compared with the normal control

KEY:

A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)
C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg
H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

The result of the effect of methanol extracts of P. nitida leaf, seed and stem-bark on the total WBC count on phenyl hydrazine induced haemolytic anaemia in wistar rats is presented in Table 1. In day 7, the mean values of the WBC were significantly ($p<0.05$) higher in groups D, E, F, H, and I compared with group A (normal control), although not statistically ($p>0.05$) different from the mean value obtained in group B. In day 14, the mean value was significantly ($p<0.05$) higher in group B compared with treatment groups and the normal control, although the TWB count did not differ significantly ($p>0.05$) from the mean value in group F, whereas in day 21, the TWB count were significantly ($p<0.05$) lower in groups D, E, F, G, and H compared with the normal control.

Table 4.6: The effects of methanol extracts of *P. nitida* leaf, seed and bark on the absolute lymphocyte count on phenyl hydrazine induced haemolytic anaemia in wistar rats.

	A	B	C	D	E	F	G	H	I	F value	P value
Treatment											
Group											
Day 7	8.88 ± 0.71*	13.95± 1.16#	13.23± 1.50	16.28± 1.04#	14.33± 1.62#	15.25± 1.51#	12.08 ±2.23	16.00± 1.87#	13.33± 1.44#	2.239	0.056
Day 14	9.48 ± 0.50*	12.53± 0.75#	10.68± 0.81	11.60 ± 0.20	7.85 ± 0.55*	12.97± 0.84#	10.15 ± 0.15	9.63 ± 1.10*	10.15 ± 0.75	3.427	0.014
Day 21	5.78± 0.46*	8.40± 0.72#	9.55± 1.71	7.95 ± 2.17	8.23 ± 1.08	7.28 ± 1.18	6.38 ±0.24	6.10 ± 1.03	8.73 ± 0.43	1.231	0.319

*p<0.05 when compared with the negative control; #p < 0.05 when compared with the normal control group.

KEY:

A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)
C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg
H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

The result in Table 4.6 showed that the absolute mean values of lymphocyte count in groups D, E, F, H, and I were statistically ($p>0.05$) the same compared with the group B, but were significantly ($p< 0.05$) higher than the mean value of the normal control group in day 7, while in day 14, the mean value was significantly ($p<0.05$) increased from 9.48 ± 0.50 $\times 10^3/mm^3$ in the normal control group to 12.53 ± 0.50 $\times 10^3/mm^3$ in group B and 12.97 ± 0.84 $\times 10^3/mm^3$ in group F. There was significant ($p<0.05$) reduction in group E, and H compared with the normal control. In day 21, there was significant ($p<0.05$) increase in group B compared with the normal control group.

Table 4.7: The effects of methanol extracts of *P. nitida* leaf, seed and bark on the absolute monocyte count on phenyl hydrazine induced haemolytic anaemia in wistar rats.

	A	B	C	D	E	F	G	H	I	F value	P value
Treatment											
Group											
Day 7	1.08± 0.11*	2.50 ± 0.11#	2.10 ± 0.27	2.98 ± 0.58#	2.95 ± 0.72#	2.55 ± 0.44#	2.40 ± 0.33#	2.75 ± 0.43	2.58 ± 0.40#	1.864	0.108
Day 14	0.60 ± 0.12	0.45 ± 0.10	0.38 ± 0.03	0.55 ± 0.05	0.40 ± 0.10	0.37± 0.07*	0.50± 0.20	0.43± 0.09	0.55± 0.15	0.701	0.687
Day 21	0.23 ± 0.08	0.20 ± 0.04	0.23 ± 0.03	0.45 ± 0.19	0.58 ± 0.14	0.18 ± 0.03#	0.28 ± 0.14	0.18 ± 0.03	0.43 ± 0.05	2.119	0.069

*p<0.05 when compared with the negative control; #p < 0.05 when compared with the normal control group.

KEY:

A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)
C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg
H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

The result in Table 4.6 showed that the absolute mean values of lymphocyte count in groups D, E, F, H, and I were statistically ($p>0.05$) the same compared with the group B, but were significantly ($p< 0.05$) higher than the mean value of the normal control group in day 7, while in day 14, the mean value was significantly ($p<0.05$) increased from 9.48 ± 0.50 $\times 10^3/mm^3$ in the normal control group to 12.53 ± 0.50 $\times 10^3/mm^3$ in group B and 12.97 ± 0.84 $\times 10^3/mm^3$ in group F. There was significant ($p<0.05$) reduction in group E, and H compared with the normal control. In day 21, there was significant ($p<0.05$) increase in group B compared with the normal control group.

Table 4.7: The effects of methanol extracts of *P. nitida* leaf, seed and bark on the absolute monocyte count on phenyl hydrazine induced haemolytic anaemia in wistar rats.

	A	B	C	D	E	F	G	H	I	F value	P value
Treatment											
Group											
Day 7	1.08± 0.11*	2.50 ± 0.11#	2.10 ± 0.27	2.98 ± 0.58#	2.95 ± 0.72#	2.55 ± 0.44#	2.40 ± 0.33#	2.75 ± 0.43	2.58 ± 0.40#	1.864	0.108
Day 14	0.60 ± 0.12	0.45 ± 0.10	0.38 ± 0.03	0.55 ± 0.05	0.40 ± 0.10	0.37± 0.07*	0.50± 0.20	0.43± 0.09	0.55± 0.15	0.701	0.687
Day 21	0.23 ± 0.08	0.20 ± 0.04	0.23 ± 0.03	0.45 ± 0.19	0.58 ± 0.14	0.18 ± 0.03#	0.28 ± 0.14	0.18 ± 0.03	0.43 ± 0.05	2.119	0.069

*$p < 0.05$ when compared with the negative control; #$p < 0.05$ when compared with the normal control group.

KEY:

A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)
C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg
H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

The mean values of the absolute monocyte count in day 7 were significantly ($p<0.05$) higher in groups D, E, F, G, and I than in the normal control group, although not significantly ($p>0.05$) different between the extract treated groups and the untreated group. In day 14, the mean value was significantly ($p<0.05$) lower in group F compared with group B whereas, in day 21, the mean value was significantly ($p<0.05$) decreased in the seed extract at 200 mg/kg compared with the normal control group.

Table 4.8: Table 4: Effects of methanol extracts of *P. nitida* leaf, seed and bark on the absolute granulocyte count on phenyl hydrazine induced hemolytic anaemia in Wistar rats.

	A	B	C	D	E	F	G	H	I	F value	P value
Treatment											
Group											
Day 7	1.15 ± 0.17	0.85 ± 0.10	0.55 ± 0.12	1.48 ± 0.61	1.50 ± 0.72	0.80 ± 0.15	1.68 ± 0.33	1.53 ± 0.25	0.95± 0.17	1.214	0.328
Day 14	0.30 ± 0.11	0.20 ± 0.07	0.10 ± 0.04	0.20 ± 0.00	0.15 ± 0.05	0.10 ± 0.00	0.15 ± 0.05	0.15 ± 0.05	0.3 ± 0.20	0.931	0.515
Day 21	2.50± 0.93*	7.83 ± 3.07#	4.73 ± 2.24	0.88± 0.17*	1.18± 0.22*	1.28± 0.29*	1.50± 0.35*	1.78± 0.75*	4.00± 1.33	2.63	0.029

* p<0.05 when compared with the negative control; # p<0.05 when compared with the normal control group.

KEY:

A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)
C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg
H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

The result presented in Table 4.8 showed the results of the methanol extracts of P. nitida leaf, seed and bark on the absolute granulocyte count on phenyl hydrazine induced hemolytic anaemia in Wistar rats. The result showed that in day 7 and 14 of blood collection and evaluation, there was no significant (p>0.05) difference across the treatment groups compared with the untreated group, although the rats in group C recorded the least count of absolute granulocyte count in day 7 and 14, but in day 21, the mean value of the absolute granulocyte in group B was significantly (p<0.05) higher compared with the normal control group. The mean value in the normal group was not significantly different (p>0.05) from the mean values obtained in groups D, E, F, G, and H.

Table 4.9: Effects of methanol extracts of P. nitida leaf, seed and bark on the relative lymphocyte count on phenyl hydrazine induced hemolytic anaemia in Wistar rats

	A	B	C	D	E	F	G	H	I	F value	P value
Treatment											
Group											
Day 7	79.78± 2.05	80.38 ± 1.59	83.28 ± 1.31	78.78 ± 4.98	77.90± 3.86	82.28 ± 1.58	73.33 ± 3.96	78.53 ± 1.56	79.60 ± 1.94	1.004	0.456
Day 14	91.38± 0.99	95.10± 1.07	95.80± 0.77#	94.45± 0.45	93.45± 1.55	96.47± 0.58#	94.15± 2.05	94.33± 2.11	92.35± 3.25	1.266	0.32
Day 21	70.35± 5.11	55.03 ± 9.14	69.25± 10.52	85.00± 2.69*	82.15± 2.75*	83.35± 2.67*	78.25± 4.37*	78.70± 6.04*	68.75 ± 6.28	2.455	0.039

*p < 0.05 when compared with the negative control; # p<0.05 when compared with the normal control.

KEY:

A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)
C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg

H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

The relative lymphocyte count presented in Table 4.9 showed no significant ($p>0.05$) difference in day 7 of blood collection and evaluation while in day 14, the rats in group C that received the reference drug, and group F recorded significantly ($p<0.05$), higher mean values of the relative lymphocyte count compared with normal control. In day 21, the mean values of the extract groups D to H were statistically ($p>0.05$) the same but were significantly higher than the untreated group.

Table 4.10: The effects of methanol extracts of *P. nitida* leaf, seed and bark on the relative monocyte count on phenyl hydrazine induced haemolytic anaemia in wistar rats.

Treatment Group	A	B	C	D	E	F	G	H	I	F value	P value
Day 7	9.68± 0.60*	14.50± 0.84#	13.30± 1.00	14.23 ± 2.30#	15.13 ± 1.82#	13.70 ± 1.32	15.1± 1.65#	13.45± 1.10	14.98± 1.22#	1.443	0.224
Day 14	5.75± 0.69	3.38 ± 0.53	3.30 ± 0.51	4.15 ± 0.45	4.55 ± 1.05	2.80 ± 0.40#	4.45 ± 1.65	4.25 ± 1.47	4.75 ± 1.45	0.975	0.486
Day 21	2.50± 0.51	1.48 ± 0.32	1.78 ± 0.43	4.73± 1.04*	5.95± 1.65*#	2.33 ± 0.26	4.28± 1.44*	2.25 ± 0.35	3.35 ± 0.55	2.952	0.017

*$p < 0.05$ when compared with the negative control; #$p < 0.05$ when compared with the normal control.
KEY:
A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)
C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg
H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

In Table 4.10 The result presented showed significant ($p<0.05$) recovery in the relative monocyte count in groups D, E, G, and I, then in the normal control group in day 7, although not significantly ($p>0.05$) different from the relative number in the untreated group, while in day 14, there was a significant ($p<0.05$) reduction in 200 mg/kg *P. nitida* seed group compared with the normal control group. In day 21, the mean relative values counted in groups D, E, and G, were significantly ($p<0.05$) higher than the mean value in the untreated group B. this suggest significant ($p<0.05$) recovery in the relative count for a duration of time (21 days treatment.

Table 4.11 The effects of methanol extracts of *P. nitida* leaf, seed and bark on the relative granulocyte count on phenyl hydrazine induced haemolytic anaemia in wistar rats.

Treatment Group	A	B	C	D	E	F	G	H	I	F value	P value
Day 7	10.5± 1.82	5.13 ± 0.96	3.43 ± 0.41#	7.00 ± 2.68	6.98 ± 2.29	4.28 ± 0.54#	11.58± 3.12*	8.03± 2.08	5.43 ± 0.77	2.152	0.065
Day 14	2.88 ± 1.06	1.53 ± 0.56	0.90± 0.29#	1.40 ± 0.00	2.00 ± 0.50	0.73± 0.18#	1.40 ± 0.40	1.43 ± 0.64	2.90 ± 1.80	1.08	0.419
Day 21	27.15± 4.86	43.50 ±9.42#	28.98± 10.91#	10.28± 2.04*	11.9± 1.31*	14.33± 2.52*#	17.48± 3.69*	19.05± 6.26*	27.90 ± 6.63#	2.972	0.016

*$p < 0.05$ when compared with the negative control; #$p < 0.05$ when compared with the normal control group.
KEY:
A Normal control (Distilled water, 5 ml/kg)
B Phenyl h+distilled water, 5 ml/kg)

C Positive control (Bunto, 5 ml/kg)
D *P. nitida* leaf 200 mg/kg
E *P. nitida* leaf 400 mg/kg
F *P. nitida* seed 200 mg/kg
G *P. nitida* seed 400 mg/kg
H *P. nitida* stem-bark 200 mg/kg
I *P.nitida* stem-bark 400 mg/kg

In table 4.11, the result showed the effect of methanol extracts of *P. nitida* leaf, seed and seed- bark on the relative granulocyte count. In day 7, there was significant ($p<0.05$) reduction of the relative number of granulocytes counted in the standard drug group and *P. nitida* seed at 200 mg/kg compared with the normal control, whereas, the mean value was significantly ($p<0.05$) higher in *P. nitida* seed at 400 mg/kg than the mean value obtained in the untreated group. This implies that the methanolic seed extract at the highest dose (400 mg/kg) significantly ($p<0.05$) increased the granulocyte cells counted, although the effect was not sustained at the 14th day of treatment compared with other extract groups. In day 14, the mean values significantly ($p<0.05$) decreased in group C and F compared with the normal control group. In day 21, the mean value in counted in group C, F, and I were significant ($p<0.05$) higher than the number counted in the normal control whereas the mean number counted in groups D, E, F, G and H were significantly ($p<0.05$) lower than the mean value obtained in the untreated group B. This significant ($p<0.05$) increase in the untreated group could suggest increased immature cell production (granulocytosis) stimulated by the erythropoietin as compensatory effect.

Discussion

The goal of the current study was to examine the hematinic and hemopoietic characteristics of P. nitida leaf, seed, and stem-bark extracts in albino Wistar rats that had been rendered hemolytically anemic by phenylhydrazine. According to the study, phenyl-hydrazine (PHZ) induction led to slight increases in the mean TWBC count values. The quick increase in TWBC mean values after PHZ administration is consistent with two processes: first, membrane damage that makes erythrocytes vulnerable to macrophage recognition and destruction; this occurs through the activation of B lymphocytes, which causes an amnestic response. [11,12,13]. The TWBC count in the untreated group was significantly high throughout the study period, although it was not statistically ($P > 0.05$) different from the value in the standard hematinic group. This suggests that the P. nitida methanolic leaf, seed, and stem-bark extracts could have restored the PHZ-induced elevated TWBC better than the standard hematinic used in this study [14]. After treatment with the various parts of the plant extracts, the mean count was gradually returned to the normal range.

The body's immune system's indicators against infection and foreign substances include the white blood cells, lymphocytes, monocytes, and granulocytes [15].

Therefore, it was discovered that the various plant extracts of P. nitida, when given at 400 mg/kg/body weight, were more efficient than 200 mg/kg dosages in reducing the leucocytophilic effect of phenylhydrazine, as seen by the significant return to normal values of these parameters. When the leucophilic effects of the three different plant extracts were compared, it was found that the stem-bark extract, which demonstrated a significant return to normal at the low dose, was not as effective at stabilizing the elevated TWBC and its indices as the leaf and seed extracts, which performed at 200 and 400 mg/kg body weight.

Additionally, it was noted that a longer treatment period along with a higher dosage of P. nitida leaf and seed extracts caused the total white blood cell count and differential counts to return to almost normal, suggesting that these effects were dependent on both dose and duration. These results demonstrate that PHZ may improve rat immune system response in addition to its known hemolytic impact, suggesting an immunological component in the ensuing anemia [16, 17]. It is significant to remember that the phyto-components of the plant extracts may be responsible for these reported effects.

Conclusion

All things considered, this study examined how Picralima nitida affected the leucophilic properties of Wistar rats that were treated with phenylhydralazine and alloxan. The results show great therapeutic potential and support the traditional usage of Picralima nitida for its characteristics. In rats treated with phenylhydralazine to produce anemia, picralima nitida substantially improved hematological indices and had noteworthy hematinic benefits. The plant extract's traditional use as a blood-boosting agent was supported by its effective counteraction of the hematological abnormalities caused by phenylhydralazine.

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