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Original Research Article

Lupinus arboreus Leaf Extract Modulates Haematosuppression by Cyclophosphamide Toxicity in Wistar Rats

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ABSTRACT

Lupinus arboreus has been reported to possess potent immunomodulatory, anti-inflammatory, and antimicrobial effects. This study aimed to investigate the impact of *Lupinus arboreus* leaf extract on haematosuppression by cyclophosphamide toxicity in Wistar rats. Crude 70% ethanol extract of the plant leaf was used for this study. Wistar rats (38), divided into various groups, were used for the study. Twenty-five rats were grouped into 5 groups (I-V) of 5 rats each as experimental groups. The lethality studies followed a modified Lorke's method, consisting of 13 rats, divided into 2 phases. Phase I rats contained 3 groups of 3 rats each, and phase 2 had 4 groups of 1 rat each. The negative control group (I) received only feed and water. Group II received 30 mg/kg of cyclophosphamide, while groups III and IV received 200 and 400 mg/kg of the extract plus 30 mg/kg of cyclophosphamide, respectively. Group 5 received 30 mg/kg of cyclophosphamide and 0.23 mL/kg of Bioferon (standard haematinic). Cyclophosphamide 30 mg/kg was administered to induce leukopenia in all test groups, except group I, for the final 4 days (days 24–28). The extract/drug was administered orally once daily for 28 days. Animals were sacrificed under chloroform anaesthesia after the duration of treatment, and blood samples were collected via ocular puncture in an EDTA container to measure haematological parameters. Treatment with cyclophosphamide resulted in a significant decrease ($p < 0.05$) in white blood cell, red blood cell, platelet, and antioxidant levels compared to the control. Similarly, treatment with the extract reversed the adverse effects of cyclophosphamide on white blood cells, red blood cells, and platelets and increased antioxidant levels in the research animals. The efficacy of the leaf extract of *Lupinus arboreus* may be attributed to its phytoconstituents.

Keywords: Cyclophosphamide, *Lupinus arboreus*, Chikadoma plant, Haematosuppression

Introduction

Lupinus arboreus (L.), family Fabaceae, is often cultivated as an ornamental plant in Nigeria, commonly known as Chikadoma. The pharmacological properties of the plant have been significantly researched. [1,2] In Nigerian traditional medicine, the leaves of Chikadoma have been used in the treatment of various illnesses, including scabies, skin abnormalities, scald heads, and other cutaneous diseases. [1, 2] Babatunde *et al.* assessed the topical antimicrobial and anti-inflammatory properties of the plant leaf extract, which showed potent anti-inflammatory and antimicrobial properties. [3]

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Its use as a dermal formula for topical application was safe and did not cause erythema or oedema.

The ethnopharmacological potential of the Chikadoma plant, from the southern Nigerian rainforest, was evaluated in a study.[4]

According to the study, plants in the Fabaceae family have a wide range of secondary metabolites with significant medicinal benefits, including antinociceptive and antioxidant actions. In a study by Okolo *et al.,* the methanol leaf extract of the Chikadoma plant was found to prevent malaria, a tropical disease caused by *Plasmodium falciparum*. [5] The results showed that the extract inhibited the growth of *Plasmodium falciparum* at the mature schizont stage. The extract's IC50 after 40 hours of incubation was 3.0 mg/mL, indicating that it has significant *in vitro* antimalarial activity against *P. falciparum.* Another study assessed the anti-arthritic properties of the methanol extract of Chikadoma leaves (MECL) in animals with arthritis induced by complete Freund's adjuvant (CFA). [6] The study's findings demonstrated the possible anti-arthritic properties of the methanol extract from Chikadoma leaves. Ohadoma & Amazu evaluated the bactericidal activity of the plant leaf in another study, which revealed the presence of phenolic acid in the plant. [7]

This current study aims to evaluate the immunomodulatory effects of the plant leaf extract on the haematosuppression in cyclophosphamide-induced toxicity in Wistar rats.

Materials and Methods

Collection and identification of plant material.

The leaves of the plant were collected on January 6, 2025, from

Mgbakwu (latitude 6° 16′ 20″ N, longitude 7° 3′ 21″ E, and coordinates 6.27234° N, 7.05605° E), Akwa, in Anambra State, and identified by Dr Ike Chibueze, a plant taxonomist at the Botany Department, Nnamdi Azikiwe University, Anambra State, Nigeria, where a voucher specimen number, UNIZIK-BOT-2025-3119, was assigned and deposited at Nnamdi Azikiwe University herbarium.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of the College of Medicine, Chukwuemeka Odumegwu Ojukwu University's Faculty of Basic Medical Science, Uli campus (Reference Number: COOU//REC/MM64/039). We followed the National Institutes of Health's guidelines for laboratory animal management and care when handling and treating the Wistar rats. [8]

Preparation of extract

The leaves of freshly harvested *Lupinus arboreus* (Chikadoma plant) were cleaned with tap water and dried for two weeks. The dried plant sample was ground into powder, and 890 g of powdered material was soaked in 2.4 L of 70% ethanol for 24 hours at room temperature (25°C). To ensure proper extraction, the mixture was stirred intermittently. It was filtered using a porcelain cloth and Whatman No. 1 paper. The filtrate was dried in a water bath (40ºC) to yield about 91 g of a reddish-brown sticky extract. The extract was reconstituted to a concentration of 1 g/mL in 1 mL of distilled water before administration. The extract was stored at 4°C until it was used. [9-14]

Phytochemical Analysis

The extract's phytochemical analysis was conducted using the techniques previously described [15, 16, 17], with minor adjustments. After preheating the oven to 2000°F, it was heated to 3300°F for five minutes at a rate of 30°F per minute. The detector operated at a temperature of 3200°C. By comparing the area and mass of the newly identified phytochemicals with those of the internal standard, the phytochemicals were identified. The different phytochemical concentrations are shown in µg/g.

Lethal dose (LD_{50}) of the extract.

With a few minor modifications, the extract's lethality was assessed using Lorke's technique. [18]

This approach consisted of two steps.

First Step

Nine (9) male rats were employed in this phase. The rats were divided into 3 groups of 3 rats each. The leaf extract was administered at 50, 500, and 700 mg/kg to each animal group. The animals were kept under observation for a whole day to track both mortality rates and behaviour.

Second Step

Four (4) male rats were divided into four (4) groups of one (1) rat each throughout this phase. After administering 1250, 2500, 3750, and 5000 mg/kg of the leaf extract, respectively, to the rats, they were monitored for a day to check for any indications of acute intoxication or death.

The following formula was used to determine the extract's LD_{50}:

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

Where;

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

Experimental Animal Groupings

Twenty-five (25) Wistar rats, weighing between 110.5 and 225.3 g, were divided into 5 groups of 5 rats each. They were obtained from the Nnamdi Azikiwe University animal house in Awka, Nigeria. All animals were housed in appropriate and comfortable wire-mesh cages with normal lighting and temperature (between 25°C and 29°C). They were fed a typical rat pelleted diet and had unlimited access to tap water. The animals were allowed to acclimatise for a duration of two weeks.

The rat groupings are as follows:

Group 1 (negative control group) received water and feed only.
Group 2 (cyclophosphamide only group) received 30 mg/kg cyclophosphamide.
Group 3 (low-dose extract group) received a combination of 200 mg/kg of the extract alongside 30 mg/kg of cyclophosphamide.
Group 4 (high-dose extract group) received a combination of 400 mg/kg of the extract alongside 30 mg/kg of cyclophosphamide.
Group 5 (standard drug group) received a combination of 0.23 mL/kg of bioferon and 30 mg/kg of cyclophosphamide.

Leukopenia was induced in all test groups except group 1 by administering cyclophosphamide at 30 mg/kg on the final 4 days (days 24-28) of treatment. The extract and drug were administered orally once daily for a period of 28 days.

Collection of Blood Sample

After 28 days of extract/drug administration, the animals were sacrificed at the Department of Physiology, Chukwuemeka Odumegwu Ojukwu University, via chloroform anaesthesia, and blood samples were collected by ocular puncture using a heparinised capillary tube. The blood sample was collected into an EDTA container and centrifuged for 30 minutes at 3000 rpm to extract the serum, which was then used for the haematological test. [19-22]

Determination of Haematological Parameters

Determination of Pack Cell Volume / Hematocrit %

A capillary tube was placed in the microhaematocrit rotor, which was filled to three-quarters of its capacity with well-mixed EDTA blood. The empty end was sealed, and the tube was centrifuged for five minutes. Using the microhaematocrit reader, the PCV was read as soon as centrifuging was complete. [11]

Determination of total white blood cells counts Unit $X10^{9}/L$

The test tubes were filled with 0.38 mL of the dilution fluid using a pipette. This was mixed with 0.02 millilitres of thoroughly mixed EDTA blood, and the counting room was assembled. A Pasteur pipette was used to remix the diluted blood sample. After that, the sample was placed within one of the chambers' grids. The chamber was left unattended for twenty minutes to give the white blood cells time to settle. The cells in the chamber's four huge squares were counted using the X10 objective lens, and the number of white cells per litre was recorded. [11]

Determination of thrombocyte count unit $X10^{9}/L$

Using a pipette, the test tube was filled with 0.38 millilitres of dilution fluid. After adding 0.02 mL of thoroughly mixed EDTA blood, the sample was poured into the counting chamber. The chamber was set up on moist tissue in a Petri dish and sealed with a lid to maintain the fluid after 20 mm of undisturbed time to prevent drying out. The platelets were counted in the tiny squares, and the number of platelets per litre was reported after examination with an X10 objective lens. At the end of a dry-clean slide, a drop of blood was placed. Keeping the spreader at an angle of about 30 degrees, the drop of blood was spread by pulling the spreader back to touch it, allowing the blood to run along the edge to form a film that was 40 to 50 mm long (two-thirds of the slide). The spreader's end was cleaned and wiped. The slide was waved back and forth to allow the film to air-dry immediately. When completely dried, the dry film was protected from dust and insects. It was fixed in pure methanol.[11]

Leishman Staining Technique

After the undiluted Leishman stain was applied to the slide, it was allowed to mix for 2 minutes. To ensure that the stain and water were thoroughly mixed, twice as much pH 6.5 buffered water was added to the stain, and the mixture was then blotted for 8 mm. After allowing it to air-dry, the slide was cleaned with tap water and placed in a draining rack. During the examination, an oil-immersed objective lens was employed. [11]

Determination of RBC count (procedure) $X10^{12}/L$

Four millilitres of diluting fluid were placed into a test tube. After carefully mixing the EDTA blood, 0.02 mL was added and swirled.

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After the counting chamber was assembled and filled with a well-mixed sample, it was left unattended. The combination was examined using an x10 objective lens. The number of red blood cells per litre was determined by counting the red blood cells in the tiny squares. N x 201 x 109/0.2 x 0.1 is the RBC count. Where N was the number counted, 201 was the diluting factor, 0.2 was the area, and 0.1 mm was the chamber's depth. [11]

Determination of Oxidative Markers
Malondialdehyde (MDA)
After mixing 1.6 mL of Tris-KCl buffer with an aliquot of 0.4 mL of supernatant, 0.5 mL of 30% TCA was added. The liquid was then heated for an hour after the addition of 0.5 mL of 0.75% TBA. This was centrifuged at 4,000 rpm after being cooled on ice. Following the collection of the clear supernatant, the absorbance at 532 nm was measured using d/w as a blank. [23]

GSH (Reduced Glutathione level, µg/mL)
After mixing 0.2 mL of the sample with 1.8 mL of distilled water, 3 mL of the precipitating solution was added. The mixture was then centrifuged for 10 minutes at 4000 rpm. It was then allowed to stand for another 5 minutes. One millilitre of filtrate was combined with 4 mL of 0.1 M phosphate buffer. Lastly, 0.5 millilitres of DTNB were added. Mix and immediately begin reading absorbance at 412 nm every 30 to 60 seconds for 3-5 minutes at room temperature. [23]

Catalase Estimation (µ/g)
The sample (0.2 mL) was combined with 1 mL of phosphate buffer containing 100 mM H_2O_2 and then incubated. Mix and immediately begin reading absorbance at 240 nm every 10 to 15 seconds for 1-2 minutes at room temperature. [23]

Superoxide Dismutase (u/mL)
After 0.2 mL of the sample was diluted in d/w to produce a 1:10 dilution, 200 µL of the diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2). The reaction was then initiated by adding 0.3 mL of freshly prepared 0.3 mM epinephrine after the liquid had been quickly stirred by inversion. Mix and immediately begin reading absorbance at 420 nm every 15 seconds for 1-2 minutes at room temperature. [23]

*Glutathione –S- Transferase (*µg/mL)
In the assay, the tubes were marked as 'test' and 'blank'. 30 µL of GSH (0.1 M) was pipetted into each tube. Each tube was filled with 150 µL of CDNB (20 nm). 2.82 mL of 0.1 M phosphate buffer was added to the blank tube, and 2.79 µL was added to the test tube. The test-labelled tube was filled with 30 µL of the sample. Mix and immediately record absorbance at 340 nm every 15 seconds for 2-3 minutes at room temperature. [23]

Glutathione Peroxidase
The test combination has a total volume of 2.0 mL and is composed of 0.5 mL of sodium phosphate buffer, 0.1 mL of sodium azide, 0.2 mL of reduced glutathione, 0.1 mL of H_2O_2, 0.5 mL of a 1:10 sample dilution, and 0.6 mL of distilled water. To halt the process, 0.5 mL of 10% TCA was added to the tubes after they had been incubated for three minutes at 37°C. To determine how much glutathione was left, 1 mL of the supernatant was combined with 4.0 mL of disodium hydrogen phosphate and 1 mL of DTNB. The colour developed was measured at 340 nm. [23]

Statistical Analysis
Version 25 of the Statistical Package for the Social Sciences (SPSS) was used to analyse the data from the study. Both post hoc LSD and ANOVA were used to analyze the data. At $p < 0.05$, the data were deemed statistically significant.

Results and Discussion

The lethality results showed that, even at a level of 5000 mg/kg, the extract's LD_{50} did not cause any harm in rats. The results in Table 1 revealed a significant difference ($p < 0.05$) in body weight/kg of the cyclophosphamide group 2 rats (213.60 ± 0.20) compared to the control group (183.60 ± 0.01). The percentage difference in body weights/kg reduced significantly ($p < 0.05$) in the cyclophosphamide group 2 rats (33.97%) when compared to the 200 mg/kg of the extract + 30 mg/kg of cyclophosphamide group 3 rats (55.13%), 400 mg/kg of the extract + 30 mg/kg of cyclophosphamide group 4 rats (40.39%), and 0.23 mL/kg of bioferon + 30 mg/kg of cyclophosphamide group 5 rats (59.62%). The study animals' cyclophosphamide therapy may have caused anorexia, gastrointestinal mucosal damage, protein catabolism, muscle wasting from systemic oxidative stress, and cytokine activation, which could have contributed to the study's body weight drop. Through the production of reactive oxygen species that outweigh the body's natural antioxidant defences, cyclophosphamide causes oxidative stress in the Wistar rats used in this study. Weight loss and systemic toxicity are both influenced by this oxidative imbalance.

Table 1: Values of body weight of the extract of the Chikadoma plant in study animals.

Groups	Initial body weight/g	Final body weight/g	Difference in body weight/g	% Difference
Negative control group	152.40±2.42	183.60±0.01	31.20±0.00	-
Cyclophosphamide only group	197.00±5.83[a]	213.60±0.20[a]	20.60±0.13[a]	**33.97**
Low-dose extract group	185.00±2.92	199.00±0.11	14.00±0.33	**55.13[b]**
High-dose extract group	183.00±5.83[b]	201.60±0.20	18.60±0.13	**40.39**
Standard drug group	165.40±3.22[b]	177.00±0.04[b]	12.60±0.02[b]	**59.62[b]**

KEY: Values are presented as mean ± sem. n 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to the cyclophosphamide group.

But by scavenging free radicals and boosting antioxidant enzymes, the plant leaf extract, which is rich in phytochemicals, may have had the opposite impact, lowering lipid peroxidation and ameliorating the Wistar rats' weight loss. The results of Babatunde *et al.'s* study, [4] which examined the ethno-pharmacological potential of Chikadoma plant leaves and showed their substantial therapeutic effects, including phytochemicals and antioxidant properties, are consistent with this discovery.

The result on Table 2 demonstrated that WBC counts significantly decreased ($p<0.05$) in the cyclophosphamide group 2 (7.42 ± 0.16 x109/L) in contrast to the control group (15.03 ± 3.01 x109/L), but rose significantly ($p<0.05$) in the 400 mg/kg of the extract + 30 mg/kg of cyclophosphamide group 4 rats and 0.23 mL/kg of bioferon + 30 mg/kg of cyclophosphamide group 5 rats (20.06 ± 1.09 x109/L, 16.22 ± 0.08 x109/L, respectively) in contrast to the cyclophosphamide group 2 rats (7.42 ± 0.16 x109/L). In Table 2, RBC counts significantly increased ($p<0.05$) in the 200 mg/kg of the extract + 30 mg/kg cyclophosphamide group, in the 3 rats (7.45± 3.33 x1012/L), in contrast to the cyclophosphamide group of 2 rats (2.77 ± 1.02 x1012/L). Platelet counts significantly decreased ($p<0.05$) in cyclophosphamide group 2 rats (178.55 ± 0.50 x109/L), in contrast to control group 1 (205.00 ± 06.77 x109/L); however, they rose significantly ($p<0.05$) in test groups 4 & 5 (210.92 ± 0.33 x109/L, 199.50 ± 03.00 x109/L) in contrast to group 2 (178.55 ± 0.50 x109/L).

Cyclophosphamide is a member of the alkylating agent class, which is a subclass of antineoplastic or cytotoxic medications. It is frequently used in cancer treatment and as an immunosuppressant. Cytochrome P450 enzymes in the liver activate cyclophosphamide, resulting in the formation of phosphoramide mustard, an active metabolite, and acrolein, a poisonous byproduct. Phosphoramide mustard causes DNA cross-linking, alkylation of guanine N-7 positions, leading to impaired DNA replication and apoptosis. Since it is non–cell cycle–specific but most toxic to rapidly dividing cells, it damages not only malignant cells

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but also normal proliferating cells, particularly bone marrow stem cells. Bone marrow has high mitotic activity and continuous production of leukocytes, erythrocytes, and thrombocytes. Cyclophosphamide suppresses hematopoiesis, leading to leukopenia (decreased WBCs, immunosuppression, and increased infection risk), anaemia (reduced RBCs, persistent fatigue, and pallor), and thrombocytopenia (decreased platelets and increased bleeding tendency). Thus, its blood cell–damaging effect is essentially due to bone marrow suppression or myelotoxicity from DNA cross-linking in progenitor cells.

Table 2: Values of some hematological parameters of study animals

Groups	WBC ($x10^9$/L)	RBC ($x10^{12}$/L)	Platelets ($x10^9$/L)
Negative control group	15.03 ± 3.01	6.11 ± c	205.00 ± 06.77
Cyclophosphamide only group	7.42 ± 0.16[a]	2.77 ± 1.02	178.55 ± 0.50[a]
Low-dose extract group	13.10 ± 1.20	7.45± 3.33[b]	191.08 ± 0.44
High-dose extract group	20.06 ± 1.09[b]	3.46 ± 0.27	210.92 ± 0.33[b]
Standard drug group	16.22 ± 0.08[b]	5.02 ± 1.10	199.50 ± 03.00[b]

KEY: Values are presented as mean ± sem. n 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to the cyclophosphamide group.

In the current investigation, male Wistar rats that had been exposed to cyclophosphamide-induced toxicity were assessed for their ability to recover. The findings showed that administering the extract to the animals significantly protected them from the damaging effects of cyclophosphamide.

It was observed that the cyclophosphamide-treated rats had significantly lower leukocyte, erythrocyte, and thrombocyte counts compared to the control rats. The decreased levels of leukocyte, erythrocyte, and thrombocyte counts observed in the study are consistent with earlier findings of immunosuppression caused by cyclophosphamide in mice. The co-administration of graded doses of the extract and cyclophosphamide resulted in significantly higher white blood cell and platelet counts, as well as significantly higher red blood cell counts, as compared to the cyclophosphamide-induced toxicity group, according to this study. The pattern found in this investigation aligns with a prior publication, which describes the protective effects of plant extracts, such as *Moringa oleifera* leaf, against cyclophosphamide-induced immunosuppression. [24] Although an IL-2 assay was not performed in this study, its physiological role in haematopoiesis has been suggested; as a result, cyclophosphamide treatment affects IL-2, which is involved in the maturation and development of WBCs. Other studies have demonstrated that flavonoids and phenolics can promote haematopoiesis.

The toxicity of cyclophosphamide to normal cells has been shown to diminish when flavonoids are combined with chemoprotective drugs. [25, 26] Additionally, an increase in WBC may be a sign that the plants' leaves improved the animal's resistance to infection, which could explain the antimicrobial activity of the plants. [2, 7] The allegedly increased platelet and red blood cell counts could indicate that the extract has coagulating and antianemic properties. This is because platelets' main job is to identify injured blood vessel endothelium, which builds up at the site of injury and eventually triggers blood clotting to seal the wound. Additionally, they are a component of the innate and adaptive immune systems. Through interactions with leukocytes, they contribute to the start of inflammation and are also involved in the development of tumours and atherosclerosis. Therefore, the primary cause of the elevated platelet levels may be this reason. The results of the RBC count and the increase in platelet count both indicate the absence of any anaemic potential in the extract. As a result, incidences of a decreased platelet count have been linked to anaemia. [24]. As a result, the findings clearly imply that the extract may operate as a naturally occurring haemato-stimulant to counteract the haemato-suppression brought on by cyclophosphamide. Combining the extract with cyclophosphamide for administration modifies blood parameters and may help treat intracellular bacterial and viral infections, promote wound healing, and treat anaemia.

The result in Table 3 demonstrated that MDA significantly increased ($P<0.05$) in cyclophosphamide group 2 rats (0.60±0.03 nmol/mL) in contrast to group 1 (0.50±0.40 nmol/mL), but decreased significantly ($p<0.05$) in the 400 mg/kg of the extract + 30 mg/kg of cyclophosphamide group 4 rats & 0.23 mL/kg of bioferon + 30 mg/kg of cyclophosphamide group 5 rats (0.49±0.22 nmol/mL, 0.41±0.00 nmol/mL) in contrast to group 2 (0.60±0.03 nmol/mL). SOD significantly decreased ($p<0.05$) in cyclophosphamide group 2 rats (0.27±0.00 U/mL), in contrast to control group 1 (0.42±0.10 U/mL), but increased significantly ($p<0.05$) in the 400 mg/kg of the extract + 30 mg/kg of cyclophosphamide group 4 rats (0.40±0.20 U/mL) in contrast to group 2 (0.27±0.00 U/mL). GSH significantly increased ($p<0.05$) in the 200 mg/kg of the extract + 30 mg/kg cyclophosphamide group 3 rats & 400 mg/kg of the extract + 30 mg/kg of cyclophosphamide group 4 rats (1.15±0.03 Umol/L, 1.04±0.10 Umol/L) in contrast to the cyclophosphamide group 2 rats (0.92±0.02 Umol/L).

Table 3: Values of oxidative markers of the extract of the study animals

Groups	MDA nmol/mL	SOD U/mL	CAT Umol/L	GSH Umol/L
Negative control group	0.50±0.40	0.42±0.10	3.88±0.00	1.22±0.50
Cyclophosphamide only group	0.60±0.03[a]	0.27±0.00 [a]	2.62±0.10	0.92±0.02
Low-dose extract group	0.55±0.00	0.30±0.00	2.88±0.05	1.15±0.03[b]
High-dose extract group	0.49±0.22[b]	0.40±0.20[b]	2.75±1.00	1.04±0.10[b]
Standard drug group	0.41±0.00[b]	0.32±0.50	3.15±0.00	0.97±0.30

KEY: Values are presented as mean ± sem. n 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to the cyclophosphamide group.

Glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) are examples of endogenous antioxidants. It's possible that the cyclophosphamide therapy in this study triggered acrolein metabolism, which lowers SOD, CAT, and GSH, while increasing reactive oxygen species (ROS), oxidative stress, lipid peroxidation (MDA), and tissue damage. It's possible that cyclophosphamide metabolites led to an excess of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide anion, which depleted the research animals' GSH pools and inactivated their enzymes.

One indicator of lipid peroxidation is MDA. Reactive oxygen species (ROS) produced by cyclophosphamide attack membrane polyunsaturated fatty acids, raising MDA levels and reflecting oxidative damage to the rats' cell membranes in this investigation. However, the plant leaf extract examined in this study is abundant in flavonoids and polyphenols, which have antioxidant qualities. As a result, it scavenges free radicals by directly reducing ROS, lipid peroxidation, and MDA, as well as by increasing the levels of antioxidant enzymes and GSH. This is consistent with research on the documented phytochemical analyses of the plant leaves. [4, 7]

Conclusion

This study demonstrated that the treatment with cyclophosphamide resulted in a significant decrease in white blood cells, red blood cells, platelets, and antioxidant levels in Wistar rats. However, treatment with the extract ameliorated the harmful effects of cyclophosphamide on white blood cells, red blood cells, and platelets and improved antioxidant levels of the research animals. The efficacy of the leaf extract from the Nigerian Chikadoma plant may be attributed to its

phytoconstituents. Future research should be carried out to elucidate the molecular mechanism and validate the efficacy of the leaf extract in human clinical trials..

Conflict of Interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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