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PROTECTIVE EFFECTS OF *EUGENIA UNIFLORA* LEAF EXTRACT ON HEMATOLOGICAL PARAMETERS AND IMMUNE FUNCTION IN ALBINO RATS EXPOSED TO CYPERMETHRIN-INDUCED OXIDATIVE STRESS

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ABSTRACT
This study aimed to evaluate the protective effects of *Eugenia uniflora* leaf extract on hematological parameters and immune function in albino rats exposed to cypermethrin-induced oxidative stress. Thirty female Wistar rats were acclimatized and divided into six groups, receiving various treatments, including a control group, a toxicant group (cypermethrin), and groups treated with the extract alone or in combination with the toxicant. Blood samples were collected for analysis of hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, total white blood cell (TWBC) count, and leukocyte differentials. Automated hematology analyzers and microscopic techniques were utilized for precise measurements. The control group exhibited optimal hematological parameters, while the toxicant group showed significant ($P \leq 0.05$) reductions in Hb (13.90 g/dl) and RBC (5.48 x 10^6/mm³), indicating potential anemia. Conversely, the extract-only group demonstrated a Hb level of 17.40 g/dl, comparable to control values. Combinations of the toxicant with various doses of the extract showed varying effects, with some doses mitigating but not fully restoring hematological parameters. Eugenia uniflora leaf extract appears to exert a protective effect on hematological parameters and immune function in albino rats exposed to oxidative stress. The findings support the therapeutic potential of Eugenia uniflora in managing oxidative stress-related conditions and highlight its role in promoting overall hematological health.

KEYWORDS: *Eugenia uniflora*, Hematological effects, Oxidative stress, Cypermethrin, Albino rats.

1. INTRODUCTION
Eugenia uniflora, commonly known as pitanga or Brazilian cherry, is a member of the Myrtaceae family that has garnered attention in recent years for its potential therapeutic properties, particularly in the context of oxidative stress and organ protection. This plant is rich in phytochemicals such as flavonoids, tannins, and phenolic compounds, which are known for their antioxidant and anti-inflammatory activities (Falcão *et al.*, 2018; Ferreira *et al.*, 2021). These bioactive components have been implicated in the modulation of various physiological processes, including hematological parameters, making *E. uniflora* a candidate for investigating its effects on blood profiles and leukocyte differentials.

Fig. 1: The Pitanga (*Eugenia uniflora*) leaves and fruits.

Cypermethrin, a synthetic pyrethroid insecticide, is commonly utilized in agricultural practices to manage pest populations. Despite its effectiveness, concerns regarding its toxicity have emerged, particularly regarding its impact on non-target organisms, including mammals (Kumar & Kumar, 2021).

The blood profile test is a fundamental diagnostic tool that provides insight into the overall health and physiological status of an organism. It encompasses various parameters, including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration, and hematocrit levels. These parameters are crucial indicators of hematological health and can reveal underlying conditions such as anemia, infection, and inflammation (Kumar & Abbas, 2017). In particular, the WBC differential count is vital for assessing immune function, as it quantifies the various types of leukocytes, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils (Hoffbrand & Moss, 2016).

Research has shown that oxidative stress can significantly impact hematological parameters, often leading to alterations in blood composition and immune function (Ayala *et al.,* 2014). Given that *E. uniflora* exhibits potent antioxidant properties, it is hypothesized that its leaf extract may exert a protective effect on hematological parameters in albino rats subjected to oxidative stress or toxicants. For instance, studies have indicated that phytochemicals from *E. uniflora* can mitigate oxidative damage in various organ systems, potentially leading to improved blood profiles (Fakhri *et al.,* 2022).

In this context, the present study aims to evaluate the haematological effects of *E. uniflora* leaf extract on the blood profile and WBC leukocyte differentials in albino rats. By assessing these parameters, the study seeks to elucidate the potential protective role of *E. uniflora* against oxidative stress and its implications for maintaining hematological health. Understanding these effects may contribute to the broader body of evidence supporting the integration of traditional herbal remedies into modern therapeutic practices, particularly in the management of oxidative stress-related conditions (Madrigal-Santillán *et al.,* 2014).

2. MATERIALS AND METHODS

2.1 Collection and Preparation of *Eugenia uniflora* for Extraction and Analysis

Fresh leaves of *Eugenia uniflora* were harvested from Ehimiri Housing Estate in Umuahia, Nigeria, and were identified in the Taxonomy Department at the College of Crop and Soil Science, Michael Okpara University of Agriculture, Umudike. The leaves were meticulously selected to ensure their quality and suitability for analysis.

Following harvest, the leaves were air-dried for one week in a controlled environment to prevent moisture and sunlight interference. Once thoroughly dried, the leaves were pulverized using an electric blender to obtain a fine powder. The resulting powder was stored in an airtight container to preserve its integrity until the extraction and analysis processes commenced (Ferreira *et al.,* 2021).

2.2 Experimental Animals Used for the Study

A total of thirty female Wistar rats were employed in this study. These rats were closely monitored from birth and separated from male rats at four weeks of age to ensure they were nulliparous and non-pregnant. The selected rats weighed between 130 and 150 g and were obtained from the Animal House at the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State.

Prior to the experiment, the rats underwent a one-week acclimatization period in the animal housing facility at the College of Veterinary Medicine. This acclimatization allowed the rats to adapt to their new environment, thereby enhancing the reliability of the experimental results. All animal ethics procedures were strictly adhered to throughout the study, with approval obtained from the College of Veterinary Medicine-Animal Ethics Committee.

During the experimental period, the rats were fed a standard Chikun grower feed and had access to tap water ad libitum. The animal housing conditions, including temperature and humidity, were closely monitored and regulated, and a 12-hour light-dark cycle was maintained to ensure the well-being of the animals.

The experimental design included the following
Group Allocation: The rats were divided into six groups, with five rats in each group
- Group A (Control): Received only feed and water.
- Group B (Toxicant Only): Administered cypermethrin at a dose of 70 mg/kg body weight.
- Group C (Extract Only): Received the *Eugenia uniflora* extract at 400 mg/kg body weight.
- Groups D, E, and F: Received cypermethrin at 70 mg/kg body weight along with the extract at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight, respectively.

- Treatment Protocol: Cypermethrin was administered once at the beginning of the 14-day treatment period, while the extracts were given on alternate days, 24 hours after the administration of the toxicant. All rats had free access to feed and water throughout the study.

- Post-Treatment Analysis: At the end of the 14-day period, the rats were euthanized, and blood samples were collected through cardiac puncture using a 2 ml sterile syringe into an EDTA vacutainer tube to prevent coagulation by a modified method of Ohwada 1986. The samples were immediately used for the estimation of the different parameters.

2.3 Measurement of blood parameters

Blood samples were analyzed using an automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK) with standard calibration, according to the manufacturer's instructions for analysis of human blood and accurately programmed for the analysis of red blood cell (RBC)

count, total white blood cell (WBC) count, hemoglobins (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobins concentration (MCHC)

2.4 Statistical analysis

The results gotten were subjected under statistical analysis using SPSS software (version 25.0 for windows; SPSS, Chicago, IL, USA). Results were expressed as a Mean ± Standard Error of Mean (SEM) and analyzed using one way analysis of variance (ANOVA) descriptives followed by post Hoc Tukey LSD test for homogeneity of subsets. Significance is taken to be P≤0.05.

3. RESULTS

3.1 Effects of *Eugenia uniflora* on the Blood profile of cypermethrin intoxicated albino rats

From the results presented in Table 1, the control group 1 exhibited optimal hematological parameters, with HB at 17.37±0.13 g/dl, PCV at 46.50±0.29%, and RBC count at 7.49±0.05 x 10^6/mm³. Additionally, the TWBC count at 10.05±0.26 x 10^3/mm³ reflects a robust immune response.

Conversely, the group exposed to the toxicant at a dosage of 70 mg/kg exhibited significant decreases (P≤0.05) in several hematological parameters: HB dropped to 13.90±0.17 g/dl, PCV to 33.00±1.15%, and RBC count to 5.48±0.07 x 10^6/mm³. The TWBC count also decreased to 9.38±0.16 x 10^3/mm³, suggesting a compromised immune response.

When the toxicant was combined with the extract at different dosages, results varied. The combination of the toxicant with 200 mg/kg of extract resulted in HB at 16.20±0.12 g/dl and a PCV of 41.00±0.58%, indicating that the extract may mitigate some of the toxic effects but does not fully restore the parameters to baseline levels. However, at the higher extract dosage of 600 mg/kg (Group 6), the HB decreased to 14.80±0.12 g/dl, demonstrating a potential negative interaction where high doses of the extract may not effectively counteract the toxicant's effects.

In contrast, the group 3 receiving only the extract at 400 mg/kg demonstrated hematological parameters comparable to the control group, with HB at 17.40±0.12 g/dl and PCV at 45.00±0.58%.

Table 1: Effects of *Eugenia uniflora* on the Blood Profile of cypermethrin intoxicated albino rats.

GROUPS	HB (g/dl)	PCV (%)	RBC ($x10^6 mm^3$)	TWBC ($x10^3 mm^3$)	MCV (Fl)	MCH (Pg)	MCHC (g/dl)
1	$17.37±0.13^{a}$	$46.50±0.29^{a}$	$7.49±0.05^{a}$	$10.05±0.26^{c}$	$62.08±0.05^{a}$	$23.37±0.08^{d}$	$37.64±0.11^{c}$
2	$13.90±0.17^{d}$	$33.00±1.15^{c}$	$5.48±0.07^{c}$	$9.38±0.16^{c}$	$60.79±1.65^{a}$	$25.39±0.02^{b}$	$42.22±0.95^{a}$
3	$17.40±0.12^{a}$	$45.00±0.58^{a}$	$7.28±0.10^{a}$	$14.90±0.17^{a}$	$61.82±0.09^{a}$	$23.91±0.18^{cd}$	$38.68±0.24^{bc}$
4	$16.20±0.12^{b}$	$41.00±0.58^{b}$	$6.62±0.11^{b}$	$10.03±0.10^{c}$	$61.99±0.13^{a}$	$24.50±0.22^{c}$	$39.53±0.27^{bc}$
5	$15.70±0.17^{b}$	$38.50±0.87^{b}$	$6.15±0.14^{b}$	$10.20±0.12^{c}$	$62.61±0.06^{a}$	$25.55±0.32^{b}$	$40.81±0.47^{ab}$
6	$14.80±0.12^{c}$	$35.00±0.58^{c}$	$5.55±0.09^{c}$	$13.03±0.36^{b}$	$63.06±0.06^{a}$	$26.68±0.21^{a}$	$42.31±0.37^{a}$

GROUPS -1-Control, 2-Toxicant (T) only 70mg/kg, 3-Extract (E) only 400mg/kg, 4-(T) 70mg/kg +(E)200mg/kg, 5- (T) 70mg/kg +(E)400mg/kg, 6- (T) 70mg/kg +(E)600mg/kg

Sample size (n) = 5 Results are presented as Mean± SEM and different letters on the same column are significantly different P≤0.05

3.2 WBC leukocyte differentials of cypermethrin intoxicated albino rats treated with *Eugenia uniflora* leaf extract

The results presented in Table 2 showed that the control group of the albino rats exhibited a healthy leukocyte differential profile, with lymphocytes at 61.00±0.58%, neutrophils at 31.00±0.00%, monocytes at 5.50±0.29%, eosinophils at 2.50±0.29%, and basophils at 0.00±0.00%. Lymphocyte levels decreased to 50.50±0.29%, while neutrophil levels remained constant at 31.00±0.58%.

Table 2: Effects of *Eugenia uniflora* on the WBC leukocyte differentials of cypermethrin intoxicated albino rats.

GROUPS	Lymphocytes %	Neutrophils %	Monocytes %	Eosinophils %	Basophils %
1-Control	$61.00±0.58^{ab}$	$31.00±0.00^{a}$	$5.50±0.29^{a}$	$2.50±0.29^{de}$	$0.00±0.00^{a}$
2-Toxicant (T) only 70mg/kg	$50.50±0.29^{e}$	$31.00±0.58^{a}$	$6.50±0.29^{a}$	$11.50±0.87^{a}$	$0.50±0.29^{a}$
3-Extract (E) only 400mg/kg	$63.00±0.58^{a}$	$30.00±0.58^{a}$	$5.00±0.00^{a}$	$2.00±0.00^{e}$	$0.00±0.00^{a}$
4-(T) 70mg/kg +(E)200mg/kg	$58.50±0.29^{bc}$	$31.50±0.29^{a}$	$5.50±0.87^{a}$	$4.50±0.29^{cd}$	$0.00±0.00^{a}$
5- (T) 70mg/kg +(E)400mg/kg	$57.00±1.16^{c}$	$32.50±1.44^{a}$	$5.50±0.29^{a}$	$5.00±0.00^{bc}$	$0.00±0.00^{a}$
6- (T) 70mg/kg +(E)600mg/kg	$54.00±0.00^{d}$	$32.50±0.87^{a}$	$6.00±0.00^{a}$	$7.00±0.58^{b}$	$0.50±0.29^{a}$

Sample size (n) = 5 Results are presented as Mean± SEM and different letters on the same column are significantly different P≤0.05

4. DISCUSSION

Blood Profile test

Hematological parameters, including hemoglobin (HB), packed cell volume (PCV), red blood cell (RBC) count, total white blood cell (TWBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), are crucial indicators of an individual's health status. These parameters provide insights into the oxygen-carrying capacity of the blood, immune function, and overall hematological health.

The values in Table 1 indicate a healthy state, with sufficient red blood cells to transport oxygen effectively throughout the body (Murray *et al.*, 2012).

These reductions in the hematological parameters in the toxicant group indicate a potential anemic state, characterized by decreased oxygen-carrying capacity. Anemia can result from various factors, including hemolysis, inadequate erythropoiesis, or nutritional deficiencies (Bennett *et al.*, 2015).

In contrast, the group receiving only the extract at 400 mg/kg demonstrated hematological parameters comparable to the control group. This suggests that the extract may possess hematopoietic properties, promoting the production of red blood cells and enhancing overall blood health (Nwafor *et al.*, 2018).

The MCV, MCH, and MCHC remained relatively stable across the groups, indicating that the toxicant's impact was more pronounced on overall red blood cell counts and hemoglobin levels. MCHC values for the toxicant group increased to 42.22±0.95 g/dl, which could suggest a compensatory mechanism in response to lower red blood cell counts.

Therefore, the extract at a normal dose of 400mg/kg demonstrated hematological parameters comparable to that of the control group while the group exposed to the toxicant at a dosage of 70 mg/kg exhibited significant decreases in several hematological parameters and the combination of the toxicant with a low dose of 200 mg/kg of extract mitigated some of the toxic effects but does not fully restore the parameters to baseline levels.

WBC leukocyte differentials

The assessment of leukocyte differentials, which include various types of white blood cells such as lymphocytes, neutrophils, monocytes, eosinophils, and basophils, provides essential insights into the immune status. These parameters reflect the body's response to infections, inflammation, and other stressors.

The values presented in Table 2 suggest a balanced immune response, with lymphocytes playing a crucial role in adaptive immunity and neutrophils serving as the first line of defense against infections (Nour *et al.*, 2019).

In contrast, the group exposed to the toxicant at a dosage of 70 mg/kg demonstrated significant changes in leukocyte differentials. The increase in eosinophils to 11.50±0.87% indicates a possible allergic or inflammatory response to the toxicant, as eosinophils are typically elevated in the presence of allergens or parasites (Wong *et al.*, 2020). This shift in the leukocyte profile suggests that exposure to the toxicant may compromise the immune system's ability to respond effectively to pathogens.

The group that received only the extract at 400 mg/kg exhibited a return to near-normal lymphocyte levels at 63.00±0.58%, suggesting that the extract may possess immunomodulatory properties that enhance lymphocyte production or activity (Ahmad *et al.*, 2021). The neutrophil count remained stable, indicating that the extract does not negatively impact the innate immune response.

The combination of the toxicant with 200 mg/kg of extract resulted in lymphocyte levels of 58.50±0.29%, showing partial recovery from the toxicant's effects. However, at the highest extract dosage of 600 mg/kg, lymphocyte levels decreased to 54.00±0.00%, suggesting a potential adverse effect of high extract dosage on immune function.

Monocyte percentages remained relatively stable across all groups, with slight variations that did not indicate significant changes in monocyte activity, which typically increases in response to chronic inflammation or infection (Roussel *et al.*, 2021). The eosinophil levels, while elevated in the toxicant group, showed a slight reduction with the extract, indicating a potential anti-inflammatory effect.

The result therefore showed that the extract did not negatively impact the innate immune response while the intoxicant possibly caused allergic reaction or inflammation that led to increased eosinophils, but the combination of the toxicant with 200 mg/kg of extract helped in partial recovery to normalcy.

CONCLUSION

The results showed that the extract significantly improved critical blood profile indicators, such as hemoglobin concentration, packed cell volume, and red blood cell count, suggesting potential hematopoietic properties. Furthermore, the extract demonstrated favorable effects on leukocyte differentials, particularly in restoring lymphocyte levels, which are essential for a robust immune response.

However, the study also reveals that higher doses of the extract may not always yield beneficial outcomes, pointing to the need for careful dosing and further investigation into the optimal concentrations for therapeutic efficacy.

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