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BIOCHEMICAL EVALUATION OF THE HEPATOPROTECTIVE AND NEPHROPROTECTIVE EFFECTS OF *EUGENIA UNIFLORA* LEAF EXTRACT AGAINST CYPERMETHRIN-INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT
This study investigates the biochemical impact of *Eugenia uniflora* leaf extract on liver and kidney function tests, antioxidant activity, and lipid profiles in Wistar rats exposed to cypermethrin, a widely used synthetic pesticide known for its neurotoxic effects. The study utilized Lorke's method to estimate the LD_{50} of the extract and cypermethrin, establishing the safety thresholds for subsequent experimentation. A total of 30 rats were divided into six groups to assess the ameliorative effects of varying doses of the leaf extract (200, 400, and 600 mg/kg) against a single dose of cypermethrin (70 mg/kg). Biochemical analyses of serum were conducted to evaluate liver enzymes (ALT, AST, and ALP), renal markers (creatinine and urea) and antioxidant status (MDA, CAT, SOD, and GSH). Results indicated that cypermethrin exposure significantly elevated liver enzymes and renal markers, indicating potential organ damage. Conversely, administration of *E. uniflora* extract, particularly at 200 mg/kg, demonstrated liver and kidney protective effects, evidenced by normalized enzyme levels and enhanced antioxidant activities. These findings highlight *Eugenia uniflora* as a promising candidate for mitigating oxidative stress and organ toxicity, reinforcing the need for further research on its therapeutic potential and optimal dosing strategies. This study contributes valuable insights into the integration of traditional herbal remedies in contemporary health management strategies, particularly regarding liver and kidney health.

KEYWORDS: *Eugenia uniflora*, Hepatoprotective, Nephroprotective, Cypermethrin, Oxidative stress.

1. INTRODUCTION
Eugenia uniflora, commonly known as pitanga or Brazilian cherry, is a member of the Myrtaceae family, indigenous to the tropical regions of South America, particularly Brazil. This plant has been historically utilized in traditional medicine for its numerous health benefits, including antipyretic, anti-inflammatory, and antirheumatic properties (Consolini & Sarubbio 2002; Falcão *et al.*, 2018). Recent studies have begun to explore the phytochemical constituents of *E. uniflora*, revealing a rich array of bioactive compounds, including flavonoids and phenolic compounds, which are known for their antioxidant activities (Ferreira *et al.*, 2021).

Among the commonly used insecticides is Cypermethrin, recognized as a significant environmental contaminant. Its potent insecticidal properties have led to its application in both residential and commercial settings (Wei *et al.*, 2023). Cypermethrin is known for its high biodegradability and comparatively lower toxicity, being particularly effective against a wide range of insect species. However, instances of toxicity in animals have also been documented. Cypermethrin is toxic and has been associated with various health issues, including hepato-renal dysfunction, testicular cancer, impaired motor activity, and neurotoxic effects, attributed to its ability to cross the blood-brain barrier (Ileriturk *et al.*, 2022).

The application of pesticides is projected to increase, potentially impacting the health of non-target species, including humans. Research has linked pesticide usage to various human cancers. These chemicals are prevalent in the agricultural and horticultural sectors, with the majority of individuals encountering them primarily through their food sources. The degradation of pesticides occurs at a notably slow rate, which raises concerns

about exposure through skin contact and ingestion (Abdou *et* al., 2012).

The liver plays a crucial role in metabolizing Cypermethrin, which is considered a secondary target organ. The metabolites of Cypermethrin can induce oxidative stress, leading to damage in liver cells and the release of reactive oxygen species (ROS). This oxidative stress can result in DNA damage, protein oxidation, and lipid peroxidation, further triggering liver inflammation and apoptosis. The subsequent activation of inflammatory processes and apoptosis exacerbates the damage to DNA, proteins, and lipids, perpetuating a cycle of oxidative harm.

Oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, is implicated in the pathogenesis of various diseases, including liver and kidney disorders (Halliwell & Gutteridge, 2015). The liver plays a critical role in metabolic processes and detoxification, while the kidneys are essential for the regulation of fluid and electrolyte balance and the excretion of waste products. Disruptions in the function of these organs can lead to significant health complications, making their assessment through biochemical markers crucial (Guerra Ruiz *et al.,* 2021).

The evaluation of liver and kidney function tests, alongside lipid profile assessments, provides valuable insights into the physiological effects of potential therapeutic agents. Elevated levels of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) can indicate hepatocellular injury, while markers such as serum creatinine and urea are essential for evaluating renal function (Gounden *et al.,* 2024).

This study aims to investigate the effects of *E. uniflora* leaf extract on liver and kidney function tests, together with the antioxidant activity. By assessing the serum concentrations of liver enzymes, renal markers, and antioxidant parameters, this research seeks to elucidate the potential protective effects of *E. uniflora* against oxidative stress and its implications for maintaining organ health. The findings from this study may contribute to the growing body of evidence supporting the therapeutic use of natural products in managing oxidative stress-related conditions, thereby reinforcing the importance of integrating traditional medicinal practices with contemporary scientific research.

2. MATERIALS AND METHODS

The materials used in this research were *Eugenia uniflora* leaf extract, ethanol (E-Merck), ethylene diamine tetraacetic acid (E-Merck), alanine transaminase activity assay kit (Sigma Aldrich), aspartate transaminase activity assay kit (Sigma Aldrich), urea assay kit (Sigma Aldrich), creatinine assay kit (Sigma Aldrich).

The following equipment was utilized for this research: Multifunction Electric blender (Silvercrest), Bio-RAD C.1000 Touch Thermal Cycler (Upland, CA, USA), Benchtop Variable Transilluminator M-26V/PIN 95-0458-02 (Cambridge, UK), Soxhlet extractor, B.BRAN Centrifuge (B.BRAN Scientific and Instrument Company, England), Digital Monochrome Printer Model P95DW (Mitsubishi Electric, Malaysia), Biorad DNA Electrophoretic System Model No: Power Pac Basic, Serial No: 041BRI12306 (Singapore), Thermo Scientific Nanodrop 2000 Spectrophotometer UV-Vis Spee, Serial: J206, Model El12352 (USA), Thermo Scientific Sorvall Legend Micro 21 Centrifuge, Label No: 50137357, Cat 75002435 (Osterode am Harz, Germany), Thermo Scientific Rotary Evaporator Model R-JOO (USA), Gas Chromatography-Mass Spectrometry Analyzer (Agilent MassHunter GCMS system model 5977).

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).

250g of the leaf powder was weighed, soaked with 1000ml of ethanol for 5 days (stirred every day), and filtered. The residue was squeezed, soaked with 500ml ethanol for 3 days (stirred every day), and filtered (the maceration procedure was repeated several times until clear extract was obtained). The extract was collected and evaporated with a rotary evaporator until a viscous extract was obtained (Zhang *et al.,* 2018).

2.2 Experimental Animals Used for the Study

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 140g±10g 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 (Guerra Ruiz *et al.*, 2021).

By utilizing well-controlled conditions and standard procedures, this study aims to accurately assess the effects of *Eugenia uniflora* leaf extract on liver and kidney function and antioxidant activity.

2.3 Treatment of Experimental Rats Exposed to Cypermethrin with the Extracts

A total of thirty rats were selected for this study, aimed at investigating the ameliorative effects of *Eugenia uniflora* extract on the toxic effects of cypermethrin. 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. One milliliter of blood was dispensed into plain test tubes without anticoagulants to allow clotting. Following clotting, the samples were centrifuged to separate the serum.

The blood samples obtained were centrifuged at 3000 rotations per minute for 15 minutes. Serum obtained were used for various biochemical analyses, including urea, creatinine, albumin, aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

The biochemical analysis of hepatoprotective activity parameters (alanine transaminase and aspartate transaminase) and the biochemical analysis of nephroprotective activity parameters (urea and creatinine) were carried out by the standard method from Sigma Aldrich.

Statistical analysis

Statistical analysis was done using SPSS software (version 25.0 for windows; SPSS, Chicago, IL, USA). Results are 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 (fisher 1935) significance is taken to be $P \leq 0.05$.

3. RESULTS

Table 1: Liver and Kidney function test.

GROUPS	T.Protein (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (Mg/dl)	Urea (Mg/dl)	Creatinine (Mg/dl)
1-Control	8.3±0.15^{a}	52.2±0.57^{c}	38.2±0.33^{d}	66.4±0.51^{ab}	0.4±0.01^{d}	20.2±0.02^{c}	1.1±0.01^{c}
2-Toxicant(T) only 70mg/kg	5.4±0.08^{d}	56.5±1.51^{b}	43.4±0.56^{b}	65.6±1.44^{ab}	0.6±0.00^{ab}	22.5±0.53^{ab}	1.2±0.01^{ab}
3-Extract (E) only 400mg/kg	8.0±0.09^{a}	52.4±0.18^{c}	36.3±0.41^{e}	65.7±0.25^{ab}	0.4±0.03^{d}	20.2±0.31^{c}	1.0±0.03^{c}
4-(T) 70mg/ kg+ (E)200mg/kg	6.8±0.10^{b}	56.0±0.27^{bc}	40.5±0.00^{c}	65.2± 0.03^{b}	0.5±0.01^{c}	21.0±0.23^{bc}	1.2±0.01^{bc}
5-(T)70mg/ kg+(E) 00mg/kg	6.4±0.06^{c}	57.9±1.30^{b}	42.3±0.60^{b}	66.4±1.10^{ab}	0.6±0.00^{bc}	21.5±0.06^{bc}	1.2±0.02^{bc}
6-(T)70mg /kg+(E)600mg/kg	6.1±0.04^{c}	69.3±0.61^{a}	45.2±0.00^{a}	69.1±0.26^{a}	0.6±0.01^{a}	23.3±0.56^{a}	1.3±0.02^{a}

Sample size (n) = 5 Results are presented as Mean± SEM and P≤0.05

The experimental results presented in Table 1, showed a clear disparity in biochemical markers between the control group and those exposed to the toxicant. The control group exhibited a total protein level of 8.3±0.15 g/dl, while the group exposed to the toxicant at a dosage of 70 mg/kg showed a significant reduction to 5.4±0.08 g/dl.

The control group had a stable AST level of 52.2±0.57 U/L and ALT levels of 38.2±0.33 U/L. Conversely, the toxicant group exhibited a slight increase in AST to

56.5±1.51 U/L and ALT to 43.4±0.56 U/L, indicating potential liver damage or stress (Lala *et al.*, 2023). Notably, the combination of the toxicant with the extract at varying doses produced mixed results of dose dependent increase in the parameters apart from a dose dependent decrease in the total protein level, with the highest extract dosage (600 mg/kg) yielding the most significant elevation in AST to 69.3±0.61 U/L and ALT to 45.2±0.00 U/L, indicating a possible exacerbation of liver stress. Bilirubin remained relatively low across all groups but were slightly elevated in the toxicant group (0.6±0.00 mg/dl).

Furthermore, the renal function markers, urea and creatinine, were evaluated. The control group maintained stable urea levels at 20.2±0.02 mg/dl, while the toxicant group displayed an increased urea level to 22.5±0.53 mg/dl.

The result also showed that the control group and extract group had similar results across all the parameters checked.

Table 2: Antioxidant test.

GROUPS	MDA (nm/g protein)	CAT (IU/g protein)	SOD (IU/g protein)	GSH (µg/L)
1-Control	5.71±2.68^{b}	7.98±2.56^{a}	1.87±0.18^{c}	62.64±0.12^{a}
2-Toxicant (T) only 70mg/kg	12.40±0.12^{b}	2.60±0.60^{a}	2.04±0.11^{bc}	55.98±0.60^{a}
3-Extract (E) only 400mg/kg	51.34±12.37^{a}	2.35±0.03^{a}	2.69±0.17^{a}	55.36±10.33^{a}
4-(T) 70mg/kg +(E)200mg/kg	11.71±0.80^{b}	7.46±2.75^{a}	2.38±0.05^{abc}	86.16±15.14^{a}
5- (T) 70mg/kg +(E)400mg/kg	13.35±2.76^{b}	2.73±0.63^{a}	2.30±0.08^{abc}	63.68±5.53^{a}
6- (T) 70mg/kg +(E)600mg/kg	17.52±0.05^{b}	1.23±0.51^{a}	2.48±0.06^{ab}	69.30±4.69^{a}

Sample size (n) = 5 Results are presented as Mean± SEM and P≤0.05

The biochemical results presented in Table 2 indicate the effects of *Eugenia uniflora* leaf extract on various antioxidant markers in rats exposed to the toxicant cypermethrin. The measured parameters include Malondialdehyde (MDA), Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione (GSH), each providing insights into oxidative stress and antioxidant defense mechanisms.

The extract alone exhibited extremely high MDA levels (51.34 nm/g protein) when compared to the other groups, however, the combination of cypermethrin with lower doses of the extract (200 mg/kg) resulted in reduced MDA levels (11.71 nm/g protein), indicating that the extract may have protective effects against lipid peroxidation at this dosage.

CAT levels were significantly lower in the toxicant-only group (2.60 IU/g protein) when compared to the group given the toxicant and the low dose of extract at 200 mg/kg which improved CAT activity to 7.46 IU/g protein, indicating that the extract may enhance the antioxidant defense system at a low dose. However, higher doses of the extract (400 mg/kg and 600 mg/kg) resulted in reduced CAT activity.

There was a slight difference in SOD levels across groups where the groups given the toxicant with the extract showed slight dose dependent increases in the SOD levels.

GSH levels were highest in the toxicant + extract (200 mg/kg) group (86.16 µg/L), in contrast, the extract alone had lower GSH levels (55.36 µg/L).

4. DISCUSSION

4.1 Liver and Kidney function test

Total protein levels are indicative of nutritional status and liver function; lower levels may suggest compromised liver health or malnutrition, both of which can have dire implications for overall health (Dutta *et al.*, 2021; Dufour *et al.*, 2000).

Liver enzymes, specifically AST and ALT, serve as critical indicators of liver health. (Fakhri *et al.*, 2022).

The alkaline phosphatase (ALP) levels, crucial for bile duct health and liver function, showed minimal variation across groups, suggesting that the toxicity primarily impacts other liver functions rather than biliary excretion (Lowe *et al.*, 2023). Bilirubin levels, a byproduct of hemoglobin breakdown and a marker for liver function, remained relatively low across all groups but were slightly elevated in the toxicant group (0.6±0.00 mg/dl), hinting a potential disruption in bilirubin metabolism (Lala *et al.*, 2023).

The elevation in urea signifies possible renal impairment or increased protein catabolism due to toxic stress (Ryabova *et al.*, 2023). Creatinine levels remained relatively consistent across groups, suggesting stable renal function, although slight increases were noted in the toxicant group which may suggest mild renal impairment. (Kasote *et al.*, 2015).

The introduction of the extract appeared to offer some protective effects against the toxicant, although results varied with dosage. For instance, the extract at 400 mg/kg combined with the toxicant showed improvements in AST and ALT levels compared to the toxicant alone, suggesting a potential hepatoprotective effect of the

extract (Fakhri *et al.*, 2022; Madrigal-Santillán *et al.*, 2014). However, the highest extract dosage led to increased enzyme levels, indicating that dosage optimization is crucial for therapeutic efficacy.

4.2 Antioxidant test

MDA is a marker of lipid peroxidation and oxidative stress. Higher MDA levels indicate increased oxidative damage. Cypermethrin's neurotoxic effects promote lipid peroxidation (Ayala *et al.*, 2014).

The result suggests that the extract may contribute to oxidative stress under certain conditions and may possibly have a pro-oxidant effect, indicating that further investigation into the conditions affecting the extract's properties is necessary. However, the combination of cypermethrin with lower doses of the extract (200 mg/kg) resulted in reduced MDA levels (11.71 nm/g protein), indicating that the extract may have protective effects against lipid peroxidation at this dosage.

Catalase (CAT) is an enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen, playing a crucial role in the antioxidant defense system.

Catalase levels were significantly lower in the toxicant-only group (2.60 IU/g protein), highlighting impaired antioxidant defenses due to cypermethrin exposure. The administration of *Eugenia uniflora* extract at 200 mg/kg improved CAT activity to 7.46 IU/g protein, indicating that the extract may enhance the antioxidant defense system. However, higher doses of the extract (400 mg/kg and 600 mg/kg) resulted in reduced CAT activity, which could indicate a potential inhibitory effect or depletion of the enzyme due to excessive oxidative stress (Kasote *et al.*, 2015)

Superoxide Dismutase is an important antioxidant enzyme that catalyzes the conversion of superoxide radicals into hydrogen peroxide and oxygen, thus protecting cells from oxidative damage. There was a slight difference in SOD levels across groups where the groups given the toxicant with the extract showed slight dose dependent increases in the SOD levels. This suggests that the extract may not significantly influence SOD activity, or that the activity of this enzyme is maintained even under oxidative stress conditions induced by cypermethrin (Zandi & Schnug 2022).

Glutathione is a key antioxidant that protects cells from oxidative stress. Its levels indicate the overall antioxidant capacity of the body.

GSH levels were highest in the toxicant + extract (200 mg/kg) group (86.16 µg/L), indicating that this dosage may effectively enhance the overall antioxidant capacity of the rats, counteracting the effects of cypermethrin. In contrast, the extract alone had lower GSH levels (55.36 µg/L), suggesting that the extract's protective effects are most pronounced when combined with a toxicant, likely due to a synergistic interaction (Fakhri *et al.*, 2022).

The results suggest that *Eugenia uniflora* leaf extract has potential therapeutic effects in mitigating oxidative stress induced by cypermethrin. The protective effects are particularly evident at lower doses of the extract, which enhance antioxidant enzyme activity and increase GSH levels, thereby reducing MDA levels.

However, the high levels of MDA associated with the extract alone warrant caution. Further investigation is needed to determine the optimal dosage and conditions under which the extract can be beneficial without contributing to oxidative stress.

5. CONCLUSION

The combined administration of the toxicant with *Eugenia uniflora* leaf extract produced varying effects depending on the dosage, the highest extract dosage appeared to exacerbate liver stress rather than mitigate it, as indicated by increased AST and ALT levels.

Furthermore, the toxicant is seen to compromise kidney function, while the extract showed protective effects at a lower dosage, as evidenced by reduced Malondialdehyde (MDA) levels and improved catalase (CAT) activity pointing to enhanced antioxidant defense. All these highlights the importance of dosage when evaluating the therapeutic efficacy of natural extracts in mitigating oxidative stress and organ damage.

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