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Changes in Oxidative Stress Biomarkers in Wistar Albino Rats due to the Oral Administration of Aqueous Stem Extract of *Cissus populnea* (Okoho).

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Abstract

Cissus populnea is a plant with evidence of ethnomedicinal uses. However, a dearth of information exists on changes in oxidative stress parameters due to chronic administration of the plant's aqueous stem extract. The present study evaluates changes in oxidative stress parameters in Wistar albino rats due to oral administration of the aqueous stem extract. Forty animals (20 males and 20 females), 8 groups (n=5) (4 males, 4 females) were given daily oral doses (125mg/kg, 250mg/kg and 500mg/kg body) of the extract for 28 days were used. The control groups received distilled water. Tissues from the liver, heart, kidney, lungs, and testes (males) and ovaries (females) of the animals were used for the determination of malondialdehyde, reduced glutathione and the activities of catalase and superoxide dismutase. In the males, the extract led to a significant ($p< 0.05$) reduction in the hepatic malondialdehyde while the level in other organs remained unaltered ($p> 0.05$). In the females, administration of 500 mg/kg of extract led to a significant ($p< 0.05$) increase in both hepatic and renal malondialdehyde levels, but a significant ($p> 0.05$) reduction in the cardiac malondialdehyde level, remained significantly ($p> 0.05$) unchanged in other organs. Though the extract caused a significant rise ($p< 0.05$) in glutathione and activities of catalase and superoxide dismutase in some of the organs, malondialdehyde was not affected at doses below 500 mg/kg. Thus, the extract did not cause oxidative stress in the animals at low doses. The findings support the potential use of Cissus populnea in managing oxidative stress-related conditions, particularly at lower doses where it appears to enhance antioxidant defences without causing oxidative damage.

Keywords: Aqueous extract, *Biomakers, Cissus populnea,* oxidative stress, whister rat

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INTRODUCTION

Oxidative stress is a complex and evolving concept that has gained clarity over time. According to Ji and Yeo (2021), it refers to the imbalance between oxidants and antioxidants, leading to an excessive accumulation of reactive oxygen species (ROS). These ROS, which include hydrogen peroxide (H2O2), hydroxyl radicals (OH^-), nitric oxide (NO), organic hydroperoxides (ROOH), and superoxide anions ($O2^-$) (Schieber & Chandel, 2014), are natural byproducts of aerobic metabolism. However, when not adequately neutralized by antioxidants, ROS can cause significant damage to lipids, proteins, and deoxyribonucleic acid (DNA), contributing to the development of various diseases.

Recently, there has been growing interest in the use of medicinal plants for their therapeutic properties, beyond their role as food sources (Adeniran & Akindele, 2024). Among these plants is *Cissus populnea,* a liana from the Vitaceae family (Amplidaceae). Widely utilized in ethnomedicine, *C. populnea* has various traditional applications. In Nigeria, for example, the Idoma people of Benue State use the stem bark as a soup condiment known as Okoho, while the Yoruba refer to it as Orogbolo, Ogbolo, or Ajara, and the Hausa call it Daafaaraa (Burkill, 2000 as cited in Achikanu & Ani, 2020). Beyond its culinary uses, the plant's aqueous stem extract is traditionally employed for treating male infertility, skin diseases, boils, and urinary tract infections (Kone et al., 2004).

Several studies have explored the medicinal potential of *Cissus populnea.* Ojekale et al. (2015) demonstrated the spermatogenic properties of the aqueous stem extract in Wistar rats, suggesting its potential use in treating male infertility. Similarly, Aondoaseer et al. (2021) reported the hypoglycemic effects of the extract in diabetic rats, indicating its potential as an antidiabetic agent. Moreover, Aletan et al. (2022) found that the stem extracts of *C. populnea* possess moderate antioxidant abilities in vitro, further supporting its use in traditional medicine.

Despite these promising findings, there is a notable scarcity of information regarding the in vivo effects of *C. populnea* on oxidative stress biomarkers. While oxidative stress biomarkers are critical tools for assessing disease progression and the impact of therapeutic interventions, the effects of chronic administration of *C. populnea* stem extract on these biomarkers remain largely unexplored. This study addresses the significant gap in the current literature by investigating the in vivo effects of *Cissus populnea* aqueous stem extract on oxidative stress biomarkers in Wistar rats. Unlike previous studies that have focused primarily on in vitro antioxidant activity or specific therapeutic properties, this research evaluates the chronic administration of the extract and its impact on oxidative stress parameters across multiple organs. The novelty of this work lies in its focus on the effects of *C. populnea* stem extract on oxidative stress biomarkers in Wistar rats, providing valuable insights into the potential therapeutic applications of this plant in managing oxidative stress-related conditions.

MATERIALS AND METHODS

Plant material collection

Stem parts of *C. populnea* bearing the leaves were purchased from Mushin market in Mushin Local Government Area of Lagos State, Nigeria. The plant was identified by a taxonomist at Forest Herbarium, Forest Research Institute Ibadan with Voucher number FHI 1133642.

Pre-Extraction Treatment
The stem was washed and cut into pieces spread on brown paper and allowed to air dry in a shade for 3 weeks. The dried pieces were then crushed using a local mortar to a coarse powder.

Preparation of the extracts
The coarse powdered sample was used for extraction. A quantity 500g of the sample was soaked with in 5 litres of distilled water, and allowed to stand for 72 hours at room temperature (25^{o} C) with intermittent shaking. The extract was filtered with Whatman filter paper (No 1), the filtrate was then concentrated to dryness in water bath at 70 ^{o}C; then labelled and stored until use for administration.

Preliminary phytochemical screening
The plant extract was subjected to photochemical screening analysis using the methods of Sowofora (1993)

Experimental Animals
The animals were obtained from the animal house, Department of Pharmacognosy, University of Lagos, Lagos State, Nigeria. The animals were acclimatized for 7days before the experiment, during which they were fed with pelletized animal feed and clean water. The NIH Guide for the care and use of laboratory animals (National Institute of Health, 2011) was strictly followed. The research was approved by the NOUN Research and Ethical committee (ETC/2023/NOUN/08/001).

A total of 40 Wistar albino rats (20 males and 20 females) weighing between 160 to 180 g were used for the study. The animals were weighed and randomly grouped into 8 (4 males and 4 females) groups of 5 animals per group. Three groups from each sex (Groups 2,3 and 4) were given daily doses of the plant extract (125, 250 and 500 mg/kg) respectively in 1 ml of distilled water while the control group (Group 1) for each sex was given 1ml distilled water for 28 days. These doses were chosen based the on pharmacological active doses which were effective in the spermatogenic effect (Ojekale et al., 2015) and antidiabetic effect (Aondoaseer et al., 2021) of the extract. At the end of the 28 days treatment period, the animals were deprived feed but had free access to drinking water for 24 hours before being anaesthetized under inhaled chloroform. The abdomen and the thorax were opened and the organs (liver, lungs, kidney, heart, testis (males) and ovaries (females)) were quickly removed and placed in sterile containers. A portion of 0.5g of tissue of each organ was homogenised in 4.5 ml of buffer solution (ice-cold phosphate buffer, pH 7.4) using homogeniser. The resulting homogenates were centrifuged in sterile bottles at 15,000 rms for 10 mins in a centrifuge. The resulting supernatant collected and stored at 4^{o} C until required for the biochemical investigations.

Determination of Superoxide Dismutase activity
Superoxide Dismutase activity was determined by the ability of the enzyme to inhibit the auto-oxidation of epinephrine. This was determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

Determination of Catalase activity

Catalase activity was determined according to Sinha et al. (1972). It was assayed colourimetrically at 620nm and expressed as μmoles of H_2O_2 consumed/min/mg protein at 25^0C. The reaction mixture (1.5ml) contained 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml of tissue homogenate and 0.4ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

Determination of Reduced Glutathione

The reduced glutathione (GSH) content of liver tissue as non-protein sulphydryls was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.

Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100^oC for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- a complex of 1.56×10^5 $M^{-1}CM^{-1}$

Statistical analysis

The results from the investigations were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the differences between groups then Tukey's multiple comparison test was used where significant differences existed. Differences in means were considered significant at $P < 0.05$. All analyses were performed in R statistical programe version 4.3.0

RESULTS

The results of phytochemical screening of *C. populnea* revealed the presence of important phytochemical constituents such as; tannin, phenols, phlobatannins, alkaloids, saponins, flavonoids and cardiac glycosides.

The liver of the mice in the control groups showed higher antioxidant response and oxidative stress characteristics when compared to the treatment groups. The liver assay shows a decreased trend in the enzyme activities and oxidative stress of mice administered 12mg/Kg, 250mg/Kg and 500mg/Kg of the extracts (Figure 1 a, b, c & d). Generally, the 125mg/kg had a higher oxidative influence on the mice than others (Figure 1 a, b, c & d). These variations were observed to be significant ($p < 0.05$) throughout the study period (Figure 1). The female mice also had a generally significant ($p < 0.05$) variation in the MDA, GSH, SOD and CAT levels (Figure 2 a, b, c & d).

An investigation into the antioxidant response pattern of the kidney assay for the male rats discloses only a significant ($p < 0.05$) difference in the SOD activities, which was in a dose-dependent trend (Figure 3b). The 125mg/Kg of the extract administered induced the highest response while at an exposure level of 500mg/Kg, the least oxidative response was obtained. In addition, observed variations in the GSH, CAT and MDA levels were made, however these

variations were not significant ($p > 0.05$) (Figure 3 a, c & d). The catalase activity in the kidney of the female mice was not significantly ($p > 0.05$) altered, while its lipid peroxidation was highest in the 500mg/Kg group (Figure 4 c & d). The extract had a significant ($p < 0.05$) effect on the superoxide dismutase of the kidney of the male mice (Figure 3 b) while a dose-dependent trend of the extract was observed on the kidney of the female mice (Figure 4 a, b, c & d).

The MDA levels and CAT activity in the heart of the male whister rats were not significantly ($p < 0.05$) altered during the study period. Moreover, GSH, SOD, and CAT in this organ varied significantly ($p < 0.05$) across the treatment groups. The SOD was in a dose-dependent trend (Figure 5b). Generally, there was a dose-dependent response in oxidative stress and antioxidant response for the heart of the female whister mice. The variations in the levels of GSH, MDA, SOD & CAT were significant ($p < 0.05$) (Figure 6 a, b, c & d). The glutathione and the SOD were significantly different for the heart (Figure 5A & B). There was a significant ($p < 0.05$) reduction in the oxidation of the exposed groups. Oxidative stress decreased with an increase in the dose of the extract in the female heart assay (Figure 6 a, c & d).

Interestingly, lipid peroxidation, GSH and CAT were not significantly ($p > 0.05$) altered in the lungs of the mice, only SOD activity varied significantly ($p < 0.05$) (Figure 7 a, b, c & d). The females had no significant changes in the MDA levels of their lungs (Figure 8 d). The levels of GSH, CAT and SOD were significantly altered ($p < 0.05$). The Biomarker, SOD was significantly altered in the lung of the mice exposed to the extract (Figure 7b). The results show no significant ($p > 0.05$) alteration in the oxidative markers (Figure 7b). The oxidative stress was significantly altered in the lungs of the female except for MDA (Figure 8d). Superoxide dismutase and Glutathione were also significantly ($p > 0.05$) different across all the groups (Figure 8 a, b & c). Surprisingly, investigation into the oxidation level and antioxidant response in the testes of the mice show no significant ($p > 0.05$) alteration in the SOD, GSH, CAT and MDA levels of the mice throughout the study period (Figure 9 a, b, c & d).

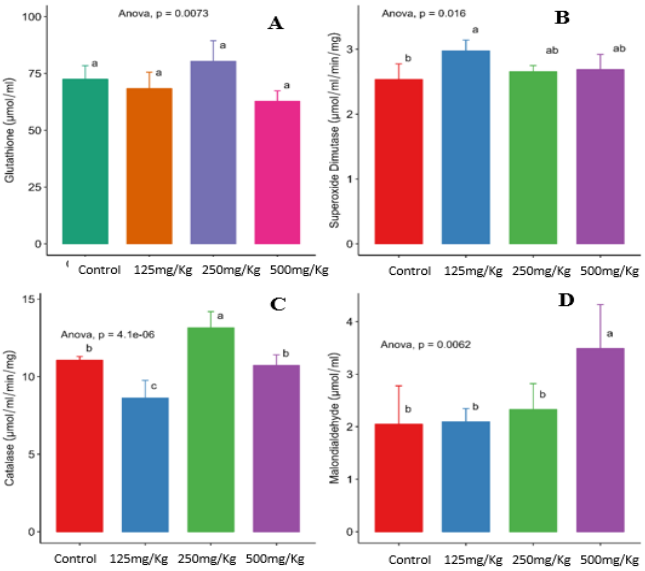

Figure 1: Oxidative stress response of male rats from the Liver

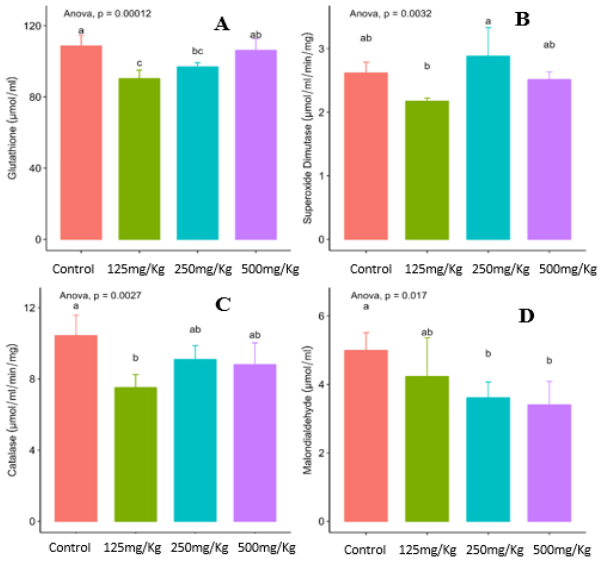

Figure 2: Oxidative stress response of female rats from the Liver

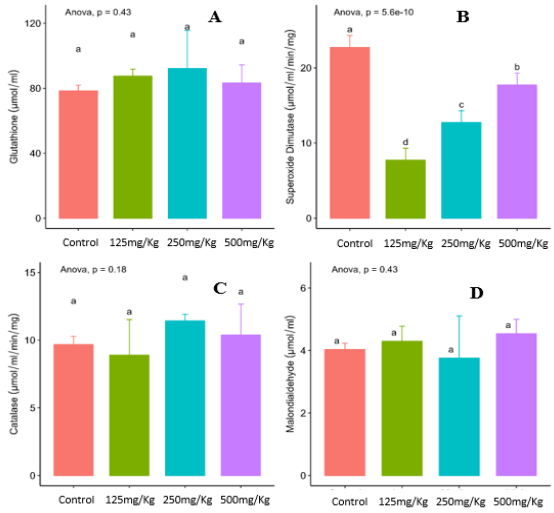

Figure 3: Oxidative stress response of male rats from the Kidney

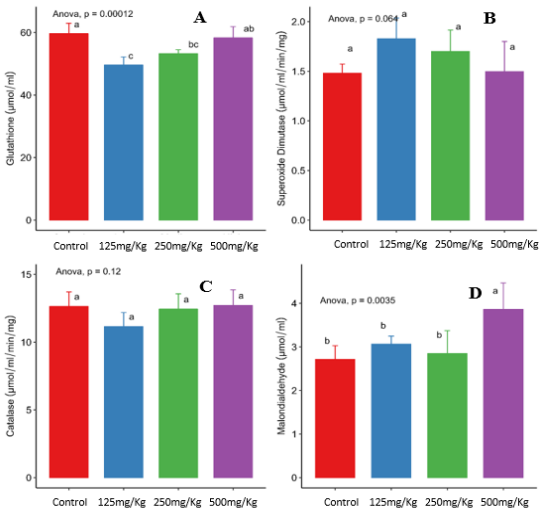

Figure 4: Oxidative stress response of female rats from the Kidney

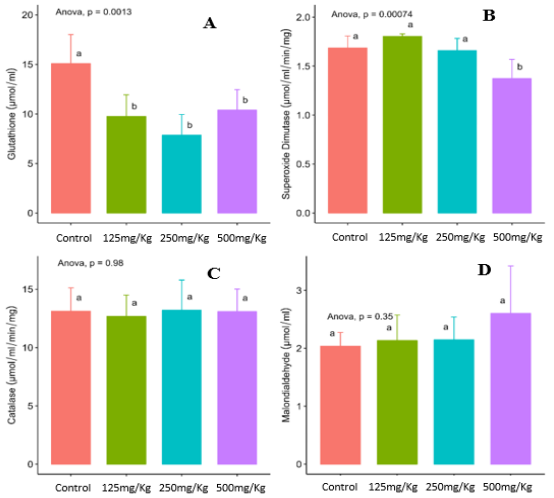

Figure 5: Oxidative stress response of male rats from the heart

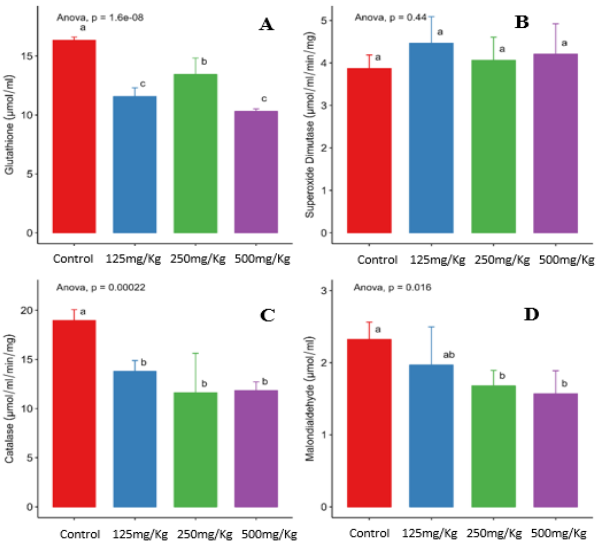

Figure 6: Oxidative stress response of female rats from the heart

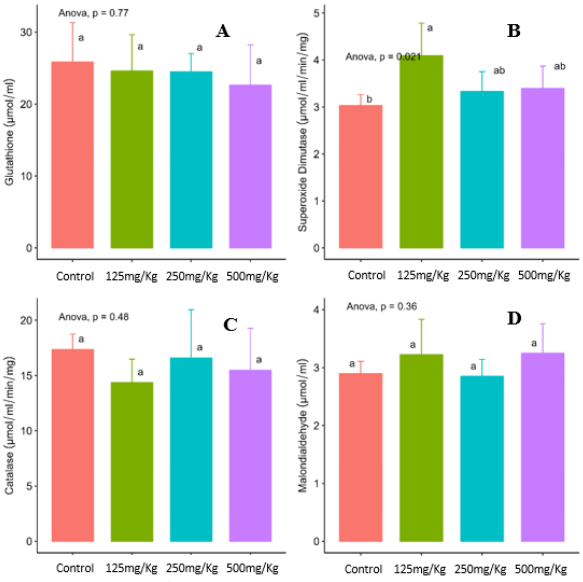

Figure 7: Oxidative stress response of male rats from the Lung

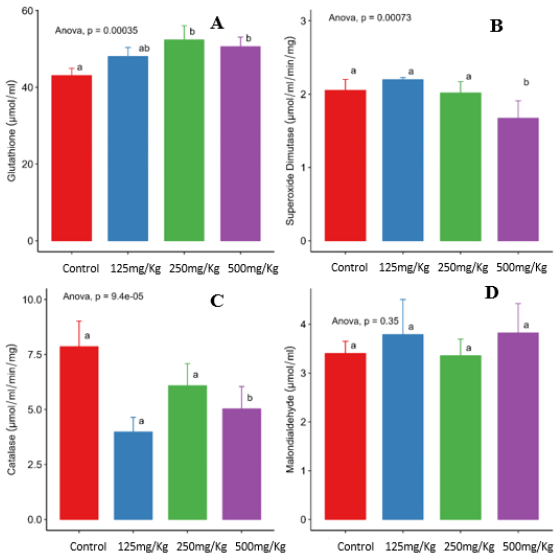

Figure 8: Oxidative stress response of female rats from the Lung

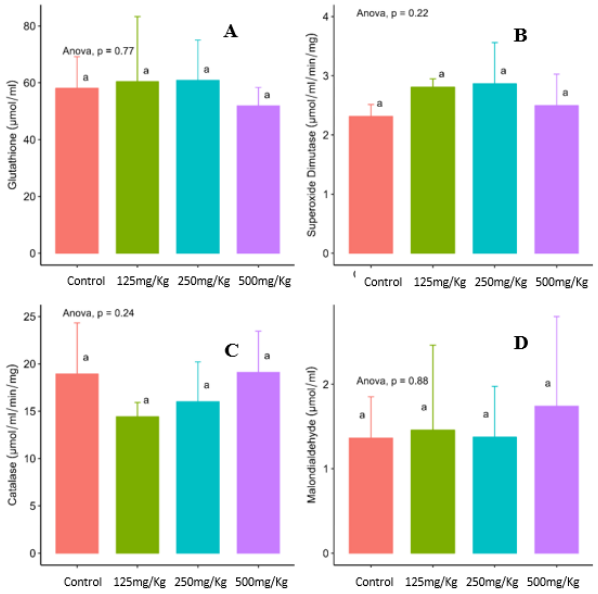

Figure 9: Oxidative stress response of male rats from the testes

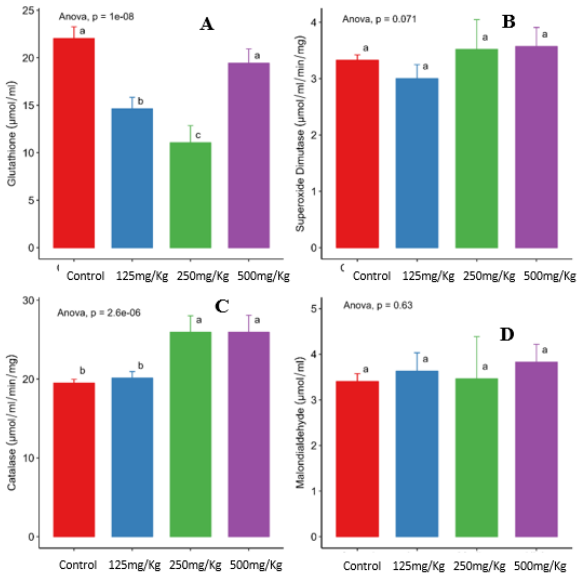

Figure 10: Oxidative stress response of female rats from the ovaries

DISCUSSION

For centuries, phytochemicals have held significant importance for communities globally. These natural metabolites have been integral to traditional healthcare systems, serving as treatments for a wide array of diseases. Additionally, phytochemicals have played a crucial role as precursor molecules in drug development processes. The isolation of bioactive compounds from plant materials remains a fundamental aspect of natural product research (Bitwell et al., 2023). The presence of important phytochemical contents such as tannins, phenols, phlobatannins, alkaloids, saponins, flavonoids, and cardiac glycosides in *Cissus pulpmela* shows that the plant can prospected for various medicinal purposes. Studies have shown that tannins, phenols, phlobatannins, alkaloids, saponins, flavonoids, and cardiac glycoside play a crucial role in protecting and enhancing health by providing antioxidant, anti-inflammatory and antimicrobial activities (Chanda, S., & Ramachandra, 2019). For instance, tannins exhibit strong antioxidant properties, which help protect cells from oxidative damage and reduce the risk of chronic diseases such as cancer and cardiovascular conditions. Phenolic compounds are potent antioxidants that mitigate oxidative stress, thus protecting against cellular damage and inflammation. They play a critical role in maintaining cardiovascular health, and reducing the risk of heart diseases by preventing the oxidation of low-density lipoprotein (Vázquez-Ruiz, et al., 2022; Lutz, et al., 2019). Additionally, phlobatannins are condensed tannins that have strong antioxidant and antimicrobial properties, aiding in the defense against microbial infections and protecting against oxidative stress. Their astringent qualities can also impact digestive health, potentially reducing the risk of gastrointestinal diseases (Prakash et al., 2013). Saponins have a beneficial impact on cardiovascular health by lowering cholesterol levels and reducing the risk of heart disease (Ibarrolla et al., 2023). They possess anti-inflammatory and immune-boosting properties, enhancing overall health and resistance to infections. Saponins also aid in nutrient absorption and promote gut health by acting as natural detergents in the digestive system (Yanza et al., 2024). In light of the foregoing, the present result demonstrates that the local variety of *Cissus pulpmela* be used for treating different ailments and advanced studies would expand the potential of applying these phytochemicals in the production of efficient and safe drugs.

In this study, the general reduction observed in the oxidative stress parameters of groups exposed to 125mg/Kg, 250mg/Kg and 500mg/Kg showed that the plant extracts reduce oxidative stress in the liver through their antioxidant properties consequently, contributing to protecting liver cells from oxidative damage and maintaining overall liver health. A study by Akomolafe et al. (2013) revealed antioxidant properties and inhibitory effect of water extractible phytochemicals from stem bark of *C. populnea* on $FeSO_4$ and sodium nitroprusside-(SNP-) induced lipid peroxidation in rat testes during an in vitro study. The results revealed that the extract was able to scavenge DPPH radical, chelate Fe (2+) and also had a high reducing power. Lipid peroxidation was highest in the female groups exposed to the 500mg/Kg of the extracts. This proved that high doses of plant extracts can induce lipid peroxidation through pro-oxidant effects, cellular toxicity, disruption of redox homeostasis, inhibition of antioxidant enzymes, accumulation of metabolites, and direct interaction with lipid membranes. These mechanisms collectively enhance oxidative stress and lead to the oxidative degradation of lipids, compromising cell membrane integrity and function. These findings showed that the kidneys of male mice were not significantly stressed. Albeit, there was a down-regulation of the activity of SOD at 125mg/Kg. Enzyme downregulation can occur due to various reasons, and one significant factor is oxidative stress. High levels of reactive oxygen species (ROS) can damage cellular components, including proteins and DNA. This damage can lead to the downregulation of enzyme production and activity as the cell prioritizes repair mechanisms. ROS can disrupt the normal functioning of cellular machinery, including enzymes, by modifying their structure and function. This can result in decreased enzymatic activity and reduced synthesis of new enzymes (Iqbal et al., 2024) (Dar et al., 2019) .

Additionally, oxidative stress can activate various signaling pathways that lead to changes in gene expression. For instance, the activation of transcription factors like NF-κB and AP-1 in response to ROS can alter the expression of genes involved in inflammation, apoptosis, and other cellular processes. These changes in gene expression can downregulate enzymes that are not immediately necessary for the cell's survival under stress conditions (Iqbal et al., 2024) (Wang et al., 2023). The administration of 125mg/Kg, 250mg/Kg and 500mg/Kg compromised the GSH levels in the heart of the male rats in contrast to the up-regulated GSH activities in the heart of the female mice. Glutathione (GSH) is a tripeptide composed of glutamine, cysteine, and glycine, and it serves several critical functions in maintaining cellular homeostasis and protecting against toxicity. Low glutathione levels compromise the body's ability to combat oxidative stress, detoxify harmful substances, and maintain cellular and immune function, leading to increased susceptibility to various diseases and toxicities. This study shows that the administration of the extracts induced oxidative damage to the organisms which corresponded with increased activity of SOD to counteract the effect of the stress. Superoxide Dismutase plays an important role in the cellular restoration of used or reduced GSH as demonstrated by other studies (Liu et al., 2022). In this study, the high GSH in the control groups which coincided with high MDA levels could imply, that this molecule was highly involved in the detoxification process during the study period. Meanwhile, the reduced activity observed in the heart may indicate the restoration of a stable physiological state with the administration of the extracts hence leading to reduced GSH levels. The extracts, therefore, maybe a good herbal remedy for heart function. The increase in oxidative stress markers at higher doses, particularly in females, raises concerns about the safety of high-dose or long-term use of the extract. This could limit its therapeutic window and suggests the need for careful dose optimization.

CONCLUSION

The identification of significant phytochemical constituents, including tannins, phenols, phlobatannins, alkaloids, saponins, flavonoids, and cardiac glycosides in *Cissus pulpmela*, as demonstrated in our study, indicates substantial potential for future drug development. The plant extract induced oxidative changes in the male and female mice in all the organs investigated. Lipid peroxidation was highest in the female groups exposed to the 500mg/Kg of the extracts. The plant extracts reduce oxidative stress in the liver through their antioxidant properties. We conclude that the 12mg/Kg of the extract may be a good herbal remedy for heart function.

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