Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
Impact Factor (RJIF): 5.94
www.plantsjournal.com
JMPS 2025; 13(5): 60-66
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Received: 24-08-2025
Accepted: 28-09-2025

Orutugu Ayibatonye Lemmy
Department of Medical Laboratory Science, School of Allied Medical Science, Bayelsa State College of Health Technology, Otuogidi-Ogbia, Bayelsa State, Nigeria

Gborienemi George Simeon
Department of Medical Laboratory Science, Faculty of Basic Medical Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria

Ferdinand Chukwuma Ezeiruaku
Department of Medical Laboratory Science, Faculty of Basic Medical Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria

Corresponding Author:
Orutugu Ayibatonye Lemmy
Department of Medical Laboratory Science, School of Allied Medical Science, Bayelsa State College of Health Technology, Otuogidi-Ogbia, Bayelsa State, Nigeria

Comparison of the effect of *Phyllanthus amarus* leaf crude methanolic extract and vitamin E on bisphenol a-induced oxidative stress in *Rattus norvegicus*

Orutugu Ayibatonye Lemmy, Gborienemi George Simeon and Ferdinand Chukwuma Ezeiruaku

DOI: https://www.doi.org/10.22271/plants.2025.v13.i6a.1977

Abstract
Bisphenol A (BPA), an endocrine-disrupting chemical, is known to induce oxidative stress and disrupt physiological homeostasis in mammals. This study compared the antioxidant effects of *Phyllanthus amarus* leaf crude methanolic extract (PAE) and vitamin E (Vit E) on BPA-induced oxidative stress in *Rats*. Twenty four adult male rats were divided into six groups: control, BPA-treated, BPA + PAE (low dose), BPA + PAE (high dose), BPA + Vit. E and PAE only. Oxidative stress biomarkers, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), were analyzed in serum. BPA exposure significantly increased MDA levels and decreased antioxidant enzyme activities (p<0.05). Both PAE and Vit E treatments mitigated these effects, but PAE showed greater efficacy in restoring antioxidant enzyme activities. The findings suggest that *P. amarus* possesses strong antioxidative properties capable of protecting against BPA-induced oxidative damage, potentially offering a plant-based alternative to conventional antioxidants like vitamin E.

Keywords: Bisphenol A, *Phyllanthus amarus*, Vitamin E, Oxidative stress, Antioxidant enzymes, *Rattus norvegicus*

1. Introduction
Bisphenol A (BPA) is an industrial compound widely used in the manufacture of polycarbonate plastic and epoxy resins commonly found in food containers, water bottles, military helmets, as dental sealants and other consumer products [1, 2]. Additional applications for this material include the production of labels, faxes, invoices and tags for the transportation of baggage [3]. This wide application both industrially and in domestic products has led to its environmental persistence and biological accumulation[4]. BPA is considered to be unstable when it is exposed to ultraviolet light or when it is dissolved in basic or acidic solutions and when it is heated repeatedly [5, 6] predicted that BPA manufacture and use is expected to be about 10.6 million tons in 2025.

Furthermore, it is presently considered to be one of the most important compounds that are produced all over the globe in the synthetics industry as well as one of the most common EDC known to disrupt metabolic processes. As to the findings of [7], the pace at which it has been integrated into the ecosystem and the food chain is around one hundred tonnes with each passing year. The high level of production and utilization that it boasts is a probable explanation for this phenomenon. In accordance with the findings of [8], the Environmental Protection Agency (EPA) of the United States of America listed it as the third most urgent environmental issue in the whole globe. Because of its EDC characteristics and widespread usage in both internal and exterior contacts with people, pervasive environmental presence and leaching potential, BPA had become a public health concern, according to [9]. Potential sources of BPA exposure according to [10] and [11] include transdermal, inhalation, and oral routes.

Chronic exposure to BPA has been associated with reproductive toxicity, metabolic disorders, cardiovascular diseases, hepatic and renal dysfunctions [12]. One of the key mechanisms underlying BPA induced toxicity is oxidative stress characterized by excessive generation of

reactive oxygen species (ROS) and depletion of antioxidant defense systems, even when it is present in low doses [9, 13].

The biological imbalance caused by oxidative stress disrupts cellular macromolecules such as lipids, proteins, and nucleic acids, leading to cell damage and organ dysfunction [14, 15, 16]. This has prompted the exploration of antioxidant compounds capable of mitigating ROS mediated toxicity. Vitamin E (α-tocopherol), a well-established lipid soluble antioxidant, has been extensively used as a reference antioxidant in experimental models for oxidative stress. It protects cell membranes by terminating lipid peroxidation chain reaction and stabilizing membrane intergrity [17, 18, 19] state that oxidative stress and age-related illnesses are two metabolic stresses that cause vitamin E levels to fluctuate together with dietary intake of vitamin E. They also stated that consumption of competing nutrients, proteins that aid absorption, individual absorption efficiency influenced by health conditions, vitamin E metabolism, lifestyle choices, gender, and genetic polymorphisms are some othe factors that affect its bioavailability.

However, synthetic antioxidants like vitamin E, though effective, are sometimes limited by narrow mechanism of action and potential dose related effect [20]. Also current toxicological research according to [21] have demonstrated that synthetic antioxidants have a negative effect on the body and are lacking in effectiveness with undesirable side effects. On the other hand, natural antioxidants are more cost-effective, culturally acceptable, possess greater antioxidant activity, and provide benefits that are available over a longer period of time. These factors, according to the authors, have led to a rise in the use of natural antioxidants, which has prompted more research into natural sources that might possess antioxidant qualities.

Therefore, medicinal plants containing diverse phytochemicals have gained attention as promising sources of natural antioxidants [21]. Among these, *Phyllantus amarus* (Family: Phyllanthaceae), traditionally used in African and Asian ethnomedicine, exhibit hepatoprotective, nephroprotective, anti-inflammatory, and antioxidant properties. The plant is rich in bioactive constituents such as phyllantin, hypophyllantin, lignans, flavonoids, tannins, and polyphenols, which contribute synergistically to free radical scavenging and the enhancement of endogenous antioxidant enzymes [22].

Previous studies have shown that *P. amarus* extract ameliorates oxidative damage induced by xenobiotics like carbon tetrachloride (CCl_4), paracetamol, and heavy metals. Yet, comparative evaluation of *P. amarus* and standard antioxidants like vitamin E against BPA induced oxidative stress remain limited. Understanding how *P. amarus* modulates oxidative biomarkers relative to vitamin E could provide evidence for its potential as a natural alternative therapy against BPA mediated toxicity.

Hence, this study was designed to compare the ameliorative effects of *P. amarus* crude methanol leaf extract and vitamin E on BPA induced oxidative stress in *Rattus norvegicus*. The finding are expected to broaden the understanding of plant based antioxidants in combating EDC-related oxidative damage and contributes to the development of safe, affordable, and accessible therapeutic strategies.

2. Materials and Methods

Research Design

This research utilized randomized subject control experimental design and observational study.

Animal Care and Treatment

Twenty four (24) adult male rats (*Rattus norvegicus*) weighing between 110-180g were purchased from the disease-free stock of the animal house, Pharmacology Department of the Niger Delta University, Amassoma and were left to acclimatize for two (2) weeks. The rats were bred in metallic cages with wire screen tops and were kept under adequate ventilation with room temperature of 25±2 ºC and adequate relative humidity with a 12hr natural light-dark cycle. Animals were allowed to feed *ad libitum* with Pellet feed (pelletized growers feed) manufactured by Grand Cereals Limited with good hygiene maintained by constant cleaning and removal of urine and faeces with spilled feed from cages daily. Animal handling was carried out according to Good Laboratory Practice (GLP) and all the animal experiments were carried out in accordance with the National Institute of Health Guide for care and Use of Laboratory Animals [23]

Selection Criteria for Rats

All the animals for the study were of appropriate specie, sex, weight and age. Rats with signs of disease (lumps, hair loss, and diarrhea, scratching all the time) were not included in the study. Appropriate protocols were observed for the study.

Ethical Clearance

This was obtained from the College of Health Sciences Ethical Committee NDU, Amassoma, Bayelsa State Nigeria.

Experimental Design

Rats were randomly divided into Six groups (n = 4 per group):

Group A: **Control:** The rats in this group served as the control (non-exposed group). They were given commercial feeds and water *ad libidium* only daily for fourteen (14) days.

Group B: (**BPA 200mg/kg):** The rats in this group were given commercial feeds and BPA (200mg/kg body weight) daily for fourteen (14) days.

Group C: **BPA (200mg/kg) and *P. amarus* leaf extract (400mg/kg):** The rats in this group were given commercial feed, BPA (200mg/kg body weight) and treated with *P. amarus* aqueous leaf extract (400mg/kg body weight) daily for fourteen (14) days.

Group D: BPA (200mg/kg) and *P. amarus* leaf extract (800mg/kg): The rats in this group were given commercial feed, BPA (200mg/kg body weight) and treated with *P. amarus* aqueous leaf extract (800mg/kg body weight) daily for fourteen (14) days

Group E: BPA (200mg/kg) and Vitamin E (100mg/kg): The rats in this group were given commercial feed, BPA (200mg/kg body weight) and treated with vitamin E standard drug (100mg/kg body weight) daily for fourteen (14) days.

Group F: Aqueous *P. amarus* leaf extract (800mg/kg): The rats in this group were given commercial feed and *P. amarus* aqueous leaf extract (800mg/kg body weight) daily for fourteen (14) days.

Chemicals and Plant Extract Preparation

Bisphenol A (BPA) and vitamin E were obtained from Sigma-Aldrich (USA). Fresh *Phyllanthus amarus* leaves were

sourced locally at Ogbia Town, in Ogbia Local Government Area of Bayelsa State and submitted to the Pharmacy Department of the Niger Delta University for authentication by a Taxonomist and a herbarium number was given as the identity of the plant. Leaves were air-dried, powdered, and extracted with 80% methanol using a Soxhlet apparatus. The filtrate was concentrated under reduced pressure and stored at 4 °C until use.

Biochemical Assays

After the treatment period, animals were sacrificed under light anesthesia, and blood samples were collected and analyzed for:

- **MDA** (lipid peroxidation marker) using thiobarbituric acid reactive substances (TBARS) method [24]
- **SOD**, **CAT**, and **GPx** levels using standard spectrophotometric methods [25, 26]

Statistical Analysis

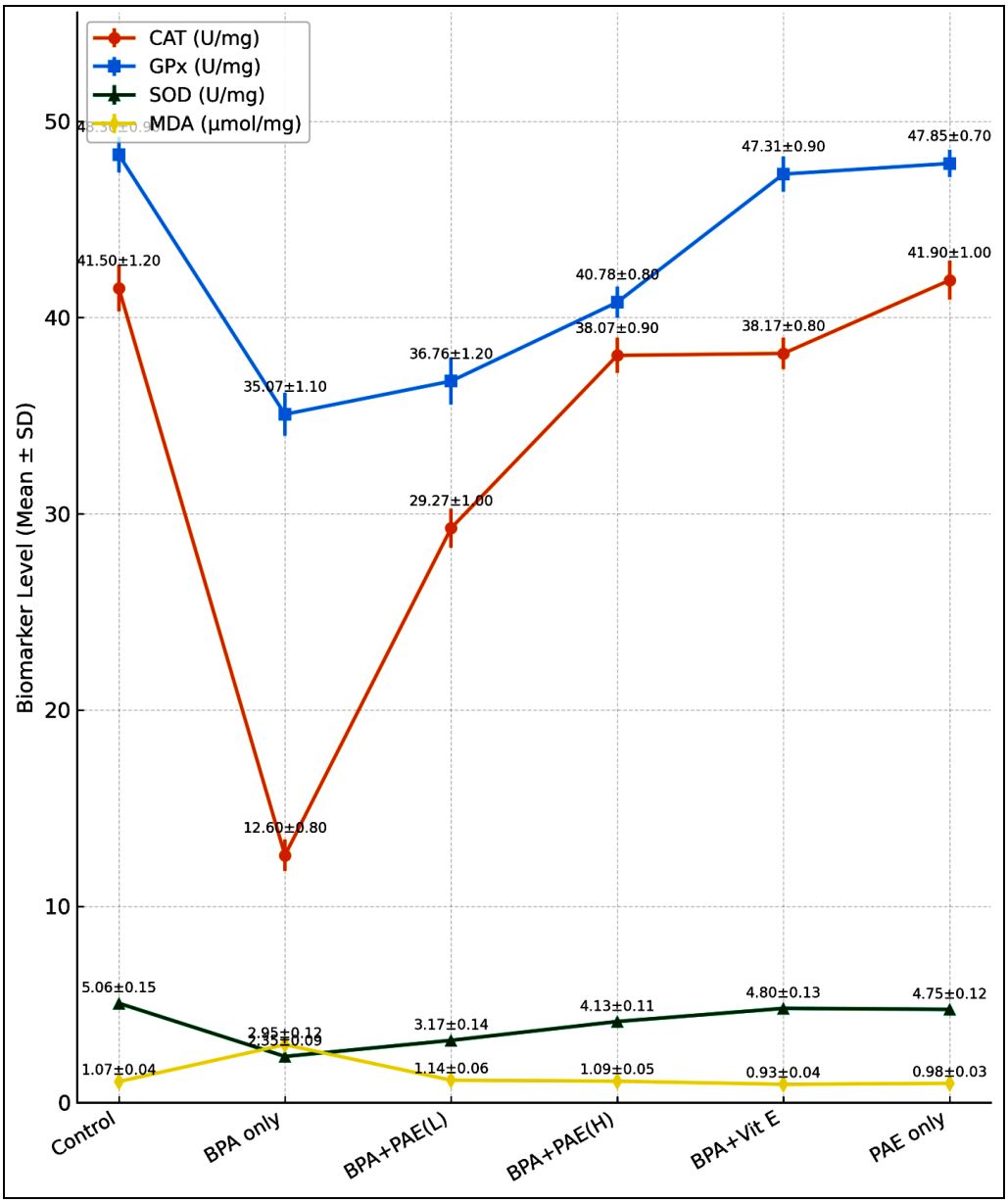
Data were expressed as mean ± SD and analyzed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$ considered significant).

3. Results

Table 1: Oxidative Stress Biomarker Levels in *Rattus norvegicus* Exposed to BPA and Treated with *P. amarus* Extract or Vitamin E

Group n	CAT (U/mg)	GPx (U/mg)	SOD (U/mg)	MDA (µmol/mg)	Remarks
GRP 1 - Control 4	41.5±1.2	48.3±0.9	5.06±0.15	1.07±0.24	Normal oxidative balance
GRP 2 - BPA only 4	12.6±0.8	35.07±1.1	2.35±0.09	1.77.±0.10	Severe oxidative stress
GRP 3 - BPA + Low *P. amarus* 4	29.27±1.0	36.76±1.2	3.17±0.14	1.14±0.12	Moderate protection
GRP 4 - BPA + High *P. amarus* 4	38.07±0.9	40.78±0.8	4.13±0.11	1.09±0.15	Strong protection
GRP 5 - BPA + Vit E 4	38.17±0.8	47.31±0.9	4.80±0.13	0.93±0.09	Strong protection
GRP 6 - *P. amarus* Only 4	41.9±1.0	47.85±0.7	4.75±0.12	0.98±0.12	Near-normal protection

Keys: Values represent mean ± SEM; n = 4 animals per group; $p<0.05$ considered significant compared to control and BPA-treated groups. CAT-Catalase, GPx-Glutathione Peroxidase, SOD-Superoxide Dismutase, and MDA-Melandoaldehyde



Key: BPA -Bisphenol A, PAE-*Phyllantus amarus* leaf extract, Vit E-Vitamin E

Fig 1: Oxidative Stress Biomarkers (BPA, PAE, Vitamin E)

Figure 1 Multi-line trend plot showing changes in oxidative stress biomarkers (CAT-Catalase, GPx-Glutathione Peroxidase, SOD-Superoxide Dismutase and MDA-Melandoaldehyde) across treatment groups. Values are expressed as mean ± SD (n=4). The Y-axis represents biomarker levels, and the lines illustrate treatment-dependent variations among control, BPA, and treatment groups

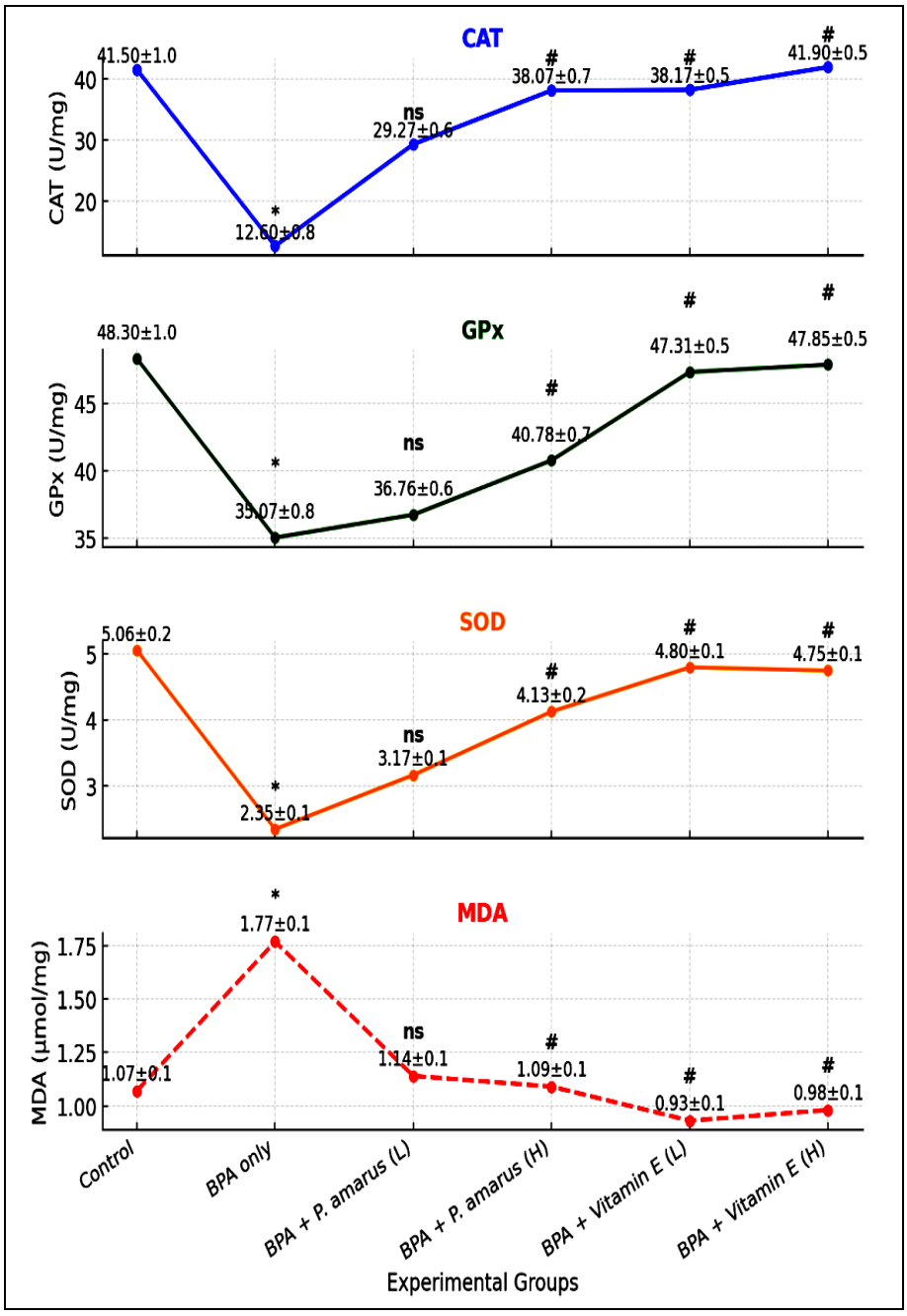


Fig 2: Effects of *P. amarus* Leaf Extract and Vitamin E on BPA-Induced Oxidative Stress Biomarkers in *Rattus norvegicus*

Figure 1 & 2: Effect of *Phyllanthus amarus* and Vitamin E on BPA-Induced Oxidative Stress Biomarkers in *Rattus norvegicus*

The chart depicts the mean ± SD values of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) across six experimental groups, revealing the biochemical response to bisphenol A (BPA) and subsequent antioxidant interventions.

In the control group, antioxidant enzymes maintained baseline activity (CAT = 41.5±1.2 U/mg; GPx = 48.3±0.9 U/mg; SOD = 5.06±0.15 U/mg), and MDA was minimal (1.07±0.24 µmol/mg), indicating a stable oxidative balance. In contrast, the BPA-only group exhibited a significant (p<0.05) decline in CAT, GPx, and SOD levels (CAT = 12.6±0.8 U/mg; GPx = 35.07±2.1 U/mg; SOD = 2.35±0.13 U/mg), alongside a marked increase in MDA (1.77±0.10 µmol/mg), confirming oxidative stress induction and compromised enzymatic defense systems. This observation aligns with prior studies showing that BPA disrupts hepatic and renal antioxidant enzyme activities through excessive reactive oxygen species (ROS) generation and mitochondrial dysfunction [27, 28].

Administration of *P. amarus* at both low and high doses significantly (p<0.05) increased antioxidant enzyme activities relative to the BPA-only group, indicating dose-dependent restoration of enzymatic antioxidant capacity. In the high-dose *P. amarus* group, CAT (38.1±1.7 U/mg) and GPx (40.8±1.5 U/mg) approached control levels, suggesting near-complete recovery of enzymatic function. The low-dose group

also demonstrated moderate improvement (CAT = 29.3±1.5 U/mg; GPx = 36.8±1.2 U/mg), corroborating reports that *P. amarus* phytoconstituents such as phyllanthin, hypophyllanthin, and quercetin enhance endogenous antioxidant defenses [29, 30]

Co-administration of vitamin E also resulted in statistically significant improvements in CAT, GPx, and SOD activities ($p<0.05$ vs. BPA), reflecting its well-established role as a lipid peroxidation inhibitor and free radical scavenger [31]. Notably, the enzymatic activities in the vitamin E group were comparable ($p>0.05$) to those of the *P. amarus*-treated rats, suggesting similar antioxidant efficacy between the natural extract and the standard reference antioxidant.

Furthermore, MDA levels declined progressively across all treatment groups, showing significant differences ($p<0.05$) compared to BPA exposure. The *P. amarus*-only group exhibited the lowest MDA value (≈ 0.98±0.07 µmol/mg), signifying potent suppression of lipid peroxidation and intrinsic antioxidant potential even in the absence of BPA challenge.

Mechanistically, the improvement in enzymatic activities suggests enhanced expression or stabilization of antioxidative enzymes, possibly mediated by phytochemicals that modulate the Nrf2/ARE pathway [32]. These findings demonstrate that P. amarus can serve as a potent natural antioxidant and hepatoprotective agent comparable to vitamin E in counteracting BPA-mediated oxidative damage.

Statistical annotations on the figure (*, #, ns) indicate significance relative to the control ($p<0.05$ = *; ns = not significant). The trends collectively confirm that both *P. amarus* and vitamin E effectively attenuate BPA-induced oxidative stress by restoring enzymatic antioxidant activities and minimizing lipid peroxidation.

Discussion

The results presented in the table show the activities of key antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) alongside malondialdehyde (MDA), a marker of lipid peroxidation, in experimental rats subjected to bisphenol A (BPA) toxicity and treatment with *Phyllanthus amarus* extract and vitamin E.

In the control group (GRP 1), high activities of CAT (41.5±1.2 U/mg), GPx (48.3±0.9 U/mg), and SOD (5.06±0.15 U/mg) with low MDA (1.07±0.24 µmol/mg) indicate a normal oxidative balance. This baseline confirms the integrity of the endogenous antioxidant defense system in the absence of toxic exposure [33].

Exposure to BPA alone (GRP 2) caused a marked decrease in CAT, GPx, and SOD activities, accompanied by a significant rise in MDA levels (1.77± 0.10 µmol/mg), indicating severe oxidative stress. This aligns with reports that BPA induces oxidative damage by generating reactive oxygen species (ROS), impairing enzymatic antioxidant systems, and promoting lipid peroxidation [34, 35]. BPA's lipophilic nature enables it to disrupt mitochondrial function and cellular redox homeostasis, thereby increasing oxidative damage to lipids and proteins [36].

Treatment with *P. amarus* at low and high doses (GRP 3 and GRP 4) showed dose-dependent improvement in antioxidant status. The low dose (29.27±1.0 U/mg CAT; 36.76±1.2 U/mg GPx; 3.17±0.14 U/mg SOD; MDA = 1.14±0.12 µmol/mg) provided moderate protection, while the high dose (38.07±0.9 U/mg CAT; 40.78±0.8 U/mg GPx; 4.13±0.11 U/mg SOD; MDA = 1.09±0.15 µmol/mg) resulted in strong protection against oxidative stress. This improvement demonstrates the antioxidant potency of *P. amarus*, attributed to its high content of polyphenols, flavonoids, and lignans such as phyllanthin and hypophyllanthin [29, 30]. These compounds can scavenge free radicals and upregulate endogenous antioxidant enzymes, mitigating BPA-induced oxidative damage.

Vitamin E treatment (GRP 5) also markedly restored antioxidant enzyme activities and reduced lipid peroxidation (MDA = 0.93±0.09 µmol/mg), indicating strong protection similar to the high-dose *P. amarus* group. Vitamin E, a well-known lipid-soluble antioxidant, interrupts lipid peroxidation chain reactions and stabilizes cell membranes [37]. The similarity in response between the vitamin E and high-dose *P. amarus* groups suggests comparable antioxidant efficacy, highlighting *P. amarus* as a potential natural alternative to synthetic antioxidants.

The *P. amarus*-only group (GRP 6) showed near-normal antioxidant values comparable to the control group, confirming that the extract itself does not impose oxidative stress but rather supports redox homeostasis. Overall, the data demonstrate that *P. amarus* exerts significant protective effects against BPA-induced oxidative damage, likely due to its synergistic bioactive phytochemicals that enhance endogenous antioxidant enzyme activities and reduce lipid peroxidation

Conclusion

The present study demonstrates that bisphenol A (BPA) exposure induces significant oxidative stress in *Rattus norvegicus*, as evidenced by elevated malondialdehyde (MDA) levels and marked depletion of endogenous antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). These biochemical alterations confirm that BPA triggers reactive oxygen species (ROS) generation, lipid peroxidation, and disruption of cellular antioxidant defense mechanisms. Co-administration with *Phyllanthus amarus* leaf crude methanolic extract and vitamin E markedly mitigated these oxidative alterations, restoring enzymatic and non-enzymatic antioxidant balance. The protective effects of *P. amarus* were comparable to those of vitamin E, with slightly greater restoration of antioxidant enzyme activity, suggesting that the plant extract exerts broader antioxidant effects due to its synergistic phytochemical constituents.

The findings highlight *Phyllanthus amarus* as a potent natural antioxidant capable of counteracting BPA-induced oxidative stress through free radical scavenging and enhancement of endogenous antioxidant enzymes. Considering the environmental prevalence of BPA and the increasing incidence of oxidative stress-related disorders, *P. amarus* presents a promising, safe, and affordable therapeutic alternative to synthetic antioxidants such as vitamin E.

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