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Morin and Vanillic Acid Alleviate DEP- and Bisphenol S–Mediated Liver Toxicity via Antioxidant Mechanisms in a Rat Model

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Abstract

Continuous exposure to endocrine-disrupting chemicals, including diethyl phthalate (DEP) and bisphenol S (BPS), is associated with oxidative stress, hepatic dysfunction, and increased risk of tissue injury. This study investigated the hepatoprotective potential of morin and vanillic acid against oxidative liver damage induced by DEP and BPS in rats. Adult Wistar rats were orally exposed to DEP (50 mg/kg) and BPS (200 mg/kg) for 21 days and co-treated with morin and vanillic acid at 25 mg/kg each. Oxidative stress was assessed by measuring hydrogen peroxide (H_2O_2), nitric oxide (NO), and malondialdehyde (MDA). Liver function indices included alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Antioxidant defenses were evaluated using superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH). Exposure to DEP and BPS significantly ($P < 0.05$) increased oxidative stress markers and liver enzyme activities while suppressing antioxidant defenses, indicating marked hepatocellular injury. Co-administration of morin and vanillic acid significantly reduced H_2O_2, NO, and MDA levels, restored antioxidant enzyme activities, and normalized hepatic biomarkers. These improvements demonstrate attenuation of the oxidative disturbances and hepatic dysfunction associated with DEP and BPS exposure under the experimental conditions investigated. Overall, morin and vanillic acid effectively attenuated DEP- and BPS-induced hepatotoxicity through antioxidant and cytoprotective mechanisms. The findings support their potential application in managing liver injury associated with endocrine-disrupting chemicals, highlighting their capacity to reduce oxidative stress and restore antioxidant defenses. This hepatoprotective potential was demonstrated in Wistar rats following the 21-day exposure period and concurrent treatment with compounds.

Keywords
Diethyl phthalate;
Bisphenol S;
Morin;
Vanillic acid;
Hepatoprotection.

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1 Introduction

Environmental contamination by endocrine-disrupting chemicals (EDCs) has become a major global health concern due to their persistence, bioaccumulation, and toxicological impacts on biological systems. Among these contaminants, bisphenol S (BPS) and diethyl phthalate (DEP) are increasingly utilized in industrial and consumer products as substitutes for more regulated compounds, thereby increasing the likelihood of chronic human exposure. BPS, chemically identified as $(HOC_6H_4)_2SO_2$, is a structural analogue of bisphenol A (BPA) in which the dimethyl methylene bridge is replaced by a sulfone group, conferring greater thermal and photostability (Qiu et al., 2019). Due to these physicochemical advantages, BPS is widely incorporated into epoxy resins, food packaging materials, thermal papers, adhesives, and polymer-based products (Herrero et al., 2018). Although initially considered a safer alternative to BPA, emerging evidence indicates that BPS possesses endocrine-disrupting, hepatotoxic, and oxidative stress–inducing properties comparable to or greater than BPA (Rochester & Bolden, 2015).

Similarly, DEP is a low-molecular-weight phthalate extensively used as a plasticizer to enhance flexibility and durability in plastics, cosmetics, food packaging, pharmaceuticals, and personal care products (Weaver et al., 2020). Human exposure to DEP occurs primarily through ingestion, inhalation, and dermal absorption, particularly from contaminated food containers and cosmetic products. Accumulating studies have demonstrated that DEP disrupts endocrine signaling, promotes reactive oxygen species (ROS) generation, and induces hepatic injury through oxidative damage and inflammatory responses (Benjamin et al., 2017).

The liver remains highly vulnerable to these xenobiotics because it serves as the principal organ for detoxification and biotransformation of environmental pollutants. Excessive ROS production during xenobiotic metabolism can overwhelm endogenous antioxidant defenses, leading to lipid peroxidation, protein oxidation, DNA damage, and hepatocellular dysfunction (Vandenberg et al., 2013).

Natural polyphenolic compounds have attracted considerable scientific interest because of their antioxidant, anti-inflammatory, and cytoprotective properties. Morin (3,5,7,2′,4′-pentahydroxyflavone) is a bioactive flavonoid abundantly present in guava leaves, almonds, apples, tea, and several medicinal plants. Morin exhibits potent free-radical scavenging, metal-chelating, anti-inflammatory, and anti-apoptotic activities, making it a promising therapeutic candidate against oxidative stress–mediated diseases (Hussain et al., 2014). Experimental evidence suggests that morin can protect hepatic tissues against chemically induced toxicity by restoring antioxidant enzyme activities and attenuating lipid peroxidation (Gutiérrez et al., 2017).

Vanillic acid (VA), chemically known as 4-hydroxy-3-methoxybenzoic acid, is a naturally occurring phenolic acid derived from dietary plants, fruits, coffee, and green tea. VA has been reported to possess strong antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and antigenotoxic properties (Williams et al., 2004). In addition, vanillic acid has demonstrated protective effects against chronic liver injury and fibrosis through modulation of oxidative stress pathways and inflammatory mediators (Itoh et al., 2009). Despite increasing evidence supporting the individual therapeutic benefits of morin and vanillic acid, limited studies have explored their combined or synergistic protective efficacy against mixed EDC-induced hepatotoxicity.

Importantly, co-exposure to multiple EDCs such as BPS and DEP reflects realistic environmental and dietary exposure patterns in humans. However, most toxicological studies have focused on single-compound

exposure, leaving a critical knowledge gap regarding combined toxicodynamic interactions and potential phytotherapeutic interventions. Therefore, this study investigated, for the first time, the synergistic hepatoprotective effects of morin and vanillic acid against DEP- and BPS-induced oxidative hepatic injury in rats. The study specifically elucidates the mechanisms underlying their antioxidant and free-radical scavenging activities in mitigating endocrine disruptor–mediated hepatotoxicity.

2 Materials and Methods

2.1 Chemicals and Reagents

Morin (Cat. No.: M2357), vanillic acid (Cat. No.: V1240), bisphenol S (BPS; Cat. No.: D5095), and diethyl phthalate (DEP; Cat. No.: D1785) were obtained from Otto Chemie Pvt. Ltd. (Mumbai, India). Dimethyl sulfoxide (DMSO) was procured from Libertas Laboratory Services Limited, Abeokuta, Nigeria. All analytical-grade reagents and assay chemicals used in this study were sourced from BDH Chemicals Ltd. (Poole, Dorset, United Kingdom), unless otherwise stated.

2.2 Experimental Animals and Ethical Approval

Twenty male albino Wistar rats weighing 150–200 g were obtained from the Animal House Facility, Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria. Animals were maintained under standard laboratory conditions at 25 ± 2°C with a 12 h light/12 h dark cycle and relative humidity of 50–60%. Rats were acclimatized for seven days prior to experimentation and had unrestricted access to standard pelletized feed and clean drinking water.

All experimental procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and adhered strictly to internationally accepted ethical principles for laboratory animal research. Ethical approval for the study was granted by the Institutional Animal Ethics Committee, Federal University of Agriculture, Abeokuta ((Approval No.: BCH/20160914).). The experimental design also incorporated the 3Rs principle (Replacement, Reduction, and Refinement) to minimize animal suffering and optimize scientific validity.

2.3 Experimental Design and Treatment Protocol

Rats were randomly allocated into four experimental groups (n = 5/group) using a simple randomization technique. Environmentally relevant doses of DEP (50 mg/kg body weight) and BPS (200 mg/kg body weight) were selected based on previous toxicological studies demonstrating endocrine-disrupting and hepatotoxic effects. Morin and vanillic acid were administered orally at 25 mg/kg body weight each due to their established antioxidant and cytoprotective potentials. All treatments were delivered once daily by oral gavage for twenty-one (21) consecutive days using 0.4% DMSO as the vehicle.

2.4 Experimental Grouping

Groups	Treatment
Group A	0.4% DMSO (Control)
Group B	DEP (50 mg/kg) + BPS (200 mg/kg)
Group C	DEP + BPS + Morin (25 mg/kg) + Vanillic acid (25 mg/kg)
Group D	Morin (25 mg/kg) + Vanillic acid (25 mg/kg)

2.5 Sample Collection and Serum Preparation

Twenty-four hours after the final treatment, animals were anesthetized intraperitoneally with ketamine/xylazine (100/7 mg/kg body weight) and sacrificed by cervical dislocation. Blood samples were collected via cardiac puncture into plain tubes and centrifuged at 5000 rpm for 10 min to obtain serum for biochemical analyses. Liver tissues were carefully excised, rinsed in ice-cold saline, blotted dry, and homogenized (10% w/v) in phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged to obtain post-mitochondrial supernatants used for oxidative stress and antioxidant assays.

2.5.1 Assessment of Oxidative Stress and Antioxidant Biomarkers

To elucidate the mechanistic basis of endocrine disruptor–induced hepatotoxicity, oxidative stress indices and endogenous antioxidant defense systems were comprehensively evaluated. Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide at 405 nm according to the method of Shangari and O'Brien (2006). Glutathione peroxidase (GPx) activity was assayed using DTNB-mediated chromogenic detection at 412 nm following the method of Rotruck et al. (1973), while reduced glutathione (GSH) concentration was estimated using Ellman's reagent (Ellman, 1959). Superoxide dismutase (SOD) activity was assessed based on inhibition of pyrogallol auto-oxidation as described by Marklund and Marklund (1974).

Reactive oxygen and nitrogen species generation was evaluated by quantifying hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels according to the methods of Junglee et al. (2014) and Rao (1997), respectively. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) concentration as thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust (1978).

2.5.2 Evaluation of Hepatic Biomarkers

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were determined spectrophotometrically using standard enzymatic procedures as indicators of hepatocellular integrity and liver function. Enzyme-specific activities were calculated according to established biochemical protocols.

2.5.3 Histopathological Examination

For histomorphological assessment, liver tissues were fixed in 10% neutral-buffered formalin, dehydrated in graded ethanol, embedded in paraffin wax, and sectioned at 5 µm thickness. Tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for pathological alterations including hepatocellular degeneration, inflammatory infiltration, necrosis, and vascular congestion.

2.5.4 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) by Duncan's Multiple Range Test (DMRT) for multiple comparison test to determine intergroup differences. Values of $P < 0.05$ were considered statistically significant. The integration of biochemical and histopathological endpoints in this study provides a novel multi-biomarker approach for evaluating the synergistic hepatoprotective efficacy of morin and vanillic acid against combined endocrine disruptor toxicity.

3.0 Results

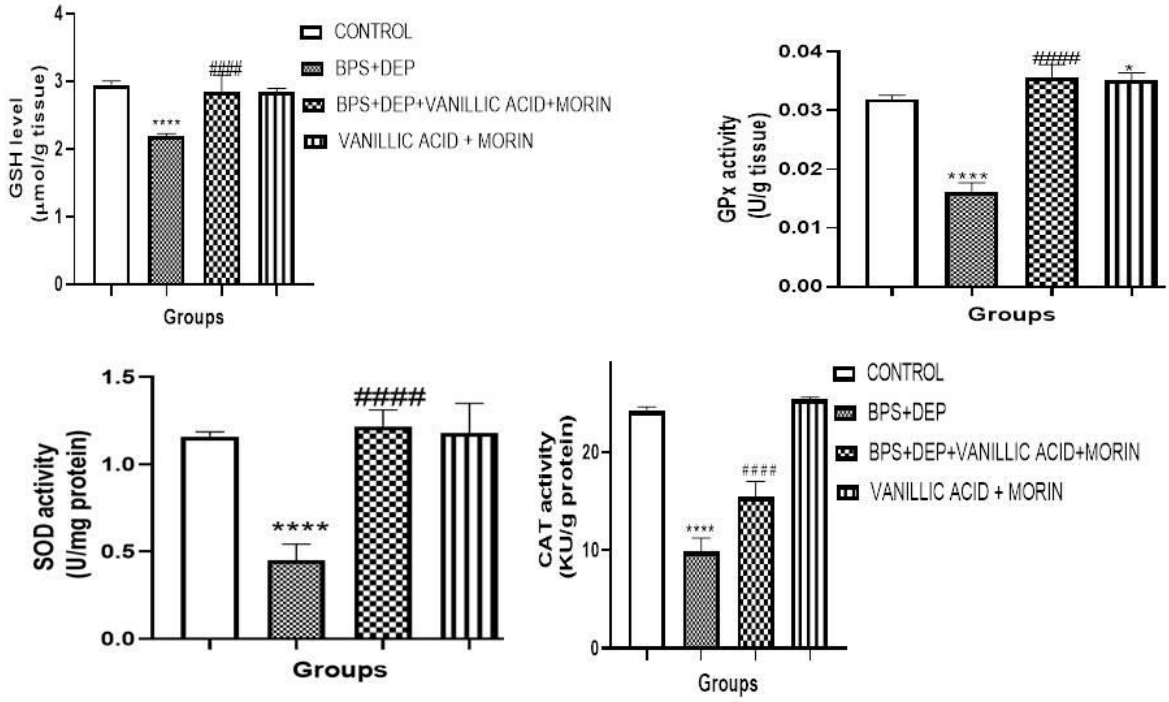


Figure 1. Protective effects of morin and vanillic acid against diethyl phthalate (DEP)- and bisphenol S (BPS)-induced suppression of antioxidant defense biomarkers in rat erythrocytes.

Reduced glutathione (GSH) level; glutathione peroxidase (GPx) activity; superoxide dismutase (SOD) activity; and catalase (CAT) activity. Data are presented as mean ± SEM (n = 5). Bars with different superscripts differ significantly at $P < 0.05$

The results presented in the **figure 1** demonstrate the effects of combined exposure to bisphenol S (BPS) and diethyl phthalate (DEP) on antioxidant defense biomarkers, as well as the protective role of morin and vanillic acid treatment. Exposure to BPS+DEP significantly ($P < 0.05$) reduced the levels and activities of endogenous antioxidants, including reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), when compared with the control group. This reduction indicates severe oxidative stress and depletion of the antioxidant defense system caused by endocrine disruptor toxicity. The marked decrease in GSH suggests impaired cellular redox balance, while the

reduction in GPx, SOD, and CAT activities reflects diminished enzymatic scavenging of reactive oxygen species (ROS).

However, co-treatment with morin and vanillic acid significantly ($P < 0.05$) restored GSH concentration and enhanced GPx, SOD, and CAT activities compared with the BPS+DEP-treated group. The antioxidant enzyme levels in the treatment groups were comparable to, or slightly higher than, those of the control group, indicating effective attenuation of oxidative damage. These findings suggest that morin and vanillic acid exert potent antioxidant and cytoprotective effects by improving endogenous antioxidant capacity and neutralizing free radicals generated by DEP and BPS exposure.

The results indicate that combined exposure to DEP and BPS induces oxidative hepatic injury through suppression of antioxidant defense mechanisms, whereas morin and vanillic acid effectively ameliorate the toxic effects by restoring redox homeostasis and enhancing antioxidant enzyme activities.

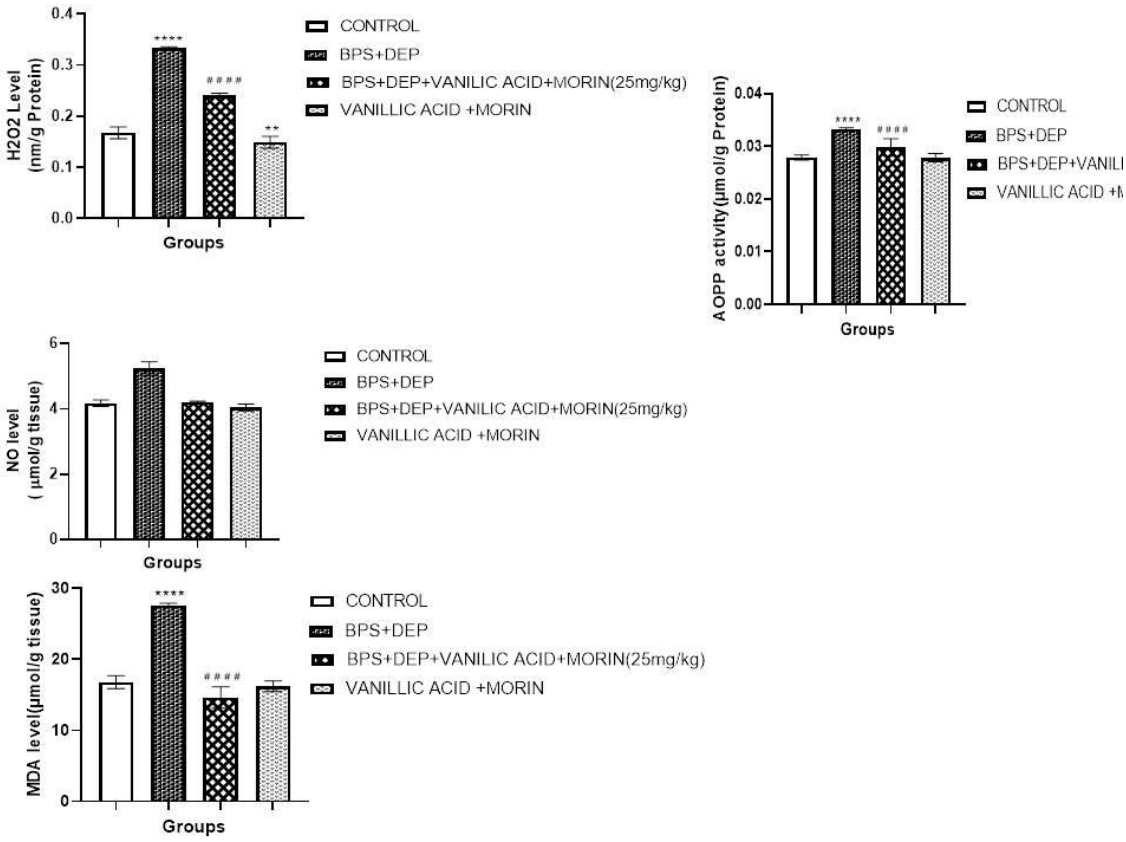


Figure 2. Protective effects of morin and vanillic acid against diethyl phthalate (DEP)- and bisphenol S (BPS)-induced oxidative stress alterations in rats.

Hydrogen peroxide (H_2O_2) level; advanced oxidation protein products (AOPP); (c) nitric oxide (NO) level; and malondialdehyde (MDA) level. Data are expressed as mean ± SEM (n = 5). Bars bearing different superscript letters are significantly different at $P < 0.05$.

Figure **2** illustrates the effects of morin and vanillic acid on oxidative stress biomarkers in rats exposed to bisphenol S (BPS) and diethyl phthalate (DEP). The biomarkers evaluated include hydrogen peroxide (H_2O_2), advanced oxidation protein products (AOPP), nitric oxide (NO), and malondialdehyde (MDA), which are established indicators of oxidative and nitrosative stress. Exposure to BPS+DEP significantly ($P < 0.05$) elevated the levels of H_2O_2, AOPP, NO, and MDA compared with the control group, indicating enhanced generation of reactive oxygen and nitrogen species, oxidative protein modification, and lipid peroxidation. The marked increase in H_2O_2 reflects excessive reactive oxygen species production, while elevated AOPP levels suggest oxidative damage to cellular proteins. Similarly, the rise in NO concentration indicates nitrosative stress and inflammatory responses associated with endocrine disruptor toxicity. Increased MDA levels further confirm membrane lipid peroxidation and hepatocellular oxidative injury induced by combined BPS and DEP exposure.

Conversely, co-administration of morin and vanillic acid significantly ($P < 0.05$) reduced the concentrations of H_2O_2, AOPP, NO, and MDA relative to the BPS+DEP-treated group. The reduction in these oxidative stress biomarkers demonstrates the potent antioxidant and free radical–scavenging activities of the phytochemicals. The treatment restored redox balance and protected cellular macromolecules from oxidative deterioration. In some parameters, the values observed in the treatment groups approached those of the control group, suggesting substantial recovery from endocrine disruptor–mediated oxidative damage. These findings indicate that BPS and DEP induce severe oxidative stress and lipid peroxidation, whereas morin and vanillic acid exert significant protective effects by attenuating reactive species generation and preserving cellular antioxidant homeostasis.

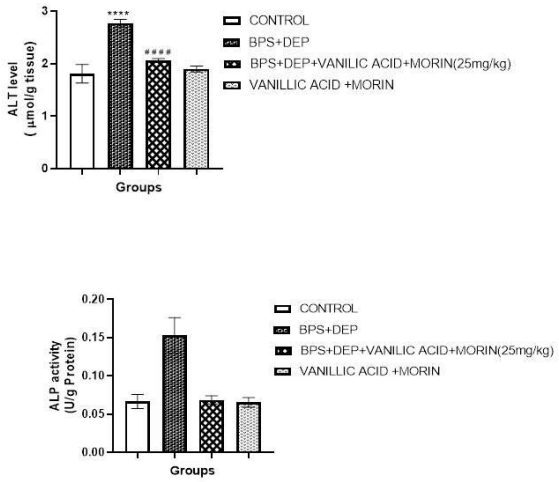


Figure 3. Hepatoprotective effects of morin and vanillic acid against diethyl phthalate (DEP)- and bisphenol S (BPS)-induced alterations in liver enzyme activities in rats.

Alanine aminotransferase (ALT) activity and alkaline phosphatase (ALP) activity. Data are expressed as mean ± SEM (n = 5). Bars with different superscript letters indicate statistically significant differences at $P < 0.05$.

Figure **3** shows the effects of morin and vanillic acid on hepatic function biomarkers in rats exposed to bisphenol S (BPS) and diethyl phthalate (DEP). The parameters evaluated include alanine aminotransferase (ALT) and alkaline phosphatase (ALP), which are sensitive indicators of hepatocellular injury and liver dysfunction. Administration of BPS+DEP significantly ($P < 0.05$) increased the activities of ALT and ALP compared with the control group, indicating hepatic membrane damage and leakage of intracellular enzymes into circulation. The elevation of ALT activity suggests hepatocellular degeneration and impaired liver integrity, while the increased ALP activity reflects possible cholestatic alterations and disruption of hepatic metabolic functions induced by endocrine disruptor exposure. These findings further confirm the hepatotoxic potential of combined BPS and DEP administration.

Conversely, co-treatment with morin and vanillic acid significantly ($P < 0.05$) attenuated the elevated ALT and ALP activities compared with the BPS+DEP-treated group. The restoration of these hepatic biomarkers toward normal values indicates substantial protection of hepatocyte architecture and membrane stability. The observed hepatoprotective effect may be attributed to the antioxidant and free radical–scavenging properties of morin and vanillic acid, which likely reduced oxidative stress–mediated cellular injury in hepatic tissues.

The results demonstrate that combined exposure to DEP and BPS induces significant liver dysfunction, whereas treatment with morin and vanillic acid effectively ameliorates endocrine disruptor–induced hepatotoxicity by preserving hepatic enzyme homeostasis and cellular integrity.

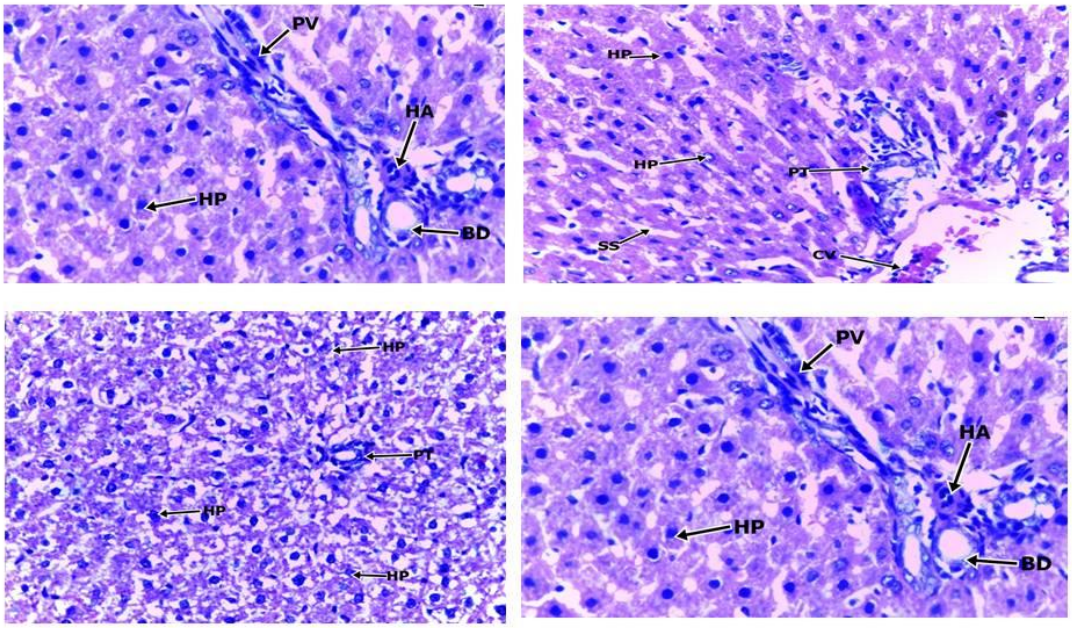


Figure 4. Histopathological evaluation of liver tissues in rats exposed to diethyl phthalate (DEP) and bisphenol S (BPS) and treated with morin and vanillic acid (H&E staining, ×400).

Group A (Control; 0.4% DMSO) showed normal hepatic architecture characterized by well-arranged hepatocytes radiating from the central vein, intact sinusoidal spaces, and preserved portal triad structures including the bile duct, hepatic artery, and portal vein. Group B (DEP + BPS) demonstrated mild hepatic alterations with distortion of hepatocyte arrangement, eosinophilic cytoplasmic changes, and moderate vascular congestion, indicative of hepatocellular stress induced by endocrine disruptor exposure. Group C (DEP + BPS + Morin + Vanillic acid) exhibited marked improvement in hepatic histoarchitecture with restoration of hepatocyte organization, preserved sinusoidal spaces, and intact portal regions, suggesting significant hepatoprotective effects of the combined phytochemical treatment. Group D (Morin + Vanillic acid only) revealed normal liver morphology comparable to the control group, with intact hepatocytes and preserved vascular structures.

Abbreviations: BD = bile duct; CV = central vein; HA = hepatic artery; HP = hepatocyte; PV = portal vein; SS = sinusoidal spaces.

4.0 Discussion

The present study demonstrates, for the first time, the synergistic hepatoprotective efficacy of morin and vanillic acid against combined diethyl phthalate (DEP) and bisphenol S (BPS)-induced oxidative hepatic injury in rats. The findings revealed that co-exposure to DEP and BPS disrupted hepatic redox homeostasis, impaired endogenous antioxidant defense systems, elevated oxidative stress biomarkers, and altered liver function indices. Importantly, combined administration of morin and vanillic acid markedly attenuated these biochemical and histopathological alterations, highlighting their potent antioxidant and cytoprotective properties against endocrine disruptor–mediated hepatotoxicity.

The liver is highly susceptible to xenobiotic-induced injury because it is the principal organ involved in the metabolism, detoxification, and biotransformation of environmental toxicants (Friedman et al., 2003). In the present study, exposure to DEP and BPS significantly elevated serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities, suggesting hepatocellular membrane damage and increased leakage of intracellular enzymes into circulation. Similar elevations in hepatic biomarkers following exposure to endocrine-disrupting chemicals have been associated with oxidative stress, inflammatory responses, and mitochondrial dysfunction (Qiu et al., 2019). However, treatment with morin and vanillic acid significantly normalized ALT and ALP activities, indicating preservation of hepatic membrane integrity and restoration of liver function. The observed hepatoprotective effect may be attributed to the ability of these phytochemicals to neutralize reactive metabolites and suppress oxidative injury.

Oxidative stress plays a central role in DEP- and BPS-induced toxicity through excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reduced glutathione (GSH) constitutes a major intracellular antioxidant that protects cellular macromolecules against oxidative damage (Pham-Huy et al., 2008). The significant depletion of GSH observed following DEP and BPS exposure in this study suggests impaired antioxidant defense and increased susceptibility of hepatic tissues to oxidative injury. Furthermore, reductions in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities indicate exhaustion of endogenous antioxidant enzymes due to overwhelming ROS production. These findings are consistent with previous reports linking endocrine disruptor exposure with antioxidant depletion and lipid peroxidation (Rochester & Bolden, 2015).

Interestingly, co-treatment with morin and vanillic acid significantly restored GSH concentration and enhanced SOD, CAT, and GPx activities. Morin, a

polyhydroxylated flavonoid, possesses strong free radical–scavenging and metal-chelating properties capable of stabilizing ROS and inhibiting oxidative chain reactions (Hussain et al., 2014). Similarly, vanillic acid has been reported to exert antioxidant and anti-inflammatory activities through modulation of cellular redox pathways and inhibition of oxidative damage (Itoh et al., 2009). The combined administration of both phytochemicals in the present study suggests a synergistic mechanism involving ROS neutralization, enhancement of endogenous antioxidant capacity, and stabilization of cellular membranes.

Elevated malondialdehyde (MDA), nitric oxide (NO), hydrogen peroxide (H_2O_2), and advanced oxidation protein products (AOPP) observed in DEP+BPS-treated rats further confirmed severe oxidative and nitrosative stress. MDA is a well-established biomarker of lipid peroxidation and membrane deterioration, while excessive NO generation contributes to nitrosative stress and peroxynitrite formation, leading to cellular dysfunction and tissue injury (Akamo et al., 2021). Increased H_2O_2 levels also indicate impaired peroxide detoxification and oxidative overload within hepatic tissues (Johnson & Giulivi, 2005). The significant reduction in these oxidative stress biomarkers following morin and vanillic acid treatment demonstrates their capacity to suppress lipid peroxidation, reduce ROS/RNS accumulation, and preserve redox equilibrium.

Histopathological observations corroborated the biochemical findings. Liver sections from DEP+BPS-exposed rats revealed distorted hepatic architecture, vascular congestion, and hepatocellular degeneration, confirming toxicant-induced hepatic injury. In contrast, rats treated with morin and vanillic acid showed marked restoration of normal hepatic morphology with preserved hepatocyte arrangement, intact sinusoidal spaces, and reduced pathological lesions. These histological improvements further support the therapeutic potential of the combined phytochemicals in protecting hepatic tissues against endocrine disruptor toxicity.

A major novel contribution of this study is the demonstration of the synergistic antioxidant and hepatoprotective effects of morin and vanillic acid against mixed endocrine disruptor exposure, which more accurately reflects real-life environmental exposure scenarios. Previous studies have largely focused on single toxicants or individual phytochemicals, whereas the present work provides integrated biochemical and histopathological evidence supporting the combined therapeutic efficacy of morin and vanillic acid against DEP- and BPS-induced hepatotoxicity. Collectively, the findings suggest that these bioactive compounds may serve as promising natural candidates for mitigating oxidative liver injury associated with environmental endocrine disruptors.

5.0 Conclusion

This study demonstrated that combined exposure to diethyl phthalate (DEP) and bisphenol S (BPS) induces oxidative stress and hepatotoxicity through disruption of antioxidant defense systems and elevation of hepatic injury biomarkers. Treatment with morin and vanillic acid significantly ameliorated these alterations by restoring antioxidant status, reducing lipid peroxidation, and preserving liver histoarchitecture. The findings highlight the synergistic antioxidant and hepatoprotective potential of morin and vanillic acid against endocrine disruptor–induced liver injury and suggest their possible therapeutic application in mitigating oxidative hepatic damage.

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Author's contribution

Eteng, Ofem Effiom conceived and designed the study, and drafted the manuscript. **Eteng, Katerine Kenyoh** participated in experimental procedures, review. **Etido, Paul** assisted with sample collection, laboratory investigations, and statistical analysis. **Grace, Ime Ekpo** participated in data acquisition. **Victor, Ekam** assisted with laboratory analyses **Godwin, Oju Igile** critical revision of the manuscript. **Eyoug Ubana** manuscript review. **Eteng, Mbeh Ubana** the final manuscript revision.

Ethical Approval

All experimental procedures involving animals were conducted in accordance with the guidelines outlined in the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH) and approved by the National Academy of Sciences (NAS). Ethical approval for this study was obtained from the Institutional Ethics Committee, Federal University of Agriculture, Abeokuta, Nigeria (Approval No.: BCH/20160914).

Reference

Qiu, W., Shao, H., Lei, P., Zheng, C., Qiu, C., Yang, M., Zheng, Y., & Mai, W. (2019). Immunotoxicity of bisphenol S via oxidative stress and inflammatory response in zebrafish. *Environmental Toxicology and Pharmacology, 65*:1–9. https://doi.org/10.1016/j.etap.2018.11.010

Herrero, P., Borrull, F., Pocurull, E., & Marcé, R. M. (2018). Bisphenol A and bisphenol S analysis in thermal paper receipts. *Analytical and Bioanalytical Chemistry, 410*(10):2479–2488. https://doi.org/10.1007/s00216-018-0939-7

Rochester, J. R., & Bolden, A. L. (2015). Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol A substitutes. *Environmental Health Perspectives, 123*(7): 643–650. https://doi.org/10.1289/ehp.1408989

Weaver, J. A., Beverly, B. E. J., Keshava, N., Mudipalli, A., Arzuaga, X., Cai, C., Hotchkiss, A. K., Makris, S. L., & Earl Gray, L. (2020). Hazards of diethyl phthalate exposure: A review of toxicological evidence. *Toxicology and Industrial Health, 36*(3):143–156. https://doi.org/10.1177/0748233720911896

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R. C., & Faisal, P. A. (2017). Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. *Journal of Hazardous Materials, 340*, 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036

Vandenberg, L. N., Ehrlich, S., Belcher, S. M., Ben-Jonathan, N., Dolinoy, D. C., Hugo, E. R., Hunt, P. A., Newbold, R. R., Rubin, B. S., Saili, K. S., Soto, A. M., Wang, H. S., & vom Saal, F. S. (2013). Low dose effects of bisphenol A. *Endocrine Disruptors, 1*(1):e26490. https://doi.org/10.4161/endo.26490

Hussain, T., Gupta, S., Adhami, V. M., & Mukhtar, H. (2014). Morin: A natural flavonoid with versatile biological and pharmacological activities. *Phytochemistry Reviews, 13*(3):541–557. https://doi.org/10.1007/s11101-014-9354-0

Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2017). Psidium guajava: A review of its traditional uses, phytochemistry, and pharmacology. *Journal of Ethnopharmacology, 117*(1):1–27. https://doi.org/10.1016/j.jep.2008.01.025

Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. (2004). Flavonoids: Antioxidants or signalling molecules? *Free Radical Biology and Medicine, 36*(7):838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Itoh, A., Isoda, K., Kondoh, M., Kawase, M., Kobayashi, M., Tamesada, M., & Yagi, K. (2009). Hepatoprotective effect of vanillic acid on liver injury induced by carbon tetrachloride. *Biological and Pharmaceutical Bulletin, 32*(7): 1215–1219. https://doi.org/10.1248/bpb.32.1215

Shangari, N., and P.J. O'Brien. (2006). Catalase activity assays. Current protocols in toxicology 27: 7-7.

Rotruck, J.T., A.L. Pope, H.E., Ganther, A.B., Swanson, D.G., Hafeman, and W. Hoekstra. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. Science 179: 588–590.

Marklund, S., and G. Marklund. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry 47: 469-474.

Junglee, S., Urban, L., Sallanon, H., Lopez-Lauri, F. (2014). Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. Am. J. Anal. Chem. 5 (11): 730

Buege, J.A. and Aust, S.D. (1978) Microsomal Lipid Peroxidation. Method Enzymology, 30: 302-310.https://doi.org/10.1016/S0076-6879(78)52032-6

Friedman, S. E., Grendell, J. H., & McQuaid, K. R. (2003). *Current diagnosis & treatment in gastroenterology* (2nd ed.). McGraw-Hill Medical.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science, 4*(2): 89–96.

Akamo, A. J., Ibegbu, D. M., Ugwor, E. I., & Ojo, O. A. (2021). Nitric oxide-mediated oxidative stress and its implication in hepatic dysfunction. *Biomedicine & Pharmacotherapy, 137*: 111234. https://doi.org/10.1016/j.biopha.2021.111234

Johnson, F., & Giulivi, C. (2005). Superoxide dismutases and their impact upon human health. *Molecular Aspects of Medicine, 26*(4-5):340–352. https://doi.org/10.1016/j.mam.2005.07.006

