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Research Article

Synergistic Effect of Morin and Vanillic Acid against Bisphenol-S and Diethylphtalate Induced-Hematotoxicity in Male Albino Rats

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Abstract

Environmentally unfriendly weapon like endocrine disruptors produces negative effect on biological system. We investigated the effect of morin and vanillic acid on diethyl phthalate (DEP) and bisphenol S (BPS)-induced hematotoxicity using a rat model. Rats were exposed to DEP (50 mg/kg) and BPS (200 m/kg) and treated with morin and vanillic acid (25 and 25 mg/kg) by oral gavage for twenty-one days, blood samples were collected for assessment of biochemical parameters (glutathione peroxidase (GPx), glutathione (GSH), malondialdehyde levels (MDA), catalase activity (CAT), nitric oxide (NO), and superoxide dismutase (SOD) respectively). Morin and vanillic acid treatment significantly ($p < 0.05$) protected red blood cells and plasma membrane architecture by abolishing the DEP+BPS-induced decrease in the activity of all the hematotoxicity. Co-treatment with morin and vanillic acid remarkably ($p < 0.05$) reversed DEP+BPS-induced decreases in glutathione levels, CAT, SOD, GPx and GSH activities in red blood cells, while attenuating DEP+BPS mediated increase in hemato-oxidative damage markers (MDA and NO levels). Collectively, the same acute doses of morin and vanillic acid might avert DEP and BPS-mediated hematotoxicity dysfunctions via its antioxidant, free-radical scavenging effect.

Keywords: *Bisphenol S; Diethyl Phthalate; Hematotoxicity; Antioxidant; Oxidative Damage*

Introduction

Morin is present in several fruits and vegetables, such as almond hulls, guava leaves (*Psidium guajava*), old fustic (*Chlorophora tinctoria*), mill (*Prunus dulcis*), osage orange (*Maclura pomifera*), *Acridocarpus orientalis*, onion, apple, and in several beverages, such as tea, red wine, seaweeds, coffee and cereal grains. Morin (3, 5, 7, 2 9, 4 9-pentahydroxyflavone) is an important phytochemical abundantly available in many plants belonging to the Moraceae family (*Morus alba*). It has the characteristic yellow colour of natural bioflavonoid [1]. Its antioxidant, anti-inflammatory and anti-proliferative potencies are well established in both *in-vivo* and *in-vitro* experiments. In addition, it represents one of the constituents of several traditional herbal medicines. Morin is readily available, with fewer side effects and robust functional properties that could explain the use of these plants in traditional herbal medicine [2]. Recent evidence has demonstrated that Morin could have a beneficial effect on several human diseases by modulating the activity of many enzymes. In some cases, Morin shows a systemic protective action, reducing negative side effects of several drugs, without interfering with their functions.

Vanillic acid (VA) is a natural compound of phenolic acids family and also a derivative of benzoic acid which is used as a flavouring agent, preservative, and food additive in the food industry. It is a form of vanillin oxide and is produced when vanillin converted to ferulic acid. VA has several pharmacological effects including anti-metastatic [3], anti-melanogenesis [4], antioxidant, anti-angiogenesis [5] and anti-apoptotic effects [6]. Recent study has shown the cardio-protective effect of VA in ischemia-reperfusion through decreasing oxidative stress and improving myocardial dysfunction [7]. Diethyl phthalate (DEP) has been found to have diverse acute and chronic toxic effects on several species at different trophic levels and endocrine-disrupting properties [8]. DEP could also be seen as a colourless, odourless, oily substance used to improve the performance and durability of many products [9]. It is added to plastic polymers as a plasticiser to help maintain flexibility. It has been used in various products, including plastic films, rubber, tape, toothbrushes, automotive components, tool handles and toys.

BPS is a synthetic organic compound widely used as a precursor in polycarbonate plastic products, epoxy resins, and various materials. Bisphenol S (BPS) has been introduced to the industry as a safe alternative to BPA; however, recent studies have shown that different BPS concentrations correlate with oxidative stress [10]. It can be directly released from factories or consumer products and is found in the environment. Consequently, global human exposure mainly results from environmental contamination and dermal contact with bisphenol-containing products or food contamination since bisphenols are primarily used in food and beverage containers [11]. Exposure to BPS for a prolonged period can cause significant alteration in the structure of body organs. DEP and BPS possess lipophilic properties capable of penetrating the membrane of red blood cells (RBCs) and initiating the production of free radicals that can cause cellular damage [12]. The functional role of RBCs is transporting oxygen from the lungs to the tissues providing all cells with the required oxygen. In the circulation, RBCs are continuously exposed to both endogenous and exogenous sources of reactive oxygen species (ROS) that can damage the RBCs and impair their function. ROS are molecules, ions, or free radicals derived from molecular oxygen. They can highly react with biological molecules such as lipids, proteins, and DNA, damaging them [12]. Hence, this study was conducted to elucidate the mechanisms in which morin and vanillic acid can synergistically mitigate the negative effect of DEP and BPS induced hematotoxicity in rat model.

Materials and Methods

Chemicals and reagents

Morin (Cat. No.: M 2357), and vanillic acid (Cat. No.: V 1240) Bisphenol S BPS (Cat. No: D 5095). Diethyl phthalate DEP (Cat. No.: D 1785) were procured from Otto Chemie Pvt Ltd (Mumbai, India).) Dimethylsulfoxide (DMSO) was purchased from Libertas laboratory services limited, Abeokuta. All other chemicals, unless otherwise specified, were products of the British Drugs House Chemicals Limited (Poole Dorset, England).

Animal care

Experimental animals, male albino Wistar rats weighing 150g - 200g, were inbred at the Animal House, Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria. They were housed in a plastic suspended cage placed in a well-ventilated, temperature-controlled (25°C) rat house with standard 12-h light/12-h dark cycles. The rats were acclimatized for one week and given standard pellet chow and fresh water ad libitum. All the animals received humane care according to the conditions outlined in the 'Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science (NAS) and published by the National Institute of Health. The institution approved this experiment with approval number: BCH/20160957.

Experimental protocol

Twenty-five rats were divided into five groups (n = 5) and treated as detailed in table 1, using a simple randomization method. Five rats per group were used based on 3R (replacement, reduction, and refinement) principles (Maestri, 2021). BPS and DEP were adminis-

tered as a mixture at toxicologically-relevant doses of 200 and 50 μg/kg body weight (b.wt.), based on previous reports by Catanese and Vandenberg (2017) and Ahmadpour., *et al.* (2021) respectively. Morin was given immediately after administration of BPS+DEP at a dosage of 25 or 50 mg/kg b.wt., based on a previous study that demonstrated the antioxidant activities of morin at these doses (Ben-Azu., *et al.* 2021). Dimethyl sulfoxide (DMSO) served as the vehicle for all treatments and administration, which lasted for twenty-one (21) days, was by oral gavage.

Groups (n = 5)	Treatment
A	DMSO (0.4% v/v)
B	DEP (50 mg/kg b.wt.) + BPS (200 mg/kg b.wt.)
C	BPS + 50 mg/kg b.w + DEP (200 mg/kg b.wt. Morin 25 mg/kg b.w+ Vanillic acid 25 mg/kg b.w)
D	Morin 25 mg/kg b.w + Vanillic acid 25 mg/kg b.w

Table 1: *Grouping of experimental animals and their treatments.*

Preparation of serum

At the end of the experiment, 24 h after the last administration, the animals were sacrificed via anaesthesia with Ketamine/Xylazine (100 and 7 mg/kg i.p., respectively) (Oguntoye and Oke, 2014) and dissected. Blood samples were collected via cardiac puncture into plain centrifuge tubes. The blood samples were centrifuged at 5000 rpm for 10 minutes. The serum, the clear supernatant, was removed and used for the biochemical analysis.

Antioxidants were determined in a suitably diluted erythrocyte lysate.

Reduced glutathione (GSH) concentration was determined spectrophotometrically at 412 nm by measuring the rate of formation of chromophoric product 2-nitro-5-thiobenzoate (TNB) as a result of the reduction of Ellman's reagent DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] by the free sulphydryl group of reduced glutathione (2GSH + DTNB → TNB + GSSG); the intensity of the yellow-colored complex formed is directly proportional to the amount of –SH groups, as described by the method of Jollow., *et al*. [13]. GSH values were expressed as μgGSH/g hemoglobin using GSH molar extinction coefficient (Σ) of 9.6 0.017 $mM^{-1} cm^{-1}$.

Glutathione peroxidase (GPx) activity was determined spectrophotometrically at 420 nm by measuring the residual GSH content during the decomposition of hydrogen peroxide using GSH as a co-factor (H_2O_2 + 2GSH →$2H_2O$ + GSSG), according to the method of Mohandas., *et al*. [14]. GSPx specific activity was expressed as Units/g hemoglobin or nmol of residual GSH/min/g hemoglobin using GSH molar extinction coefficient (Σ) of 9.6 0.017 $mM^{-1} cm^{-1}$.

Superoxide dismutase (SOD) activity was determined spectrophotometrically at 420 nm by measuring the inhibition of autoxidation of pyrogallol, a superoxide-reacting indicator molecule (SRIM) that compete with SOD for the reaction with superoxide in an alkaline medium (pyrogallol/SOD + O_2^- + 2H+ → H_2O_2 + O_2), according to the method described by Marklund and Marklund [15]. The specific activity of SOD was expressed as Units/g hemoglobin or pyrogallol 50% oxidation auto-inhibition/min/g hemoglobin using pyrogallol molar extinction coefficient (Σ) of 8.0 × 105 $M^{-1} cm^{-1}$.

Catalase activity was determined spectrophotometrically at 374 nm by measuring the rate of decomposition of hydrogen peroxide ($2H_2O_2 \rightarrow 2H_2O + O_2$), according to the method of Hadwan and Abed [16]. The specific activity of catalase was expressed as Units/g hemoglobin or mmol H_2O_2 degraded/min/g hemoglobin using the H_2O_2 molar extinction coefficient (Σ) of 43.6 $M^{-1} cm^{-1}$.

Markers of hemato-oxidative damage

Determination of total nitric oxide (NO) radical

Nitric oxide radical is produced in biological tissues by nitric oxide synthase, which metabolizes arginine to citrulline with the formation of NO via a five-electron oxidative reaction. NO is oxidized by oxygen to nitrite (NO_2^-) and nitrate (NO_3^-), which are stable final products of NO metabolism and may be used as indirect cellular markers of NO presence. The sum of cellular nitrite and nitrate levels is a measure of nitric oxide produced and hence the activity of nitric oxide synthase. Nitrate is reduced to nitrite. The nitrite reacts with a color developing agent such as Griess reagent [2% sulphanilamide in 5% phosphoric acid and 0.2% N-(1-naphthyl) ethylenediamine dihydrochloride] then generates a purple azoic compound, [$NO \rightarrow NO_2$ –, + sulphanilamide → diazonium salt, + N-(1-naphthyl)ethylenediamine → Azo dye] which gives the concentration of NO in the erythrocytes as described by Green., *et al*. (1984).

Determination of MDA in blood

The degree of erythrocytes lipid peroxidation was assayed spectrophotometrically at 532 nm by measuring malondialdehyde (MDA, an end product of cell membrane lipid peroxides/oxidative damage), as the formation of thiobarbituric acid reactive substances (TBARS) (TBA + MDA → TBA-MDA adduct) by the method of Wright., *et al*. [17]. TBARS contents were expressed as nmol MDA formed/mg hemoglobin using an MDA molar extinction coefficient (Σ) of $1.56 \times 105\ M^{-1}\ cm^{-1}$.

Histopathological examination

For histopathological examination, liver tissues were preserved in 10% neutral formalin solution. Liver tissues were embedded in Paraffin wax and 5μm sections were prepared and stained with haematoxylin and eosin (H&E).

Statistical analysis

Data were expressed as the mean ± SEM of each group. Analysis of Variance (ANOVA) was carried out to test for the level of homogeneity among the groups. Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A p-value of less than 0.05 was considered statistically significant. All the statistics were carried out by SPSS (Statistical Package for Social Sciences) software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). Graphs were plotted using GraphPad Prism 8 Software (GraphPad Software Inc., San Diego, USA).

Results

Figure 1 shows the effect of morin on glutathione (GSH) concentration in RBC of diethyl phthalate and bisphenol S-exposed rats. There was a significant decrease ($p < 0.05$) by in RBC GSH concentration in the DEP+BPS-exposed group compared to the control group. In contrast, exposed groups treated with 25 and 25 mg/kg morin and vanillic acid showed a significant increase ($p < 0.05$) in RBC GSH concentration compared to the DEP + BPS group, respectively. Further, there was a significant decrease ($p < 0.05$) RBC GSH concentration in the group treated with morin and vanillic acid compared to the control group.

Exposure to DEP and BPS inhibited RBC GPX activity compared to control. However, following treatment with 25 and 25 mg/kg morin and vanillic acid, there was a significant increase ($p < 0.05$) in RBC glutathione peroxidase (GPX) activity compared to the DEP+BPS respectively. In addition, there was also a significant increase ($p < 0.05$) RBC glutathione peroxidase (GPX) activity in the group treated with morin and vanillic acid when compared to the control group.

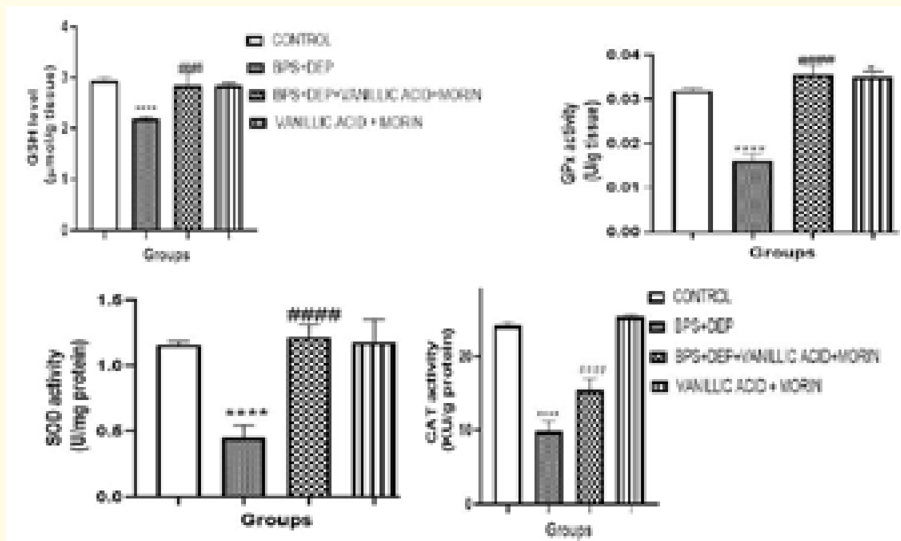

Figure 1: *Effect of morin and vanillic acid pretreatment on diethyl phthalate and bisphenol s mediated decrease in antioxidant in rats in red blood cells concentration. (a) The activities of glutathione s-transferase (b) The activities of glutathione peroxidase (c) The activities of superoxide dismutase (d) The activities of catalase. Bars represent mean ± SEM (n = 5). Bars with different letters are significantly different at P < 0.05.*

RBC SOD activity showed a significant decrease ($p < 0.05$) in rats exposed to DEP and BEP compared to the control group. However, there was a significant increase ($p < 0.05$) in RBC SOD activity in groups treated with 25 and 25 mg/kg morin and vanillic acid respectively, when compared to the DEP + BPS group. No significant difference ($p > 0.05$) was observed in the group treated with Morin and vanillic acid compared to the control group.

Similarly, RBC CAT significantly decreased ($p < 0.05$) in DEP + BPS–exposed group compared to the control group. There were significant increases ($p < 0.05$) in RBC CAT activity in exposed groups treated with 25 and 25 mg/kg morin and vanillic acid respectively, when compared to the DEP + BPS group. However, there was no significant difference in the group treated with Morin and vanillic acid when compared to the control group.

Figure 2 shows the effect of morin and vanillic acid on markers of oxidative damage in the RBCs of DEP + BPS-exposed rats. There was a significant increase ($p < 0.05$) in RBC MDA concentration in the DEP + BPS-exposed group compared to the control group. On the other hand, treatment with morin (25 and 25 mg/kg) occasioned a marked decrease in RBC MDA concentration, which followed a dose-dependent trend. Furthermore, there was no significant difference in the group treated with morin and vanillic acid when compared to the control group.

The effects of exposure to DEP + BPS and treatment with morin and vanillic acid on RBC NO concentration followed a similar trend. While there was a significant decrease ($p < 0.05$) in RBC NO concentration in the exposed group compared to the control group, treatment with morin and vanillic acid occasioned dose-dependent reduction in reactive nitrogen species (i.e. NO). Meanwhile, no significant difference ($p > 0.05$) was observed in the group treated with morin only compared to the control group.

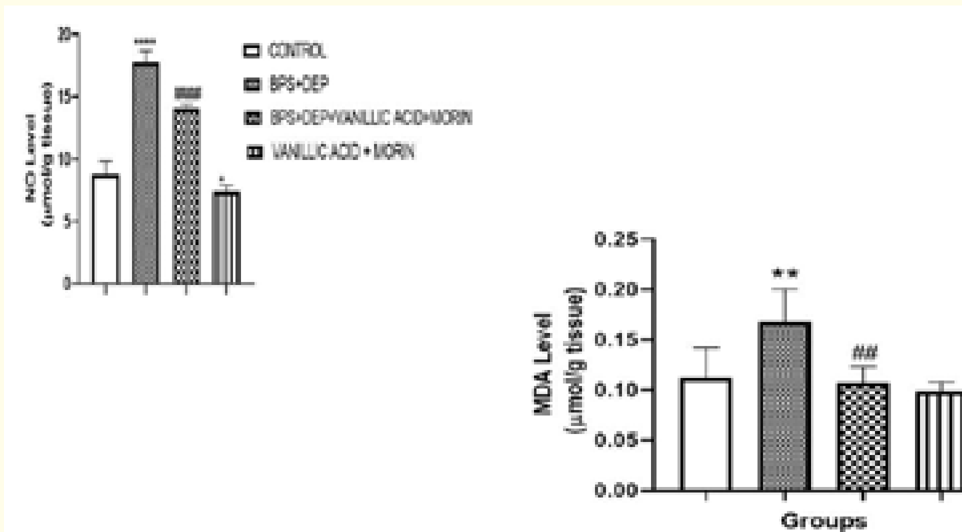

Figure 2: *Effects of morin pretreatment on diethyl phthalate and bisphenol s mediated increase in oxidative stress markers in rats on hematological parameters. (a) Nitric oxide (b) level of MDA. Bars represent mean ± SEM (n = 5). Bars with different letters are significantly different at P < 0.05.*

Figure 3 presents the results of the histopathological analysis. While no death was recorded among all groups during this study, section of liver tissues from group A (0.4% DMSO) showed a preserved architecture with a prominent central vein and hepatocytes radiating outwards. The hepatocyte has deeply stained nuclei and clear cytoplasm. The sinusoidal spaces are dilated and the portal tracts have intact limiting plates and consist of the bile duct, hepatic artery and portal vein. Section of the liver group B (50 mg/kg DEP + 200 mg/kg BPS) showed prominent congested central vein with radiating plates of hepatocytes separated by dilated sinusoidal spaces. The hepatocytes have moderate eosinophilic cytoplasm and round to oval basophilic nuclei with regular outline, fine chromatin pattern and nucleoli. The portal areas showed intact limiting plate hepatocytes and contains portal vein, hepatic artery and bile duct. Section of liver tissue from group C (200 mg/kg DEP + 50 mg/kg BPS + 25 mg/kg morin+25 mg/kg vanillic acid) showed radiating plates hepatocytes outward from the central veins. The hepatocyte is swollen with deeply stained basophilic nuclei having regular outline and clear cytoplasm. The sinusoidal spaces appeared reduced. The portal area has an intact limiting membrane and contains bile duct, hepatic artery and portal vein. The portal tract has intact limiting plate hepatocytes and contains bile duct, portal vein and hepatic artery. Section of liver tissue from group D (25 mg/kg morin and 25 mg/kg of vanillic acid) showed a preserved architecture plates of hepatocytes radiating from a central vein. The hepatocytes have moderate eosinophilic cytoplasm and round to oval basophilic nuclei with regular contour and fine chromatin pattern. The sinusoidal spaces are intact and the portal tracts has an intact limiting plate hepatocyte and contain bile duct with intact epithelial lining, hepatic artery and portal vein (Figure 3).

Discussion

Lack of adequate information and knowledge regarding the quantitative and qualitative molecular mechanisms, deter the application of phytochemicals as a therapeutic drug for many pathological complication. Morin and vanillic acid from the ancient time of human history, nature has provided the sufficient remedies in the form of herbal products for remedies and drug development which depends on the knowledge of herbal medicine [18].

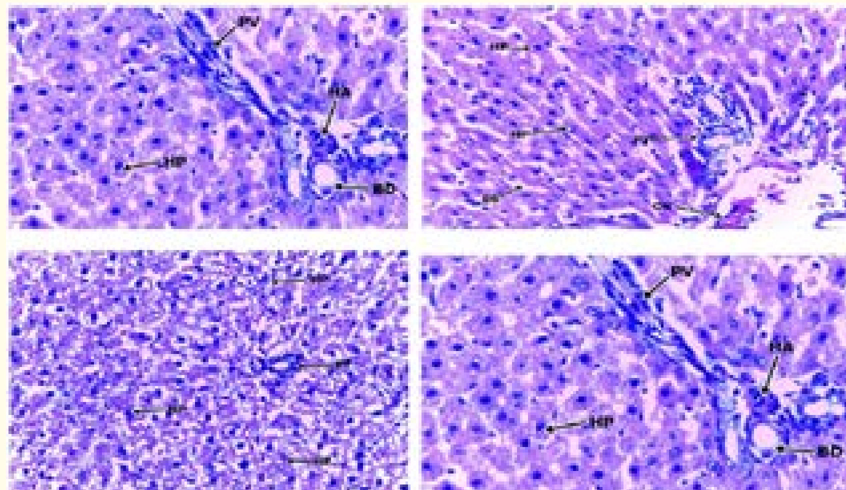

Figure 3: *Liver histology of control and DEP + BPS-exposed rats treated with morin. Group A: 0.4% DMSO; Group B: 200 mg/kg DEP + 50 mg/kg BPS; Group C: 200 mg/kg DEP + 50 mg /kg BPS + morin (25 and 25 mg/kg vanillic acid); Group D: Morin 25 mg/kg + Vanillic acid 25 mg/kg); BD = Bile Duct; CV = Central Vein; HA = Hepatic Artery; HP = Hepatocyte; PV = Portal Vein; SS = Sinusoidal Spaces. Magnification = x400 (H&E staining).*

Group A DMSO 04% (x400): *Section of liver tissues shows a preserved architecture with a prominent central vein and hepatocytes radiating outwards. The hepatocyte has deeply stained nuclei and clear cytoplasm. The sinusoidal spaces are dilated and the portal tracts have intact limiting plates and consist of the bile duct, hepatic artery and portal vein.*

Group B DEP 200 mg+BPS50 mg/kg (x400): *A section of liver tissue shows a preserved architecture plates of hepatocytes radiating from a central vein. The hepatocytes have moderate eosinophilic cytoplasm and round to oval basophilic nuclei with regular contour and fine chromatin pattern. The sinusoidal spaces are intact and the portal tracts has an intact limiting plate hepatocyte and contain bile duct with intact epithelial lining, hepatic artery and portal vein.*

Group C 200 mg/kg +BPS 50 mg/kg Morin 25 mg/kg and 25 mg/kg vanillic acid (x400): *Section of liver tissue radiating plates hepatocytes outward from the central veins. The hepatocyte is swollen with deeply stained basophilic nuclei having regular outline and clear cytoplasm. The sinusoidal spaces appeared shows reduced. The portal area has an intact limiting membrane and contains bile duct, hepatic artery and portal vein.*

Group D morin 25 mg/kg and 25 mg/kg vanillic acid (x400): *Section of the liver shows a dilated central vein and plates of hepatocytes radiating outwards. The hepatocytes have prominent amount of cytoplasm. The portal tract has intact limiting plate hepatocytes and contains bile duct, portal vein and hepatic artery.*

The present research established an inhibitory effect of morin and vanillic acid against the free radical scavenging effect and its antioxidant tendencies. Moreover, we confirmed the capability of ingesting a mixture of DEP and BPS to induce hematotoxicity in a rat's model. We effectively evaluated the effects of morin and vanillic acid on antioxidants, oxidative stress parameters. Our findings specified that morin and vanillic acid might reduce DEP + BPS-mediated hematotoxicity by acting as specific free radical scavengers, improving cellular integrity and antioxidants, and abrogating oxidative damage. These effects may thus underlie antioxidants mechanism of action on DEP and BPS-induced hematotoxicity rats.

Primarily, the RBC's primary function as oxygen conductor endangers the cell to the debilitating effects of ROS, which are formed endogenously in redox reactions and autoxidation of hemoglobin [19]. Uninhibited ROS generation leads to oxidative stress in the RBCs, resulting in several factors that add to their ageing and subsequent removal from circulation [20]. To protect against this outcome, RBCs are enriched with an array of endogenous antioxidants.

GSH plays a crucial role in the antioxidant defence system of various cell types, including RBCs [21]. It is an antioxidant in plants, animals, and some bacteria that pivots the thiol-redox control and prevents damage to cellular components caused by reactive oxygen species, including free radicals and lipid peroxides [22]. Exposure to DEP and BPS caused a significant decrease in the RBC GSH concentrations, suggesting an increased production of free radicals, most likely during the metabolism of these chemicals, resulting in cellular damage. Upon treatment of the exposed groups with morin and vanillic acid RBC GSH concentrations were significantly increased ($p < 0.05$), signifying the amelioration of oxidative stress in these groups. This effect could result from the antioxidant property of morin [23], which followed a dose-dependent trend, with the 25 mg/kg dose showing a more significant effect.

GSH is the principal non-protein thiol that protects erythrocytes ghost and its intracellular component from free radical-induced oxidation [23]. Once synthesized, GSH can be a cofactor for enzymes like GST, GPx, GSR and G6PDH. Therefore, GSH depletion could cause a decrease in GST, GPx, GSR and G6PDH activities [24]. The reduction in the activities of GSH, GPx, CAT and SOD observed in this study could directly result from DEP and BPS-induced decreases in GSH or as a result of their metabolites. Compartmentalized activities of all these endogenous antioxidant defences prevent free radical-mediated necrosis, DNA and RNA fragmentation, protein modification and lipids peroxidation of erythrocytes ghost. Also, GSH protects RBC's cytoskeleton and intracellular macromolecules against oxidation and cell injury by reacting and scavenging ROS, controlling SOD transcription and reactivating GST and GPx [25]. Thus, depletion of GSH, GST, GPx, and GSR could increase oxidative and nitrosative stress, causing alteration in various compartments, including the RBC's composition [26]. Nonetheless, morin and vanillic acid administration exerted protection by reversing DEP and BPS-induced decreases in GSH, SOD, CAT, and GPx. This effect could be by scavenging the reactive metabolites of DEP and BPS, chelating active metal ions, suppressing the oxidation of non-transition metals, supplying hydrogen atoms or electrons to stabilize the toxicants reactive metabolites and ROS produced to ensure their stability, and thereby detoxifying them.

Reactive SOD is a protective enzyme that is capable of eliminating superoxide anion radical (O^{2-}) by catalyzing its dismutation to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) [27]. The superoxide radical participates in various significant processes including lipid peroxidation, hemolysis, and ageing of red blood cells [21]. Studies have shown that deficiency of SOD may lead to Heinz bodies' formation and development of hemolytic anaemia [28]. CAT is a heme-containing redox enzyme that catalyzes the decomposition of H_2O_2 into water and molecular oxygen to protect the body from oxidative stress (free radical damage) [29]. Moreover, a significant increase in catalase activity has been proven to be inversely correlated with hemoglobin oxidation [30]. Therefore, a decreased activity of this enzyme correlates with increased methemoglobin (meth-Hb) levels in red blood cells. GPx is an enzyme with peroxidase activity and can promote the reaction of H_2O_2 with GSH to produce H_2O and GSSG, thereby protecting cell membrane structure and function from damage [31]. A decrease in CAT and/or GPx activity is associated with increased hydrogen peroxide content in the cell [32]. DEP and BPS-induced toxicity caused a significant decrease ($p < 0.05$) in the RBC's activities of GPX, SOD, and CAT (Figure 1). These results indicate the depleted antioxidant capacity of the RBC, possibly due to the overload of free radicals produced during DEP and BPS metabolism. Upon treatment with morin and vanillic acid this condition was ameliorated by strengthening the antioxidant defence capacity. These results possibly indicate the ability of morin and vanillic acid to form stable complexes with the metal ions in the antioxidant enzymes, thereby increasing their free–radical scavenging effects.

Red blood cells are highly-sensitive to oxygen free radicals (Halliwell, 2006). When the antioxidant system collapses, the excess ROS can assail the biomembranes' polyunsaturated membrane lipids in a process called lipid peroxidation with MDA as a primary product.

Exposure to DEP and BPS resulted in a significant increase ($p < 0.05$) in the RBC MDA concentrations (Figure 2). This finding suggests oxidative stress in the RBCs due to an imbalance of free radicals produced coupled with the depletion of endogenous antioxidants. The former is evidenced by the elevated NO levels we observed in DEP + BPS exposed group. Low levels of NO increase RBC deformability, membrane fluidity, and RBC filterability. However, elevated NO level exerts detrimental consequences such as hypotension, endothelial dysfunction, oxidative stress on the cellular environment, mitochondrial dysfunction and airway hyperresponsiveness [26]. Besides, NO readily combines with O^{2-}, another free radical, to form a stable and potent oxidant called peroxynitrite radical ($OONO^-$). Reactive nitrogen species (RNS), such as NO and $OONO^-$, interact with cellular biomolecules such as lipids, proteins, and nucleic acids following the depletion of the antioxidant defence system and elicit tissue perturbation and injury via induction of nitrosative stress. Treatment with morin and vanillic acid significantly decreased ($p < 0.05$) the MDA and NO concentration in RBC, portending the anti-lipid peroxidation and ROS/RNS-scavenging ability of morin [33], thereby preventing oxidative cellular damage to RBCs.

Lastly, a tremendous alteration of the hepatocyte architecture was observed from the histopathological examination of the liver section of the rats. Also, it was conspicuous that upon treatment with morin and vanillic acid there was a physiological recovery in the hepatic tissues (Figure 3), which provides further validation of the protective effect of morin against DEP + BPS-induced toxicity.

Conclusion

The recent study support the claims that combined doses of the same milligram of morin and vanillic acid pretreatment for 21 days could ameliorate BPS and DEP induced hematotoxicity by acting as specific ROS and RNS scavenger that preserve the cytoarchitecture of the liver in rats via it antioxidant free radical scavenging capacity. Therefore, combined extract of morin and vanillic acid demonstrated a significant degree of attenuation which could proffer a promising attribute to its strong antioxidant properties.

Conflict of Interest

The authors declare no conflict of interest.

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Ethical Approval

All the animals received humane care according to the conditions outlined in the 'Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science (NAS) and published by the National Institute of Health. The institution approved an experimental number of the researcher is BCH/20160957.

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