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Effect of Vanillic Acid and Morin on Bisphenol S and Diethyl Phthalate Induce-Nephrotoxicity in Male Rats

Ofem Effiom Eteng[1*], Nseobong Bassey[2], Eteng Ibiang Eteng[3], Ekam Patrick Okwe[3], Grace Ekpo[2], Victor Ekam[2], Eyong Ubana[2]

1Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
2Department of Biochemistry, University of Calabar, Cross River State, Nigeria.
3Collage of Nursing and Midwifery Science Itigidi, Cross River State, Nigeria.

Abstract

Endocrine disruptor chemicals (EDCs) have an external influence that has a detrimental effect on the biological system. Morin and vanillic acid has key nephron-pharmacological effects to reduce this effect. We used a rat model to study the impact of Morin and vanillic acid on diethyl phthalate (DEP) and bisphenol S (BPS)- induced nephrotoxicity. After twenty-one days of DEP (50 mg/kg) and BPS (200 mg/kg) exposure and treatment with Morin and vanillic acid (25 and 25 mg/kg), samples were taken for the evaluation of biochemical parameters, including glutathione peroxidase (GPx), glutathione (GSH), catalase activity (CAT), and superoxide dismutase (SOD). Levels of calcium, sodium, urea, and creatinine. Nitric oxide (NO), hydrogen peroxide H_20_2, and malondialdehyde levels (MDA) respectively. The kidney membrane was considerably ($P< 0.05$) preserved by morin and vanillic acid therapy. In a strikingly significant way ($P <0.05$), co-treatment with Morin and vanillic acid reversed DEP+BPS-induced reductions in glutathione levels, CAT, SOD, GPx, and GSH activities, as well as calcium, sodium, urea, and creatinine levels in the kidney, while attenuating DEP+BPS-mediated increases in nephron-oxidative damage markers (MDA, H_20_2, and NO levels). By acting as an antioxidant, scavenger of free radicals, and having nephron-pharmacological effects, morin and vanillic acid in combination at the same acute dosages may prevent DEP and BPS-mediated nephrotoxicity dysfunctions.

Keywords: Oxidative stress, Nephrotoxicity, Morin, Vanillic acid, Diethyl phthalate, Bisphenol S

Corresponding author: Ofem Effiom Eteng
E-mail ✉ ofemeffiom@gmail.com

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Introduction

Morin bio-flavonoids have a range of medicinal properties, such as anti-inflammatory, anti-hepatotoxic, and anti-ulcer actions. Despite the many different bioflavonoids, Morin was one of them to garner widespread notice in nature. Morin has a variety of pharmacological effects, including the ability to scavenge free radicals, the ability to inhibit xanthine oxidase, anti-inflammatory properties [1], the ability to protect DNA from free radical damage, and anticancer properties. Both traditional herbal remedies and diets contain morin hydrate [2].

Morin is thought to be a potential medicinal drug for a variety of ailments, most of which are brought on by free radical damage [3]. Reduced oxidative stress, decreased expression of tumor markers, and prevention of tumor development have all been seen after morin treatment.

Toxicants exposure to biological system poses or triggered free radicals generation. From a chemical standpoint oxidative stress is linked to either a rise in the generation of oxidizing species or a marked decline in the efficiency

of antioxidant defenses like glutathione. The effects of oxidative stress depend on how significant these changes are; a cell can overcome minor disruptions and return to its initial form. However, more extreme oxidative stress may result in necrosis, whereas stronger stressors may result in necrosis. Even mild oxidation can produce apoptosis [4].

Because its administration has been shown to increase the bioavailability of chemotherapeutic medications by blocking P-glycoproteins, morin serves a protective effect in lowering carcinogenic improvement [5]. Morin promotes the production of anti-oxidant proteins such as Catalase, Superoxide dismutase, and Glutathione peroxidase, according to several investigations. Numerous studies have demonstrated that phytochemical substances can protect cell viability by lowering the ROS burden in cells. Studies have shown that flavonoids can block transcription factors or regulatory enzymes that are crucial for regulating inflammatory mediators. Strong antioxidants with the capacity to lessen tissue damage are known as flavonoids.

Using a natural phytochemical substance is a growing method for treating, delaying, or curing illness. The bioactive components from Morin are being used in this most recent study on nephrotoxicity therapy. To demonstrate the impacts of the phytochemicals, the potential and constraints associated with pharmaceutical development are also investigated. This study field examines possible druggability in addition to scientific soundness [6].

The controlled activities of Morin, which function through regulating numerous cell-signaling pathways, are the mechanisms of such inflammatory and anti-oxidant qualities [7].

As a natural phenolic acid component and a benzoic acid derivative, vanillic acid (VA) is utilized in food companies as a flavor, preservative, and additive. It is a kind of vanillin oxide that is created when ferulic acid is transformed from vanillin. The pharmacological effects of VA include an anti-metastatic action [8], anti-melanogenesis [9], antioxidant, anti-angiogenesis [10], and anti-apoptotic effects [11]. Recent research has demonstrated how VA can improve cardiac dysfunction and reduce oxidative stress after ischemia-reperfusion injury [12]. It has been discovered that diethyl phthalate (DEP) has a variety of acute and long-term harmful iinfluences on various species at many trophic places, as well as endocrine-disrupting qualities [13]. DEP may also be thought of as an oily, flavorless material that is utilized to enhance the functionality and longevity of several items [14]. To keep plastic polymers flexible, it is added as a plasticizer. It has been utilized in several goods, such as toys, automobile parts, tool handles, tape, rubber, toothbrushes, and plastic films. BPS is a synthetic organic molecule that is frequently emloyd as a antecedent in materials including epoxy resins and other types of polycarbonate plastic. The industry has adopted bisphenol S (BPS) as a secure substitute for BPA, however, new research has revealed a correlation between various BPS concentrations and oxidative stress [15]. It exists in the environment and can be emitted into the air directly from manufacturing facilities or consumer goods. Due to the widespread use of bisphenols in food and beverage containers, environmental pollution, skin contact with bisphenol-containing products, and food contamination are the main causes of global human exposure [16]. The structure of bodily organs may be significantly altered by chronic exposure to DEP and BPS have lipophilic qualities that allow them to pass through kidney membranes and start the process of creating free radicals that can harm cells [17].

Free radicals, ions, or molecules produced from molecular oxygen are known as ROS. They have a strong tendency to react negatively with biological components such as lipids, proteins, and DNA. Therefore, the purpose of this study was to clarify the mechanisms by which vanillic acid and Morin work in concert to counteract the harmful effects of DEP and BPS-induced nephrotoxicity in the rat model.

Materials and Methods

Chemicals and reagents

Vanillic acid (Cat. No. V 1240), morin (Cat. No. M 2357), and bisphenol S (Cat. No. D 5095) are three examples. Dimethylsulfoxide (DMSO) was acquired from Libertas Laboratory Services Limited, Abeokuta. Diethyl phthalate DEP (Cat. No: D 1785) was purchased from Otto Chemie Pvt Ltd (Mumbai, India). Except as otherwise noted, all other compounds were made by British Drugs House Compounds Limited in Poole, Dorset, England.

Animal care

Male albino experimental animals Wistar rats were inbred in the Animal House, Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria. The rats ranged in weight from 150 g to 200 g. They were kept inside a suspended plastic cage in temperature-controlled (25 °C) rat housing with a typical 12-hour light/12-hour dark cycle. The rats were given regular pellet food and free access to fresh water after a week of acclimatization. According to the guidelines in the "Guide for the Care and Use of Laboratory Animals" created by the National Academy of Science (NAS) and distributed by the National Institutes of Health, all the animals were treated humanely. With approval number BCH/20160914, the organization gave the go-ahead for this trial.

Experimental protocol

A straightforward randomization procedure was used to allocate 25 rats into 5 groups (n = 5) and treat them as shown in **Table 1**. Based on the 3R (replacement, reduction, and refinement) principles, five rats per group were employed [18]. BPS and DEP were mixed and administered at toxicologically relevant doses of 200 and 50 g/kg body weight (b.wt.) based on earlier investigations by [19] and [20]. Vanillic acid and morin were given immediately after the injection of BPS+DEP at doses of 25 and 25 mg/kg b.wt. based on a prior work that shown that morin has antioxidant properties at these dosages [21]. All treatments were administered using dimethyl sulfoxide (DMSO), and the duration was twenty-one (21) days.

Table 1. Grouping of experimental animals and their treatments.

GROUPS (n = 5)	TREATMENT
A	DMSO (0.4% v/v)
B	DEP (50 mg/kg b.wt.) + BPS (200mg /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

Preparation of serum

The animals were slaughtered by anesthesia using Ketamine/Xylazine (100 and 7 mg/kg, respectively) [22] after the experiment, 24 hours after the previous treatment, and were dissected. Plain tubes were used to collect kidney samples. For 10 minutes, the samples were centrifuged at 5000 rpm. The clear supernatant known as the serum was taken out and utilized for the biochemical assessment.

In an erythrocyte lysate that had been properly diluted, antioxidant levels were found.

Antioxidants and oxidative stress markers

Post-nuclear supernatants from the homogenized (10% w/v) kidney organs were utilized in the following tests using a buffer (pH 7.4).

Catalase, excess hydrogen peroxide (H_2O_2) was reacted with ammonium molybdate to create catalase (CAT) activity, which resulted in a yellow complex that could be measured at 405 nm [23].

According to Glutathione Peroxidase (GPx) test, excess glutathione reacts with DTNB to generate a compound that absorbs at 412 nm [24].

The amount of reduced glutathione (GSH) was measured using the procedure described in [25].

Observing the suppression of pyrogallol auto-oxidation at 420 nm served as the basis for the superoxide dismutase (SOD) experiment, which was previously described [26].

Nitric oxide (NO) and H_2O_2 production were quantified using the techniques described in [27] and [28], respectively.

According to the technique of [29], the oxidative breakdown of lipids and proteins was measured as malondialdehyde (MDA), and MDA was identified as thiobarbituric acid reactive substances (TBARS).

Using Bethlot Searcy's approach [30], the levels of calcium, sodium, urea, and creatinine were measured to assess nephrotoxicity. According to the directions on the diagnostic kits that were acquired from Randox labs (UK), the levels of sodium ions (Na+) and calcium ions (Ca+) were measured using a direct spectrophotometric technique to determine [31].

Histopathological examination

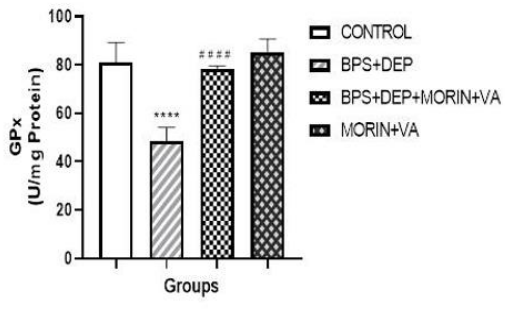
For histological investigation, kidney samples were maintained in a 10% neutral formalin solution. eosin with hematoxylin (H&E) were used to stain 5-mm slices of liver tissues that had been fixed in paraffin wax.

Statistical analysis

The data were shown as the mean and SEM for each group. The homogeneity of the groups was assessed using Analysis of Variance (ANOVA). If there was heterogeneity, the groups were separated using the Duncan Multiple Range Test (DMRT). A 0.05 p-value was deemed statistically significant. All statistics were performed using SPSS (Statistical Package for Social Sciences) software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). The graphs were made using Graph Pad Prism 8 Software (Graph Pad Software Inc., San Diego, USA).

Results and Discussion

Figure 1 depicts how exposure to diethyl phthalate and bisphenol S affected the levels of glutathione (GSH) in the kidney. When compared to the control group, the kidney GSH concentration in the DEP+BPS-exposed group decreased significantly ($p< 0.05$). As opposed to the DEP + BPS group, exposed groups treated with 25 and 25 mg/kg of Morin and vanillic acid, respectively, had a substantial rise ($p< 0.05$) in kidney GSH concentration. In addition, the kidney GSH concentration was significantly lowered ($p < 0.05$) in the group receiving Morin and vanillic acid treatment compared to the control group.



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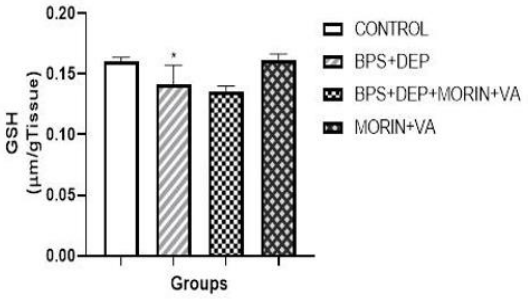

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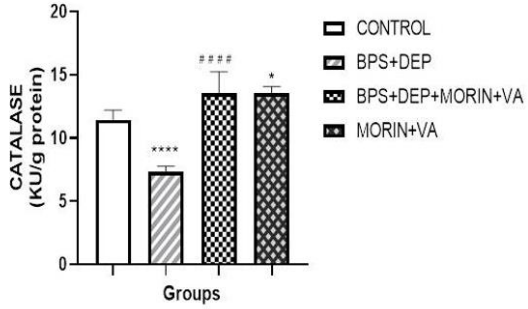

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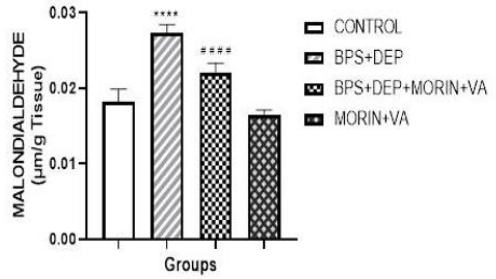
Figure 1. Effect of Morin pretreatment on diethyl phthalate and bisphenol s mediated decrease in antioxidants in rats in the kidney. (a) The activities of glutathione s-transferase (b) the activities of glutathione peroxidase (c) the activities of catalase. Bars represent mean ±SEM (n=5). Bars with different letters are significantly different at P< 0.05.

GPX activity in the kidney was decreased after exposure to DEP and BPS compared to control. However, after treatment with 25 25 mg/kg of Morin and vanillic acid, respectively, there was a substantial increase in kidney glutathione peroxidase (GPX) activity (p< 0.05) in comparison to the DEP+BPS. Furthermore, as compared to the control group, the group that received Morin and vanillic acid had a significantly higher level of kidney glutathione peroxidase (GPX) activity (p <0.05).

Rats exposed to DEP and BEP exhibited kidney SOD activity levels that were considerably lower than the control group (p 0.05). However, there was a significantly increased level of kidney SOD activity in the groups treated with 25 and 25 mg/kg of vanillic acid and morin, respectively, compared to the DEP + BPS group (p 0.05). There was no difference between the group that had Morin and vanillic acid treatment and the control group (p > 0.05).

Similar to this, the kidney CAT was considerably lower in the DEP + BPS-exposed group in comparison to control group (p <0.05). When compared to the DEP + BPS group, the exposed animals managed with 25 and 25 mg/kg of Morin and vanillic acid, correspondingly, had significantly higher kidney CAT activity (p <0.05). However, as in comparison to the control group, there was no discernible difference between the Morin and vanillic acid-treated groups.

The impact of Morin and vanillic acid on indicators of oxidative damage in the kidney of DEP + BPS-exposed rats is shown in **Figure 2.** When compared to the control group, the kidney MDA levels in the DEP + BPS-exposed group increased significantly (p <0.05). The kidney MDA concentration, on the other hand, was significantly reduced under treatment with Morin (25, 25mg/kg), and this effect was dose-dependent. In addition, there was no discernible difference between the Morin and vanillic acid-treated group and the control group.



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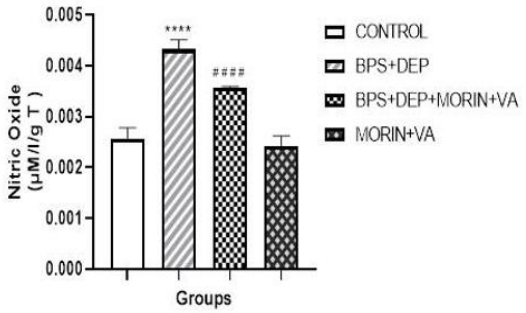

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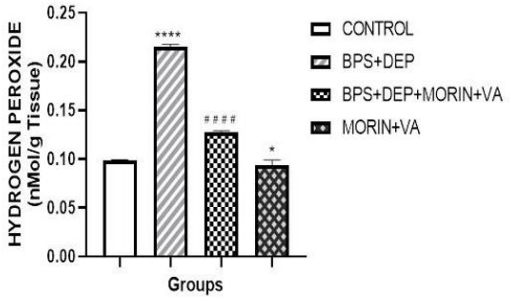

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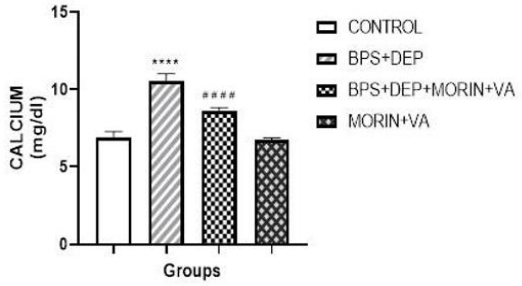
Figure 2. Effects of Morin pretreatment on diethyl phthalate and bisphenol s mediated increase in oxidative stress markers in rats on kidney parameters. (a) MDA level (b) Nitric oxide (c) Hydrogen peroxide. Bars represent mean ±SEM (=5). Bars with different letters are significantly different at P<0.05.

Similar trends were seen between the effects of therapy with vanillic acid and morin and exposure to DEP + BPS on renal NO levels. Although there was a dose-dependent

reduction in reactive nitrogen oxide (i.e., NO) following treatment with Morin and vanillic acid, the kidney NO concentration in the exposed group was considerably lower ($p < 0.05$) than that in the control group. The group that just had Morin therapy, however, did not significantly differ from the control group ($p > 0.05$).

The effects of exposure to DEP+BPS on kidney H_20_2 indicate a similar pattern of substantial increase ($P<0.05$) exposed group compared to the control group. Hydrogen peroxide is triggered treatment with Morin and vanillic acid. When compared to the control group, there was a dose-dependent reduction after treatment with Morin and vanillic acid.

Kidney creatinine, urea, calcium, and sodium levels as a result of exposure to DEP+BPS while comparing to the healthy control group, all electrolyte values are significantly higher ($P< 0.05$) in **Figure 3**. When comparing to the control group, the management usuimg 25 mg/kg of Morin and 25 mg/kg of vanillic acid causes a significant drop in renal electrolytes.



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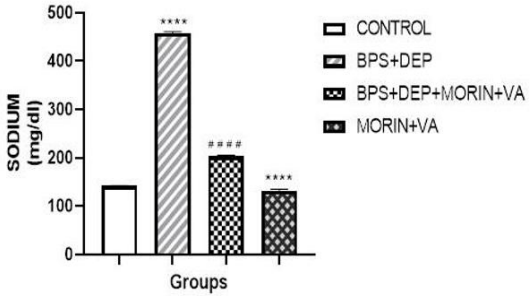

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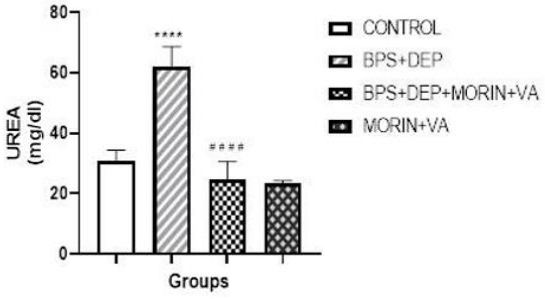

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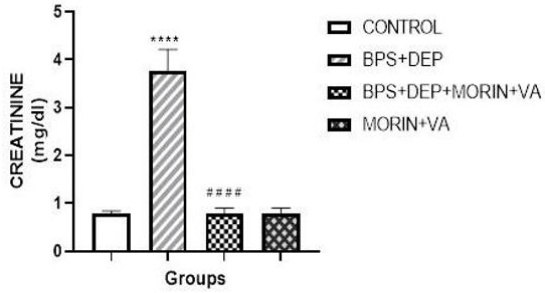
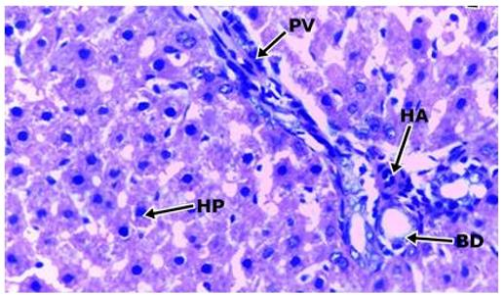


d)

Figure 3. Effect of Morin pretreatment on diethyl phthalate and bisphenol s mediated decrease in kidney function test (a) calcium (b) sodium (c) urea (d) creatinine. Bar represents mean ± SEM (n=5). Bar with different letters is significantly different at $P < 0.05$

The outcomes of the histopathological investigation are shown in **Figure 4**. The segment of kidney tissues from group A (0.4% DMSO) showed that no deaths were seen across all groups throughout this trial. The animals in the control group's kidneys were histologically examined, and the cortical and modular tubules were found to be normal without any evidence of tissue abnormalities, such as crystallization.



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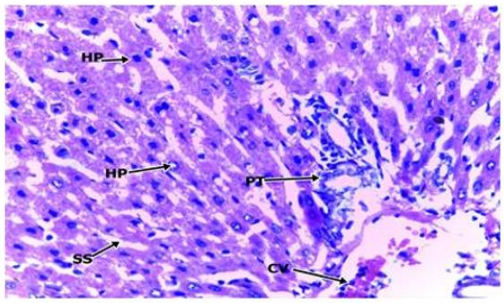

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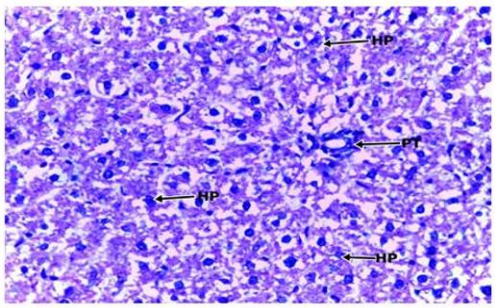

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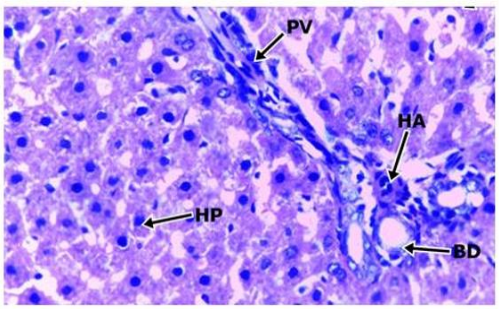

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Histopathological changes

Figure 4. kidney histology of control and DEP + BPS-exposed rats treated with morin. Group A: 0.4% DMSO; GROUP B: DEP + BPS; GROUP C: DEP + BPS + morin (25 mg/kg); GROUP D: DEP + BPS + morin (25 mg/kg and 25 Mg/kg vanillic acid changes in renal cortex tissue of control and experimental groups. H and E, ×400. Control group: renal tissue showed no sign of crystallization. DEP+BPS group: tubular crystals deposits (arrows) with secondary tubular dilatation (arrowhead), epithelial damage, and leukocyte reaction were found. In Morin and vanillic acid-treated rats, renal tissue showed small crystal formation

The subjected rats with 200 mg/kg and 50 mg/kg of DEP+BPS showed degeneration, epithelial damage, leukocyte response, and necrosis in their renal lesions. The proximal tubules, loops of Henle, distal tubules, and collecting ducts all had many crystal deposits (arrows) with secondary tubular dilatation. Treatment with 25 mg/kg each of Morin and vanillic acid brought the control group's renal architecture back to normal. After that, the crystal tubular dilatations were lessened in comparison to the normal control group, and normal tubular and collecting ducts were seen.

The results of the current investigation indicated that morin and vanillic acid had an inhibitory effect on free radical scavenging and antioxidant tendencies. We established that consuming a DEP and BPS combination might cause nephrotoxicity in a rat model. We successfully assessed the impact of vanillic acid and morin on antioxidant and oxidative stress markers. Our findings suggested that morin and vanillic acid may function as specific free radical scavengers, increasing cellular integrity and antioxidants, and minimising oxidative damage of DEP + BPS-mediated nephrotoxicity. Thus, these actions may be the basis for how antioxidants work on rats exposed to DEP and BPS-induced nephrotoxicity. The kidney is the organ most susceptible to biochemical attacks and the main site for the detoxification of foreign substances [32]. The need for long-term chemical detoxification may potentially induce oxidative stress or boost gene expression, which would explain a rise in kidney enzyme levels [33]. While treatment with vanillic acid and Morin induces a change and regulation in the biomarkers and gives a chance for the normal regulation of chemicals, exposure to DEP + BPS causes buildup in the kidney and affects the detoxification of foreign compounds. Glutathione (GSH) has to be present in sufficient quantities for the kidneys to continue operating normally. In the liver, kidney, and intestines, glutathione aids in the detoxification and elimination of poisons and toxins.

The group that got diethyl phthalate and bisphenol S had lower levels of glutathione than the control group (GSH), which indicates that the kidney is vulnerable to the buildup of toxins and other hazardous compounds. A considerable rise was seen after treatment with Morin and Vanillic acid, indicating a control in the parameters' concentration.

GSH is the main non-protein thiol that protects the kidney and its intracellular component against free radical-induced oxidation [34]. GSH can function as a cofactor for enzymes including GST, GPx, GSR, and G6PDH once it has been produced. Thus, a reduction in GST, GPx, GSR, and G6PDH activities might result from GSH depletion [35]. The reductions in GSH caused by DEP and BPS or as a result of their metabolites might be the direct cause of the decreased activities of GSH, GPx, CAT, and SOD shown in this study. All of these endogenous antioxidant defenses have compartmentalized functions that inhibit free radical-mediated necrosis, DNA and RNA fragmentation, protein alteration, and lipid peroxidation of ghost erythrocytes [36]. However, morin and vanillic acid treatment offered protection by reversing declines in GSH, SOD, CAT, and GPx brought on by DEP and BPS. It is possible to achieve this effect by scavenging the reactive metabolites of DEP and BPS, chelating active metal ions, preventing the oxidation of non-transition metals, supplying hydrogen atoms or electrons to stabilize the reactive metabolites and ROS produced to ensure their stability, and detoxifying the toxicants.

By catalysing their dismutation into hydrogen peroxide (H_2O_2) and atomic oxygen (O_2), a protective enzyme known as reactive SOD may eliminate superoxide anion radicals (O_2^-) [36]. The kidney activated a redox enzyme found in nephrons to protect the body from oxidative stress by converting H_2O_2 into water and molecular oxygen (damage from free radicals) [37]. The amount of hydrogen peroxide in the cell rises as CAT and/or GPx activity declines [38]. These findings point to the kidney's diminished antioxidant capability, which may be caused by an excess of free radicals created during the metabolism of DEP and BPS. This condition was improved by boosting the antioxidant defense capability after treatment with Morin and vanillic acid. According to these results, antioxidant enzymes may be better able to scavenge free radicals because Morin and vanillic acid can form stable complexes with the metal ions in those enzymes.

MDA levels **(Figure 2)** indicate oxidative stress in the kidney as a result of an imbalance in the production of free radicals and the reduction of endogenous antioxidants. The later is demonstrated by the increased NO amount we found in the group presented to DEP + BPS. Low NO concentrations enhance kidney filterability, membrane fluidity, and deformability. A higher-than-normal NO level, however, has negative effects on the body, including hypotension, endothelial dysfunction, oxidative stress on the cellular environment, mitochondrial malfunction, and hyperresponsiveness of the airways [39]. In addition, NO easily joins up with O_2, another free radical to create the powerful and stable peroxynitrite radical (OONO-). Following the breakdown of the antioxidant defense system, reactive nitrogen species (RNS), like nitrogen oxide and OONO-, interact with cellular macromolecules like lipids, proteins, and nucleic acids and cause tissue disruption and damage via the production of nitrosative stress. managment with Morin and vanillic acid dramatically reduced the MDA and NO levels in the kidney, indicating that Morin has the potential to scavenge ROS and RNS and inhibit lipid peroxidation [40], protecting the kidney from oxidative cellular damage.

Two amino acids link together in the presence of hydrogen peroxide, activating prostaglandins in the process. This may result in a reduction in blood pressure. A high cellular concentration of hydrogen peroxide (H_2O_2) is known to oxidize the lipids on the biological membrane and hence jeopardize the integrity of the membrane [41]. Hydrogen peroxide is a ubiquitous cellular oxidant that is primarily generated by the peroxisome.

In contrast to the control group, which showed a decrease in blood pressure below the desired level, the group given bisphenol S and diethyl phthalate had a higher level of hydrogen peroxide (H_2O_2), as shown in **Figure 2.** This increases the likelihood of oxidative stress, which leads to oxidative damage. A considerable reduction was seen after treatment with morin and vanillic acid. A kidney function test that measures creatinine serves to regulate the kidneys and facilitates the excretion of waste materials from the body. Since creatinine is an easily quantified consequence of muscle metabolism and is eliminated unaltered by the kidneys, it serves as an essential marker of kidney health [42]. When compared to the control group, the bisphenol S and diethyl phthalate group's elevated creatinine level **(Figure 3)** indicates compromised kidney function or renal disease.

Thus, abnormally high creatinine levels might be a sign of renal disease or dysfunction. When compared to the control, a substantial decline was seen after treatment with Morin and Vanillic acid. When nitrogen is combined with other elements including carbon, hydrogen, and oxygen, urea, a chemical waste product, is created. The circulation carries urea from the liver to the kidney. Urea is filtered out of the blood by healthy kidneys, along with other waste products [43, 44]. When meals high in protein, such meat, poultry, and some vegetables, are digested by the body, urea is formed. Urea is transported by the circulation to the kidneys, where it is eliminated in the form of urine together with water and other wastes.

About a million nephrons, or filtering cells, make up each kidney. A filter termed the glomerulus and a tubule are both parts of a nephron. The glomerulus filters your blood, and the tubule restores necessary chemicals to the circulation while removing waste. This is how the nephrons function.

The kidneys remove wastes and extra fluid from the body. Kidneys also remove acid that is produced by the cells of the body and maintain a healthy balance of water, salts, and minerals—such as sodium, calcium, phosphorus, and potassium—in the blood. Without this balance, nerves, muscles, and other tissues in your body may not work normally.

Wastes and excess fluid are eliminated from the body by the kidneys. The kidneys also eliminate acid that the body's cells create and keep the blood's levels of water, salts, and minerals including sodium, calcium, phosphorus, and potassium in a healthy range. Your body's neurons, muscles, and other tissues could not function properly if this equilibrium is lost.

Therefore, a rise in urea levels in the bisphenol S and diethyl phthalate-treated group **(Figure 3)**, relative to the control group, implies reduced kidney function. Via its antioxidant scavenging activity, a considerable reduction was seen after treatment with morin and vanillic acid.

Sodium supports a healthy fluid balance across the physiological systems. Hypernatremia, or a high amount of sodium in the blood, can cause high blood pressure [45]. As shown in **Figure 3**, excessive sodium secretion in the group receiving bisphenol S and diethyl phthalate hurts glomerular hemodynamics by causing hyper-filtration, raising the filtration fraction, and raising glomerular pressure. On treatment with Morin and vanillic acid, a considerable drop was seen, indicating that his antioxidant system may have favorable regulatory potentials.

Calcium is a mineral that is essential for a healthy lifestyle since it affects how cells and nerves work. A disease in which the parathyroid gland releases too much parathyroid hormone and excessive calcium secretion are both indicative of kidney stones [45]. Calcium secretion levels increased in the group receiving bisphenol S and diethyl phthalate, as indicated in **Figure 3**. When compared to the usual control group, treatment with Morin and Vanillic acid increased the amount of calcium concentration.

Finally, the outcomes of histological examinations offered affirmative proof for biochemical analysis. The kidney tissue of a healthy control animal shows typical renal diseases such as epithelial degradation, degeneration, and modular tubules without any evidence of crystallization. The possible nephroprotective efficacy of pretreatment

with morin and vanillic acid at doses of 25 mg/kg each has been demonstrated, and it has also decreased the degenerative alterations in the kidney **(Figure 4)**. The outcome is consistent with research done by [43] that showed that F. assafoetida has nephroprotective action on CCl4-induced damage in rats.

Conclusion

The study hypothesized that the synergistic impact of Morin and vanillic acid has a broad spectrum of nephron-pharmacological activities and therapeutic potentials, which might be effective as an alternative or supplementary therapy in the management of kidney stones. As a result, its antioxidant activity appears to play a significant role in mediating oxidative damage and nephrotoxicity dysfunctions through its antioxidant, free-radical scavenging action.

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