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Original Article

Antioxidative chemoprotection by *Napoleonaea vogelii* in benzene-induced leukemogenesis and hepatic dysfunction in Wistar Rat

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ABSTRACT

Exposure to benzene (BZ) is a potential risk factor for human health as it leads to hematological disorders and liver damage. This study investigates the efficacy of *Napoleonaea vogelii* (*NV*) against BZ-induced liver injury and onco-hematological initiation in Wistar rats. Rats were intranasally exposed to BZ (400 mg/kg body weight) or co-treated with *NV* (400 mg/kg body weight) for 28 days consecutively. Treatment with *NV* mitigated reduction in PCV, hemoglobin content, red blood cells and restored white blood cell counts altered in BZ exposed rats. It reduced the incidences of anisocytosis, poikilocytosis and blast frequency in blood of BZ-exposed rats. *NV* ameliorated impaired liver function by significantly reduced the activities of transaminases, alkaline phosphatase and bilirubin level relative to BZ-intoxicated rats. BZ exposure also altered redox status through significant reduction in serum total thiol, hepatic activities of superoxide dismutase, catalase and reduced glutathione level with concomitant increase in serum advanced oxidized protein products and hepatic malondialdehyde level relative to control (CTRL). However, co-treatment with *NV* re-established the serum and hepatic antioxidant status. Moreover, the increase in serum levels of proinflammatory markers: TNF-alpha, NF-KB and MPO were observed in rats exposed to BZ relative to CTRL. However, treatment with *NV* proffers abrogative influence on the surge elevation observed in TNF-alpha, NF-KB and MPO in treated rats when compared to the BZ exposed rats. Overall, *NV* protected against oxidative hepatic injury and hematological derangement caused by BZ in rats.

Keywords: benzene, *Napoleonaea vogelii,* liver injury, hematological derangement, oxidative stress

INTRODUCTION

Benzene (BZ) is an organic solvent that constitutes a global health threat to individuals as it pollutes both in indoor and outdoor air. The indoor pollution is sourced from the gases released by BZ-containing products such as furniture wax, paints and detergents while it is also commonly found in vehicle exhaust, industrial emissions, cigarette and cigarette smoke [1, 2]. Human exposure to high BZ level is prevalent due to generation of high BZ level from tobacco products such as cigarettes, pipe tobacco and water pipes [3]. One of the major routes of exposure is through contaminated air in oil refining and chemical manufacturing industries [4]. BZ-induced hematotoxicity is associated with activated signaling pathway mediated by oxidative stress in bone marrow cells [5]. The induction of toxicity by BZ is due to its

Table 1. Animal grouping and treatments

Experimental groups	Treatment and duration
CTRL	Received feed and water only.
BZ alone	Received BZ (400 mg/kg body weight) by intranasal administration every day for four weeks.
BZ + *NV*	BZ (400 mg/kg body weight) and *NV* (400 mg/kg body weight) every day for four weeks.
NV alone	*NV* (400 mg/kg body weight) every day for four weeks.

bioactivation that led to increased production of reactive oxygen species (ROS) [6, 7]. The hematotoxicity by BZ is also linked to initiation of immune oxidative stress responses that is triggered by the production of ROS [8, 9]. BZ and its metabolite were reported to induce ROS in leukemic line HL-60 cells that resulted in myelotoxicity [10, 11]. Also, exposure to BZ was reported to cause aberrant gene alteration [12]. The suppression of hematopoietic cells by BZ exposure has been associated with abnormal gene expression profiles that are involved in inflammation and DNA damage selected signal pathways [9]. Research using animal models supported the evidence that exposure to BZ can cause damage to hematopoietic system and liver organ [13]. Hepatotoxicity by BZ was associated with its metabolic transformation product that generates oxidative stress [14]. The metabolic products of BZ form covalent adducts with biological molecules in the blood and livers such as proteins and nucleic acids in liver, kidney, spleen, and blood and thus effected its toxicity [15]. Thus, the review on increase in BZ-related health issues necessitates innovative solutions.

Ethnobotanical survey for therapeutic usage of indigenous plants in combating various ailments due to environmental contaminant may be a reliable source of solution [16]. *Napoleonaea vogelii* (*NV*) belongs to leccythidaceae family and can also be found in West African countries such as Nigeria [17]. It is a valuable medicinal plant with health benefits including pain relief, fever reduction, and digestive support [18]. It possesses antidiabetic [19], anti-asthmatic and cytotoxic effects in vitro [20]. The interest on *NV* is due to its diverse medicinal properties resulted from the presence of various bioactive compounds like alkaloids, flavonoids and terpenoids [21, 22].

Similar plant has demonstrated potent antioxidant activity and cytotoxic potential on laryngeal cancer cells, rhabdomyosarcoma, breast cancer cell and cervical carcinoma lines [23]. Despite its distinct biological properties, there is little or no information on efficiency of *NV* on BZ-induced hematological alteration and liver injury. This study aims to investigate the hepatoprotective and hematopoietic potential of *NV* aqueous leaf extract in a BZ-induced toxicity in Wistar rats.

MATERIALS AND METHODS

Preparation of *Napoleonaea Vogelii* Extract

Fresh back of *NV* plants were harvested from swampy areas in Ogbomosho, thoroughly washed with tap water, cut in pieces and dried. The dried barks were ground into powder using mechanical grinder and powdered sample was extracted in 80% methanolic by cold maceration.

Chemicals and Reagents

1-chloro-2, 4-dinitrobenzene (CDNB), glutathione (GSH), 5',5'-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), epinephrine and hydrogen peroxide were purchased from Sigma® Chemical Company (London, UK). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were obtained from Fortress Diagnostics Ltd., Antrim, UK. The extracted *NV* was obtained from department of biochemistry, University of Ibadan, Ibadan, Oyo State, Nigeria. Chemicals and reagents used in this study were of analytically standard grade and got from British Drug House Poole UK.

Experimental Animals

Twenty-four albino rats weighing between 120-180 g were purchased from Temmy farms, Ogbomosho, Oyo State, Nigeria. The animals were acclimatized to laboratory conditions preceding the start of the study. The rats were contained in wire-meshed cages and provided with food and water ad libitum. They were housed at normal conditions of temperature and humidity and fed with commercial rat diet (Ladokun® Feeds, Nigeria Ltd., Ibadan, Oyo State, Nigeria).

Drug Treatment and Animal Grouping

This investigation utilized a straightforward randomized design. Following a two-week acclimation period, animals were randomly distributed into four primary experimental groups, each consisting of six subjects. The dosages of the *NV* extract and BZ were meticulously selected based on a thorough literature review [24, 25]. BZ was administered by intranasal while *NV* extract was orally administered daily for four week as outlined in **Table 1**.

Collection of Blood and Liver Samples

Twenty-four hours after the final treatment, animals received an intraperitoneal injection of 0.04% colchicine (1 mL per 100 grams of body weight) two hours before being sacrificed. Blood samples were collected via retro-orbital plexus into ethylenediamine tetraacetate bottle for hematological assessment and lithium heparinized tubes for biochemical assays, followed by sacrifice. Subsequently, livers were excised to assess enzymatic and non-enzymatic antioxidants.

Hematological assessment

Hematological analysis was carried out using blood automatic analyzer to determine hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cells (RBCs), white blood cells (WBCs), lymphocytes, monocytes, granulocytes (Gran), and platelets (PLT).

Determination of Plasma and Liver Protein Content

Protein concentrations in plasma and liver homogenate were determined using the Biuret method described by [26]. The protein levels in the samples were then calculated by referencing the standard curve generated from bovine serum albumin.

Determination of Biomarkers of Hepatotoxicity

Plasma AST, ALT, alkaline phosphatase (ALP), and bilirubin levels were determined using established methods. AST and ALT activities were measured based on the principle described by [27] and ALP activity was determined by monitoring p-nitrophenol formation at 405 nm, based on the principle described by [28]. Bilirubin levels were measured using the dimethyl sulfoxide method described by [29].

Assessment of Plasma Oxidative Stress Levels

Plasma advanced oxidation protein products (AOPP) was determined by a modified version of the method described by [30]. Briefly, 100 µl of plasma was incorporated into 400 µl of phosphate buffer saline (PBS) solution, followed by the subsequent addition of 25 µl of 1.16 M potassium iodide and, 2 minutes later, 50 µl of acetic acid. The absorbance of the combined solution was immediately measured at 340 nm against a blank solution containing 500 µl of PBS, 25 µl of 1.16 M potassium iodide, and 50 µl of acetic acid. Plasma total thiol was measured spectrophotometrically using DTNB (2,2′-dinitro 5,5′-dithiodibenzoic acid) [31].

Assay of Hepatic Antioxidant Enzymes

Reduced GSH levels were quantified using the method proposed by [32], which involves a colorimetric reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) that absorbs at 412 nm. SOD activity was assessed by [33] while catalase activity was evaluated using Singha method [34].

Assessment of Lipid Peroxidation in Liver Homogenates

Malonaldehyde (MDA) levels in liver tissue were quantified using a TBA assay, as described by [35]. Liver homogenates were reacted with TBA, resulting in a colored complex that absorbs at 532 nm. The absorbance was measured spectrophotometrically, and MDA concentrations were calculated using a standard curve, with results expressed as µmol/mg liver tissue.

Assessment of Pro-Inflammatory Cytokines and Enzymes: TNF-Alpha, NF-kB, and MPO

TNF-alpha, NF-kB, and MPO levels in liver tissue were quantified using ELISA and spectrophotometric assays. TNF-alpha and NF-kB were measured using Sandwich-ELISA kits, with optical density values proportional to their concentrations, measured at 450 nm. MPO activity was determined spectrophotometrically using O-dianisidine and hydrogen peroxide, with optical density values measured at 470 nm. The assays were performed according to the manufacturers' instructions and the method of [36].

RESULTS

Influence of *Napoleonaea Vogelii* on Hematological Parameters in Benzene Induced Hematological Derangement in Wistar Rats

Table 2 demonstrates that *NV* treatment mitigates BZ-induced hematological alterations, with enhanced PCV, HGB, and RBC counts and improves WBC count as comparable to BZ group values.

Hepatoprotective Effects of *Napoleonaea Vogelii* on Alteration of Liver Damage Biomarkers in Benzene-Induced Hematological Derangement in Wistar Rats

Table 3 shows liver function tests in Wistar rats exposed to BZ and treated with *NV*. The activities of ALT, ALP, and AST were significantly increased ($p < 0.05$) following BZ administration of 39.90%, 30.61% and 27.70, respectively. Similar trend was observed for bilirubin level where bilirubin level was increased by 54.60%. however, Co-administration of *NV* with BZ effectively restored these liver function markers to near-control (CTRL) levels, However, cotreatment

Table 2. Comparison of hematological parameters between CTRL and other groups

Groups	WBC (10×9/L)	Lym (10×9/L)	Gran (10×9/L)	RBC (10×12/L)	HGB (g/dL)	HCT (%)	MCH (pg)	PLT (10×3/uL)
CTRL	7.43 ± 1.32	6.41 ± 1.44	0.47 ± 0.73	7.93 ± 0.35	16.24 ± 0.38	46.50 ± 1.12	20.48 ± 0.73	733.00 ± 228.02
BZ	3.96 ± 0.49	3.39 ± 0.80	0.07 ± 0.04	7.54 ± 0.34	15.90 ± 1.17	47.62 ± 2.53	19.38 ± 0.50	810.00 ± 146.30
BZ + *NV*	4.59 ± 0.96	3.72 ± 0.53	0.20 ± 0.12	7.79 ± 0.44	16.60 ± 0.76	46.12 ± 3.12	19.88 ± 0.75	739.80 ± 133.43
NV	5.22 ± 1.94	5.16 ± 0.56	0.32 ± 0.30	8.38 ± 0.41	15.84 ± 1.15	46.38 ± 3.06	20.46 ± 0.47	831.80 ± 85.72

Note. Values are mean ± standard deviation

Table 3. Protective effects of changes in the plasma concentration of ALT, AST, ALP, and bilirubin in BZ group rats

Groups	ALT(U/L)	ALP (nmol/min/mg protein)	AST (U/L)	Bilirubin (µmol/l)
CTRL	20.00 ± 2.65	43.30 ± 2.75	31.48 ± 3.83	3.15 ± 0.99
BZ	27.98 ± 2.25*	59.15 ± 5.19*	40.24 ± 3.55*	4.87 ± 0.67*
BZ + *NV*	22.18 ± 2.87#	45.52 ± 2.66#	28.99 ± 3.43#	3.05 ± 0.43#
NV	20.89 ± 2.82	45.30 ± 4.04	29.20 ± 4.25	2.81 ± 0.57

Note. Data are expressed as mean ± standard deviation for six rats in each group; *Significantly different from the CTRL ($p < 0.05$); & #Significantly different from BZ group

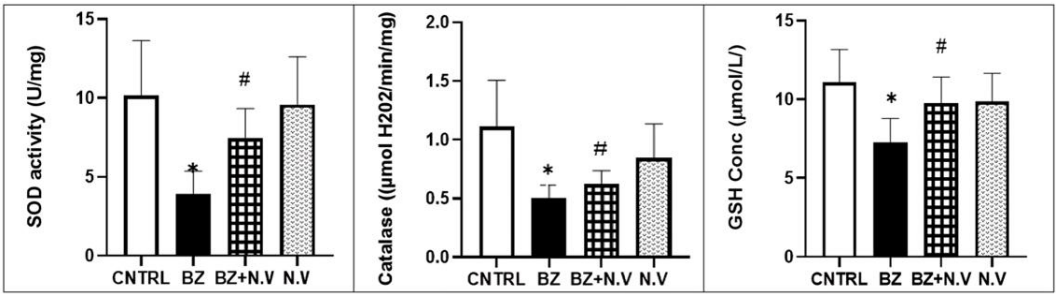

Figure 1. Protective effect of *NV* co-treatment against BZ-induced changes in hepatic biomarkers of antioxidant status in rats (data are expressed as mean ± standard deviation for six rats in each group; *significantly different from the CTRL ($p < 0.05$); & #significantly different from BZ group) (Source: Authors' own elaboration)

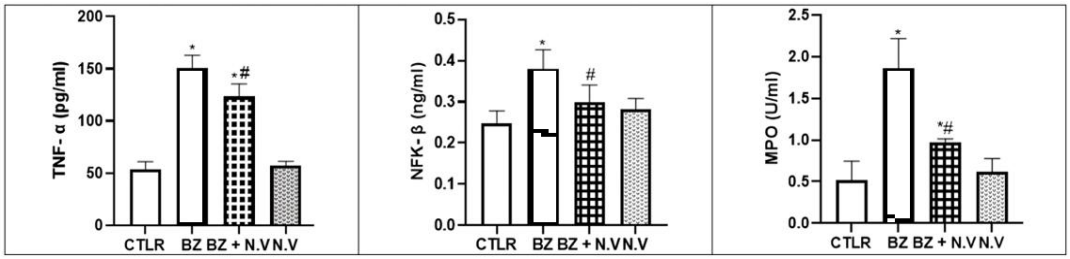

Figure 2. Effects of *NV* on hepatic inflammatory biomarkers in BZ-induced hematological derangement in Wistar rats (data are expressed as mean ± standard deviation for six rats in each group; *significantly different from the CTRL ($p < 0.05$); & #significantly different from BZ group) (Source: Authors' own elaboration)

with *NV* significantly ameliorated the BZ-induced increase in ALT, ALP, AST, and bilirubin in rats.

Napoleonaea Vogelii Treatment Protects Against BZ-Induced Alteration in Redox Status in the Liver of Rats

Figure 1 shows hepatic antioxidant status assays in Wistar rats exposed to BZ and treated with *NV*. BZ significantly decreased the activities of catalase, superoxide dismutase (SOD), and GSH level in liver tissue. However, co-administration with *NV* significantly restored these antioxidant markers, suggesting a protective effect against BZ-induced oxidative stress.

Anti-Inflammatory Effects of *Napoleonaea Vogelii* on Benzene-Induced Inflammation in Wistar Rats

Figure 2 shows inflammatory biomarker levels in Wistar rats exposed to BZ and treated with *NV*. BZ significantly increased plasma levels of nuclear factor kappa β (NFK-β), tumor necrosis factor-alpha (TNF-α), and myeloperoxidase (MPO) activity. Co-administration of *NV* with BZ significantly reduced these inflammatory markers, suggesting a protective effect against BZ-induced inflammation.

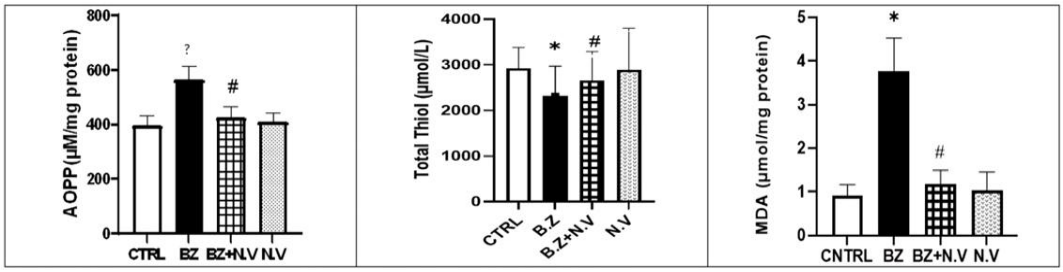

Figure 3. Effects of *NV* on oxidative stress markers in BZ-induced hematological derangement in Wistar rats (data are expressed as mean ± standard deviation for six rats in each group; *significantly different from the CTRL ($p < 0.05$); & #significantly different from BZ group) (Source: Authors' own elaboration)

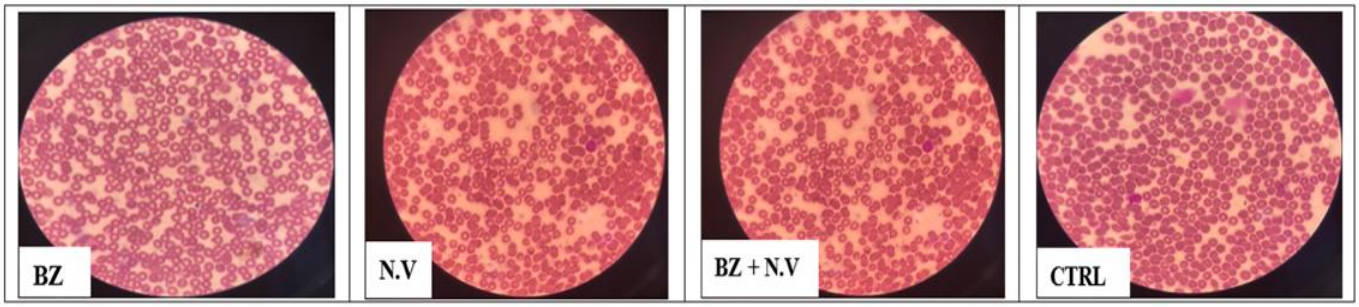

Figure 4. Blood morphology of representative of animals exposed to BZ and *NV* treatment (Source: Authors' own elaboration)

Influence of *Napoleonaea Vogelii* on Plasma Oxidative Stress Markers in Benzene-Exposed Rats

Figure 3 shows the results of oxidative stress biomarkers in Wistar rats exposed to BZ and treated with *NV*. BZ significantly decreased ($p < 0.05$) total thiol levels and concomitantly increased AOPP in plasma. However, co-administration with *NV* significantly raised the level of total thiol content and reduced the concentration of AOPP when compared to BZ group.

Moreover, the hepatic MDA content rose significantly ($p < 0.05$) in the BZ-treated rats when compared with the CTRL. However, *NV* treatment attenuated the increase in liver MDA when compared with the BZ-treated group.

Influence of *Napoleonaea Vogelii* in Benzene-Induced Blood Morphology

Figure 4 shows blood morphology of representative animals exposed to BZ and *NV* treatment. CTLR showed a normal blood cell profile, serving as a baseline for comparison. In contrast, BZ group exhibited abnormal blood cell morphology, characterized by the presence of blast cells, anisocytosis (unequal cell size), and poikilocytosis (irregular cell shape). BZ + *NV* showed a mixed response, with both normal blood cells and abnormalities, including anisocytosis and poikilocytosis, indicating a potential mitigating effect of *NV*. *NV* displayed a normal blood cell profile, similar to the CTRL group, suggesting that the plant itself is not toxic on blood cell morphology.

DISCUSSION

BZ is a common industrial chemical which is long-term exposure to it has been linked to leukemia and other blood disorders [37, 38]. The liver is particularly susceptible to BZ damage as breakdown metabolites of BZ lead to oxidative stress and DNA damage in the liver [39, 40]. The widespread exposure to BZ necessitates the urgent need for effective prevention strategies. Natural products have shown promise in therapeutic efficiency [41]. One of such plants is *NV* that has demonstrated antioxidant, anti-inflammatory and antimicrobial properties making it an attractive candidate for investigation [18, 23]. This study provides crucial insights into the mechanistic potential of protective strategies of *NV* against BZ-induced liver damage and hematological alteration in Wistar rats.

BZ exposure poses significant health risks due to its hepatotoxic and hematotoxic properties [42]. Notably, our results show significant differences in hematological parameters across CTRL and treatment groups. The CTRL group consistently demonstrates the highest counts for WBCs, lymphocytes, and Gran (**Table 2**). These cells are vital for immune function, and their reduction in treatment groups, particularly in the BZ group, suggests immunosuppressive effects of BZ [43]. This reduction can impair immune function and increase infection risk. In addition to its effects on immune cells, administration of BZ also affects RBC parameters while CTRL group maintains relatively high RBC counts. These changes mirror HGB and

HCT values that are closely tied to RBC function. BZ is known to affect erythropoiesis and potentially has impact on RBC production and lifespan. Interestingly, the group of animals that were co-administered with NV shows a significant increase in RBC count, suggesting *NV* may stimulate erythropoiesis. Moreover, mean cell HGB levels decrease across all treatment groups, with the most pronounced reduction in the BZ group. This might indicate altered HGB synthesis or RBC maturation. Such changes could affect oxygen-carrying efficiency and therefore associates BZ with heme synthesis [44]. Blood morphology analysis revealed distinct differences among treatment groups and CTRL. It has earlier been shown in our laboratory that rats exposed to BZ exhibited some morphological alterations in blood cells [45]. The BZ group showed some levels of morphological abnormalities, including abundant anisocytosis, poikilocytosis, and blast cells. Notably, co-administration of NV in BZ-exposed rats substantially reduced these abnormalities.

The elevation of liver function markers (ALT, AST, ALP, and bilirubin), as shown in **Table 3**, in BZ-exposed rats is indicative of hepatocellular damage and compromised liver function. The observed elevation of liver biomarkers due to exposure to BZ from this present research agrees with earlier observations of [46] highlighting the adverse effects of occupational BZ exposure on enzyme function. Liver damage severity is often evaluated by measuring serum transaminase (ALT and AST) levels. ALT and AST are involved in the catalysis that produce glutamate and oxaloacetate from alanine or aspartate, respectively where they transfer amino groups to α-ketoglutarate [47]. Their elevation in the serum is a clear sign of liver damage because it is consequent to hepatocyte destruction that releases its contents including ALT and AST into the extracellular space [48]. ALT is a specific liver function biomarker because of its predominant occurrence in the liver though it can also be found in other organs in minute quantities [49]. Though AST is found in multiple organs making it less specific but plasma elevation in AST has also been reported in conditions involving necrosis of hepatocytes [50]. Alkaline phosphates are produced by several parts of the body, especially the liver and its plasma elevation is also attributed to conditions such as hepatobiliary diseases [51]. The metabolic product from BZ induces lipid peroxidation in hepatic cells, compromising cellular membrane integrity and causing a significant rise in serum hepatic enzymes [52]. In our study, the BZ treated group exhibited significantly elevated plasma ALT, ALP, and AST activities compared to the CTRL group (**Table 3**). The protective efficacy of NV treatment is reflected in its ability to lower the activities of these biomarker enzymes. However, the co-administration of *NV* with BZ significantly attenuated the elevation of these liver function markers relative to BZ exclusively exposed rats, suggesting its potential to mitigate hepatocellular damage.

SOD and catalase are key antioxidant enzymes that protect cells from oxidative damage [53]. SOD converts harmful superoxide radicals into hydrogen peroxide, serving as the first line of defense against oxidative stress [54]. Catalase then breaks down this hydrogen peroxide into water and oxygen [55]. SOD is found in various cellular compartments, while catalase is primarily located in peroxisomes [56]. Together, these enzymes form a critical defense system, neutralizing ROS and maintaining cellular health [57]. Their activities are often used as indicators of oxidative stress in biological systems, playing crucial roles in aging and disease processes. As illustrated in **Figure 1**, BZ exposure caused adverse effects on antioxidant enzyme activities in rats. As expected, BZ exposure (400 mg/kg body weight, 28 days) led to decreased catalase and SOD activities in rats exposed to BZ. This finding supports the existing evidence that BZ metabolites can inactivate antioxidant enzymes, as demonstrated by [58], which suggested that decreases in catalase activity levels in livers of rats fed brominated BZ compounds could result from enzyme inactivation by specific BZ metabolites. However, co-administration of *NV* with BZ significantly restored catalase and SOD activities, suggesting its potential to bolster antioxidant defenses.

GSH is a tripeptide composed of glutamate, cysteine, and glycine. It is the most abundant non-protein thiol in cells and is particularly concentrated in the liver, which is the primary site of GSH synthesis [59]. GSH levels serve as a marker of oxidative stress and cellular health, especially in toxicological studies [60]. Consistent with these enzyme alterations, BZ exposure also led to a marked decline in GSH levels, as shown in **Figure 1**. The GSH depletion observed herein corroborates previous research on BZ toxicity and signifies a compromised antioxidant defense system. This reduction can precipitate oxidation of critical sulfhydryl-containing proteins, further exacerbating oxidative stress [61]. However, the combined administration of *NV* with BZ significantly mitigated this GSH depletion, suggesting a potential hepatoprotective effect.

The intricate relationship between MPO, TNF-α, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) plays an important role in modulating inflammation and immune responses. MPO, predominantly localized in neutrophils, generates hypochlorous acid to combat

pathogens, but its unchecked activity can contribute to tissue damage [62]. TNF-α, a potent pro-inflammatory cytokine, initiates immune responses and can induce apoptosis [63]. Furthermore, NF-κB, a transcription factor, regulates genes involved in inflammation, immune response, and cell survival [64]. These molecules interact synergistically, with TNF-α activating NF-κB, which, in turn, can upregulate MPO expression, forming a complex network of inflammatory signaling [65]. The result of this work (**Figure 2**) demonstrates that BZ exposure triggers an inflammatory response, evidenced by elevated levels of MPO, TNF-α, and NF-κB. However, co-administration of *NV* with BZ significantly mitigated these inflammatory markers, suggesting its potential as an anti-inflammatory agent.

Total thiol, MDA, and AOPP are key biomarkers in assessing oxidative stress and cellular damage [66], total thiols, comprising protein-bound and free sulfhydryl groups, are crucial for maintaining cellular redox balance [67]. MDA, a byproduct of lipid peroxidation, indicates oxidative damage to cell membranes [68]. AOPP, formed by protein oxidation, reflects protein damage due to oxidative stress [30]. The decrease in total thiol levels, as depicted in **Figure 3**, observed in the BZ group substantiates the GSH findings and furnishes additional evidence of oxidative stress. Diminished levels of protein thiols have been positively correlated with elevated lipid peroxides and AOPP in earlier studies [69]. The present study revealed the significantly increased AOPP levels as well as heightened lipid peroxidation indicated by elevated MDA levels, as shown in **Figure 3**, in BZ-exposed rats. Very importantly when *NV* was given alongside BZ, it led to a significant decrease in AOPP and MDA levels, suggesting its ability to counteract oxidative stress and related damage to both protein and lipid biomolecules.

CONCLUSION

BZ initiated leukemogenesis and induced hepatic damage through oxidative stress and inflammatory mechanisms. However, *NV* offers alleviative influence on hematological disturbance and liver dysfunctions caused by BZ exposure in Wistar rats. This may be due to its antioxidant potential and anti-inflammatory efficacy.

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AI statement: The authors stated that no generative AI or AI assisted tools were used during the study.

Declaration of interest: The authors declare no competing interest.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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