



Research Article

Mitigating methylparaben hepatotoxicity: Resveratrol's role in oxidative stress, inflammation, and apoptotic modulation

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ABSTRACT

Methylparaben (MP) is a widely used preservative in various industrial products, but its accumulation in tissues raises health concerns, including oxidative damage to vital organs like the liver. This study explores MP-induced hepatic injury and evaluates the potential hepatoprotective effects of resveratrol (RES) in a rat model, focusing on biomarkers of liver function, oxidative stress, inflammation, and apoptosis.

Rats were administered MP (200 mg/kg) alone or in combination with RES (20 mg/kg) daily for 15 consecutive days (n=8/group). Liver injury was assessed through biochemical analysis of liver function biomarkers (albumin, total protein, aspartate aminotransferase, and alanine aminotransferase), histopathological examination, and measurement of oxidative stress, inflammatory markers, and apoptotic markers.

Exposure to MP led to significant hepatic oxidative stress, as evidenced by dysregulated oxidative and antioxidant markers, increased inflammatory cytokines (tumor necrosis factor- α and interleukin-6), elevated apoptotic markers (the associated protein X and Caspase-3), and impaired liver function. This impairment was indicated by decreased serum albumin and total protein levels in the liver, along with elevated serum activities of aspartate aminotransferase and alanine aminotransferase. Histopathology revealed structural liver damage. Co-administration of RES markedly attenuated these effects, restoring oxidative balance, reducing inflammation, apoptosis, and improving liver function and histological architecture.

This study is the first to demonstrate that RES confers protective effects against MP-induced hepatotoxicity by modulating oxidative stress, inflammatory and apoptotic responses, and preserving liver function and tissue integrity. These findings highlight the therapeutic potential of RES in mitigating the adverse effects of MP and contribute valuable insights into paraben toxicology and risk assessment.

1. Introduction

Parabens, esters of p-hydroxybenzoic acid, are widely used preservatives due to their antibacterial and antifungal properties, stability, solubility, low cost, and minimal impact on texture or scent. They are found in pharmaceuticals, food, and personal care products and can enter the body via skin, ingestion, or inhalation, accumulating in blood and tissues (Chatterjee *et al.*, 2024). Parabens with shorter alkyl chains exhibit increased water solubility and enhanced skin-penetrating capability. Thus, methylparaben (MP) shows the highest water solubility and skin penetration capability among parabens (Chatterjee *et al.*, 2024). It is widely detected in environmental samples, including all water and sediment, and in around 90% of plants, fish, and invertebrates (Oliveira *et al.*, 2020). It is also found in various human specimens (Du *et al.*, 2024).

Studies have linked MP exposure to oxidative stress (Calisir *et al.*, 2025), the induction of reactive oxygen species (ROS), mitochondrial dysfunction, and endoplasmic reticulum (ER) stress (Chatterjee *et al.*, 2024; Du *et al.*, 2024). It is widespread in human populations, detected in over 97% of urine samples in the U.S. and Korea, with higher concentrations observed in females (Calafat *et al.*, 2010; Kang *et al.*,

2016). Elevated MP levels are associated with personal care product use, especially among females, as shown in studies involving Latina adolescents and Chinese populations (Berger *et al.*, 2019; Li *et al.*, 2020). These findings highlight greater paraben exposure in women, primarily from cosmetics and personal care products.

Numerous traditional remedies and natural products are believed to support liver health and protect against various disorders (McGill *et al.*, 2015). Resveratrol (RES), a natural compound and phytoalexin produced by plants in response to injury or stress (Izzo *et al.*, 2021), is one such hepatoprotective agent (Bishayee *et al.*, 2010). It has gained attention for its antiangiogenic, immunomodulatory, psychotropic, antidiabetic, and cardioprotective effects (Izzo *et al.*, 2021). The protective ability of RES against hepatotoxicity is primarily attributed to its antioxidant and anti-inflammatory properties (Jaeschke *et al.*, 2002).

This study addresses a critical gap by investigating the hepatoprotective potential of RES specifically in the context of MP-induced hepatotoxicity. It investigated MP-induced liver toxicity and provides the first comprehensive assessment of the ability of RES to mitigate hepatic injury *in vivo*. The study offers valuable toxicological insights by examining the effects of RES on MP-induced liver injury,

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highlighting its potential as a protective agent against chemical-induced hepatotoxicity. These results lay the groundwork for future investigations focusing on targeted strategies to mitigate the adverse effects of additives such as MP.

2. Materials and Methods

2.1 Animals

Forty mature female Wistar rats (200±20 g) were procured from the Animal Care Center at King Saud University. The rats were maintained in standard cages under regulated environmental conditions (25 ± 1°C temperature, 50 ± 10% humidity, and 12/12 hr light/dark cycles) with unrestricted access to normal rodent food and water. A period of one week for acclimatization was permitted prior to the start of any experimental procedures. All experimental procedures were conducted in strict accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) and received approval from the Committee of Research Ethics (Approval No.: KSU-SE-24-32) on 6 June 2024.

2.2 Experimental design

Rats were randomly assigned to five groups, with eight rats per group. Group I rats served as the control group and received no treatment. Group II rats received daily 1:1 dimethyl sulfoxide (DMSO)–distilled water injections, first subcutaneously in the inguinal region, then intraperitoneally after a one-hour interval (vehicle group). In group III, rats received intraperitoneal injections of RES at a dosage of 20 mg/kg (Gocmez et al., 2019) (RES group). In group IV, MP was administered subcutaneously bilaterally in the inguinal region daily at a dosage of 200 mg/kg (MP group) (Oliveira et al., 2020). Group V rats were given MP (200 mg/kg) one hour before RES (20 mg/kg) (MP+RES group). All treatments were dissolved in a 1:1 ratio of DMSO to distilled water to ensure proper solubility and were administered daily for 15 consecutive days to establish a consistent treatment regimen.

2.3 Biological sample collection

On the sixteenth day, the weight of each rat was recorded to be used for the calculation of relative liver weight. Rats were anesthetized intraperitoneally with 80 mg/kg ketamine and 8 mg/kg xylazine (Gaertner et al., 2008). Blood was collected via cardiac puncture, and serum was separated by centrifugation at 4,000 rpm for 10 minutes, then aliquoted and stored at -20°C. Rats were subsequently sacrificed, and livers were excised, rinsed in cold phosphate buffer saline (PBS), weighed, and documented. Relative liver weight was calculated for each animal using the following equation:

$$\text{Relative liver weight \%} = \left(\frac{\text{liver weight(gram)}}{\text{body weight on the sacrifice day(gram)}} \right) \times 100$$

The liver was divided into two portions: one was fixed in 10% formalin solution to preserve tissue structure for subsequent histopathological examination. The other was homogenized in PBS (10% w/v), centrifuged at 10,000 rpm for 20 minutes at 4°C. The resulting supernatant was then collected and stored at -80°C for subsequent analysis.

2.4 Liver function analyses

Serum alanine aminotransferase (ALT) activity (Beijing Solarbio, China), aspartate aminotransferase (AST) activity (Beijing Solarbio, China), and albumin level (HUMAN Diagnostics Worldwide, Germany) were measured. The total protein level in the liver homogenate was determined using the bicinchoninic acid (BCA) total protein assay Kit (Thermo Fisher, USA). All conducted tests were colorimetric assays. Absorbance readings were taken using a spectrophotometer: 505 nm for ALT and AST, 578 nm for albumin, and 562 nm for total protein, following the manufacturers' protocols.

2.5 Histopathological examination

The fixed liver tissues underwent a series of processing steps, which included dehydration using graded ethanol–xylene solutions, paraffin embedding, and sectioning with a microtome to a thickness of 5 µm (RM 2245, Leica, Germany). The tissue sections were then stained with hematoxylin and eosin (H&E) (Sigma Aldrich, USA), dehydrated, and mounted. Then, histological imaging was performed using an optical microscope (Nikon H550S, Japan). Subsequently, liver sections were evaluated using a histopathological scoring system, ranging from 0 to 3, by an independent specialist investigator blinded to the treatment groups. Steatosis scores were Score 0 (absent, <10%), Score 1 (mild, 10–30%), Score 2 (marked, 31–60%), and Score 3 (severe, >60%); inflammation scores were Score 0 (none), Score 1 (moderate, scattered inflammatory cells), Score 2 (marked, focal inflammation), and Score 3 (severe, diffuse inflammation); necrosis scores were Score 0 (absent, 0%), Score 1 (mild, <10%), Score 2 (marked, 10–50%), and Score 3 (severe, >50%); and fibrosis scores were Score 0 (absent), Score 1 (mild, moderately thickened central lobular vein), Score 2 (marked, markedly thickened central lobular vein), and Score 3 (severe, cirrhosis) (Veteläinen et al., 2006).

2.6 Hepatic oxidative stress status

The levels of malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) activity were measured in the supernatant of liver homogenate. All assay kits were supplied by Beijing Solarbio (China) and utilized colorimetric methods. The optical density was measured using a spectrophotometer at wavelengths of 532 nm and 600 nm for MDA, 412 nm for GSH, and 560 nm for SOD, following the instructions provided with the kits.

2.7 Hepatic inflammatory and apoptotic status

A sandwich enzyme-linked immunosorbent assay (ELISA) was carried out to quantify the levels of various markers in the hepatic tissue supernatant. For the inflammatory assessment, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured. To evaluate apoptosis, the associated protein X (Bax) and Caspase-3 (Cas-3) were analyzed. All ELISA kits were obtained from Fine Biotech (Wuhan, China) and meticulously utilized following the provider's protocols. The absorbance for all assays was measured at 450 nm using a microplate reader.

2.8 Statistical analysis

Multivariate analysis of variance was performed using Minitab 14.12.0, and other statistical analyses were conducted with Prism 10.4.2. Data were expressed as mean ± standard deviation (SD). Normality was assessed with the Kolmogorov-Smirnov test. Differences among the five experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The effect size of each factor within the ANOVA model was analyzed by calculating its eta-squared (η²) values, which are defined as the ratio of the sum of squares for the effect to the total sum of squares. Effect size thresholds were defined as small for 0.01, medium for 0.06, and large for 0.14 or higher (Lakens, 2013). Statistical significance was set at p ≤ 0.05.

3. Results

A multivariate analysis of variance was conducted to examine the effect of treatment group on the combined dependent variables: AST, ALT, albumin, total protein, SOD, MDA, GSH, TNF-α, IL-6, Cas-3, and Bax. The multivariate test statistics indicated a significant overall effect of treatment group on the combined tested markers, Wilks' Lambda = 0.02814, and p < 0.001. This result suggests that the treatment groups differ significantly when considering these dependent variables together.

The effect of RES on MP-induced hepatic injury was examined by assessing relative liver weight and several liver function markers, as

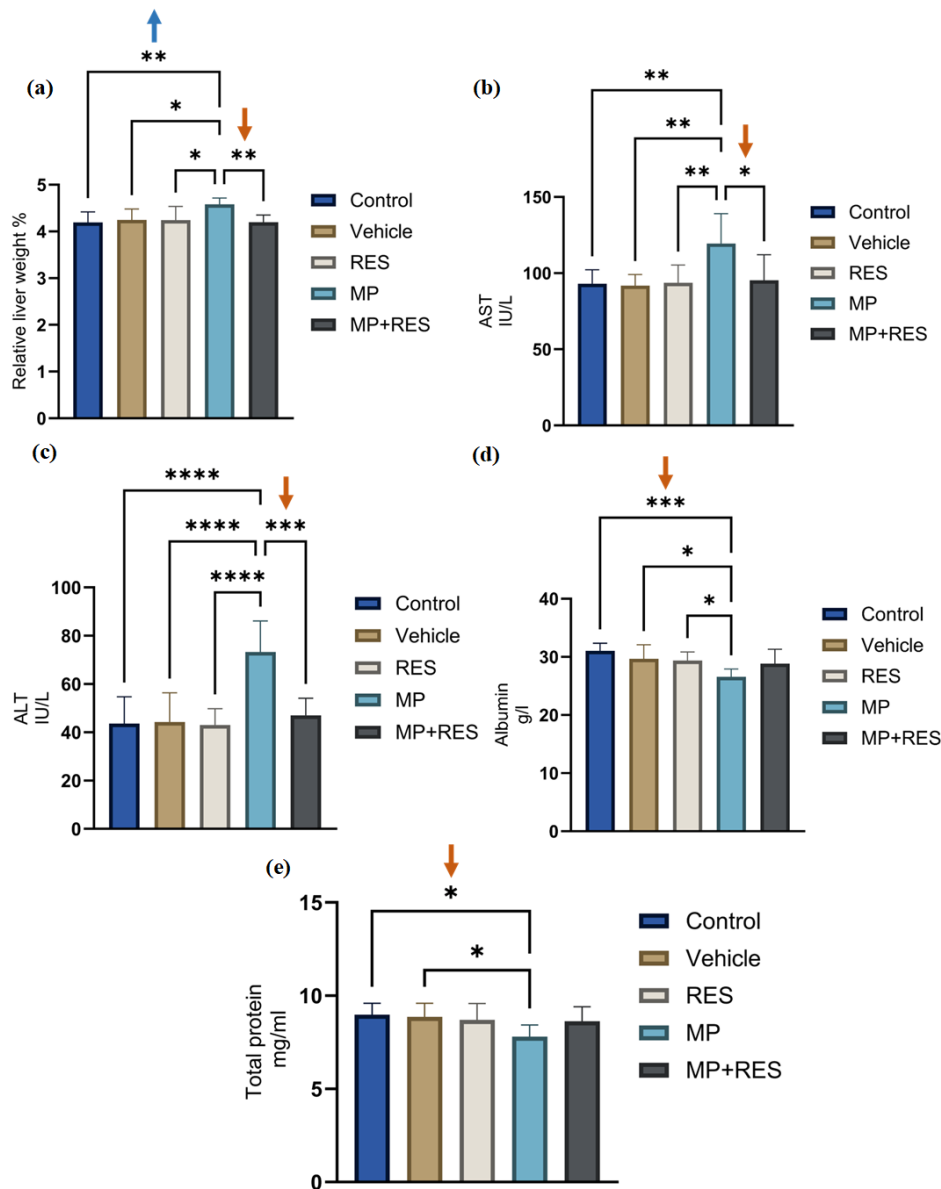


Fig. 1. The effect of RES on hepatic parameters in rats. (a) Relative liver weight was significantly increased in the MP group compared to the control. RES treatment mitigated impaired liver function induced by MP, as shown by significant reductions in serum (b) AST and (c) ALT activities. In (d) Albumin and (e) Total protein, MP exposure caused a significant decrease compared to the control. Data are presented as mean \pm SD (n=8 per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test for all comparisons. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$. Significant increases and decreases are indicated by blue upward (\uparrow) and orange downward (\downarrow) arrows, respectively. RES: resveratrol, MP: methylparaben, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

illustrated in Fig. 1. Only the relative liver weight of the MP-treated group exhibited a substantial increase compared to the control group ($p \leq 0.01$; Fig. 1a). The MP group demonstrated markedly elevated serum activities of AST ($p \leq 0.01$; Fig. 1b) and ALT ($p \leq 0.0001$; Fig. 1c) relative to the control group. Yet, albumin ($p \leq 0.001$; Fig. 1d) and total protein levels ($p \leq 0.05$; Fig. 1e) were significantly reduced in the MP group compared to the control group. Rats administered MP+RES demonstrated a statistically significant reduction in relative liver weight compared to the MP group ($p \leq 0.01$; Fig. 1a) and showed profound mitigation of hepatic function by significantly decreasing the activities of AST ($p \leq 0.05$; Fig. 1b) and ALT ($p \leq 0.001$; Fig. 1c), while also mildly increasing the levels of albumin and total protein compared to the MP group, these changes remained non-significant (Figs. 1d and e, respectively). The eta squared values for relative liver weight, AST, ALT, albumin, and total protein were 0.345, 0.398, 0.593, 0.41, and 0.272, respectively, indicating large effect sizes. A significant portion of the variability in these liver function parameters is explained by differences among the five groups, demonstrating the substantial

impact of RES treatment on mitigating MP-induced toxicity and improving liver health.

Histological staining of the liver was employed to assess the pathological changes induced by MP exposure and their response to RES treatment (Fig. 2). The control group exhibited no pathogenic alterations. It exhibited a typical structure with veins and a network of hepatocyte strands interspersed by blood sinusoids (Fig. 2a). The liver tissue of animals administered RES and the vehicle group exhibited no pathological abnormalities, maintaining a healthy structure similar to the control group (Figs. 2b and c, respectively). Conversely, liver sections from animals subjected to MP exhibited significant accumulation of infiltrative cells (Fig. 2d). The MP+RES group had improved hepatic tissue morphology and nearly alleviated the degenerative changes induced by MP (Fig. 2e). The histopathological score (Table 1) indicates that the control, vehicle, and RES-treated groups exhibited no pathological signs. In contrast, the group treated with MP showed a high pathological score, attributed to marked steatosis and inflammation, although there was only mild necrosis.

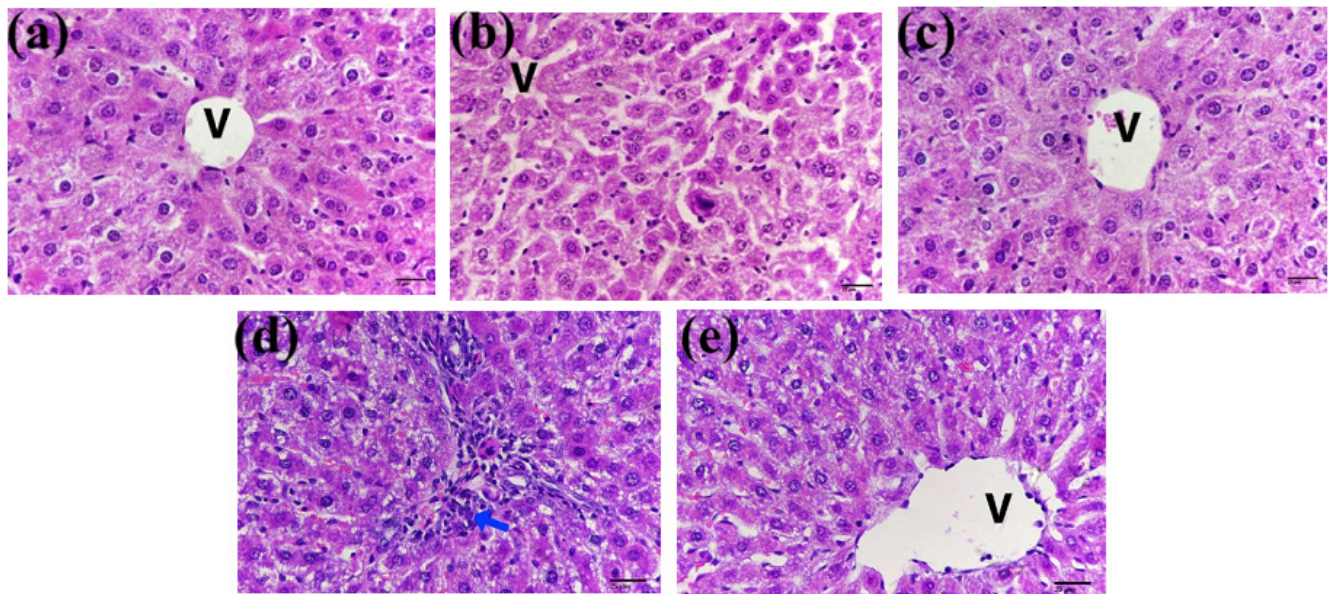


Fig. 2. Photomicrographs of liver sections stained with H&E from all groups. (a) The control rats displayed normal hepatic architecture, characterized by healthy hepatocytes and veins. (b) The vehicle group exhibited no pathological abnormalities. (c) Rats treated with RES showed no pathological features. (d) MP-treated rats presented infiltrative cells accumulation (arrow). (e) MP-RES rats appeared to maintain near-normal hepatic architecture, with healthy veins and hepatocytes. (X400, scale bar 25 μ m). H&E: hematoxylin and eosin, V: vein, RES: resveratrol, MP: methylparaben, MP-RES: methylparaben and resveratrol.

Table 1.

Histopathological scoring of hepatic injury across experimental groups. Hepatic injury was evaluated using a semi-quantitative scoring system (0–3) for steatosis, inflammation, necrosis, and fibrosis. No liver injury was observed in the Control, Vehicle, or RES groups. Marked steatosis and inflammation, along with mild necrosis, were observed in the MP group, while significant improvement was noted in the MP + RES group. Data are presented as observed scores. RES: resveratrol; MP: methylparaben.

Criteria	Control	Vehicle	RES	MP	MP+RES
Steatosis	0	0	0	2	1
Inflammation	0	0	0	2	1
Necrosis	0	0	0	1	0
Fibrosis	0	0	0	0	0
Total	0	0	0	5	2

Furthermore, the group receiving both MP and RES demonstrated a reduced pathological score, characterized by mild steatosis and inflammation, with no necrosis present.

The measured values of parameters related to oxidative stress in the liver tissues of different groups are depicted in Fig. 3. The results suggested that MP significantly decreased the activity of the SOD antioxidant enzyme in liver tissues compared to the control group ($p \leq 0.05$; Fig. 3a). Furthermore, the group administered MP exhibited a significantly elevated level of a liver lipid peroxidation marker (MDA) compared to the control group ($p \leq 0.0001$; Fig. 3b). The rats in the MP + RES group demonstrated a significant elevation in hepatic SOD activity and a significant reduction in hepatic MDA level relative to the MP group ($p \leq 0.001$ and $p \leq 0.0001$, respectively) (Fig. 3a, b, respectively). Despite the GSH level being mildly reduced in the MP-treated group, no significant differences in GSH level were seen among all groups (Fig. 3c). For oxidative stress markers, eta squared values were 0.868 for MDA and 0.489 for SOD, reflecting large effect sizes and indicating that group differences largely explain their variation. These results suggest that RES significantly modulates oxidative stress and alleviates MP toxicity. In contrast, GSH showed a moderate effect size with an eta squared of 0.07.

Two key proteins associated with inflammation were measured. Rats that received only MP exhibited significantly higher levels of the pro-inflammatory markers TNF- α ($p \leq 0.01$; Fig. 4a) and IL-6 ($p \leq$

0.001; Fig. 4b) compared to the control. However, rats that received both MP and RES had a significant reduction in the levels of TNF- α ($p \leq 0.05$; Fig. 4a) and IL-6 ($p \leq 0.05$; Fig. 4b) compared to those that only got MP. Regarding inflammatory markers, eta squared values of 0.422 for TNF- α and 0.49 for IL-6 indicate large effect sizes, with treatments accounting for 42.2% and 49% of the variance, respectively. This supports the significant efficacy of RES in reducing inflammation caused by MP toxicity.

To assess apoptosis, two primary proteins associated with apoptosis were examined. Rats treated only with MP showed significantly elevated levels of Cas-3 ($p \leq 0.001$; Fig. 5a) and Bax ($p \leq 0.001$; Fig. 5b) compared to the control group. Conversely, rats administered both MP and RES demonstrated a significant decrease in Cas-3 ($p \leq 0.05$; Fig. 5a) and Bax ($p \leq 0.05$; Fig. 5b) levels relative to those receiving only MP. In this study with five groups, eta squared values of 0.431 for Cas-3 and 0.544 for Bax also indicate large effect sizes, with treatments explaining 43.1% and 54.4% of the variance. These findings demonstrate the effective role of RES in mitigating MP-induced apoptosis.

4. Discussion

The most commonly used paraben, MP, is noted for its high water solubility and skin penetration (Oliveira et al., 2020). Nutraceuticals such as RES, a plant-derived polyphenol with potent antioxidant properties surpassing vitamin E, has been explored as a safer alternative for disease prevention and therapy (Bishayee et al., 2010). The hepatoprotective effects of RES against various toxic insults have been previously demonstrated (Ahmad and Ahmad, 2014; Lee et al., 2010; Şehirli et al., 2008). This study contributes novel evidence regarding the capability of RES to mitigate MP-induced hepatotoxicity, examining liver impairment markers, histopathological abnormalities, oxidative stress, inflammation, and apoptosis.

An increase in relative liver weight following MP exposure reflects physiological stress and potential functional impairment, aligning with prior research on the effects of MP on rodent liver, adrenal glands, and thyroid glands (Du et al., 2024; Vo et al., 2010). Elevated activities of serum AST and ALT, together with decreased serum albumin and total protein levels in the liver, further indicate hepatocyte injury and compromised synthetic function. Our results regarding this increase are corroborated by the research conducted by Du et al. (2024). The presence of AST and ALT in the bloodstream signifies hepatocyte injury

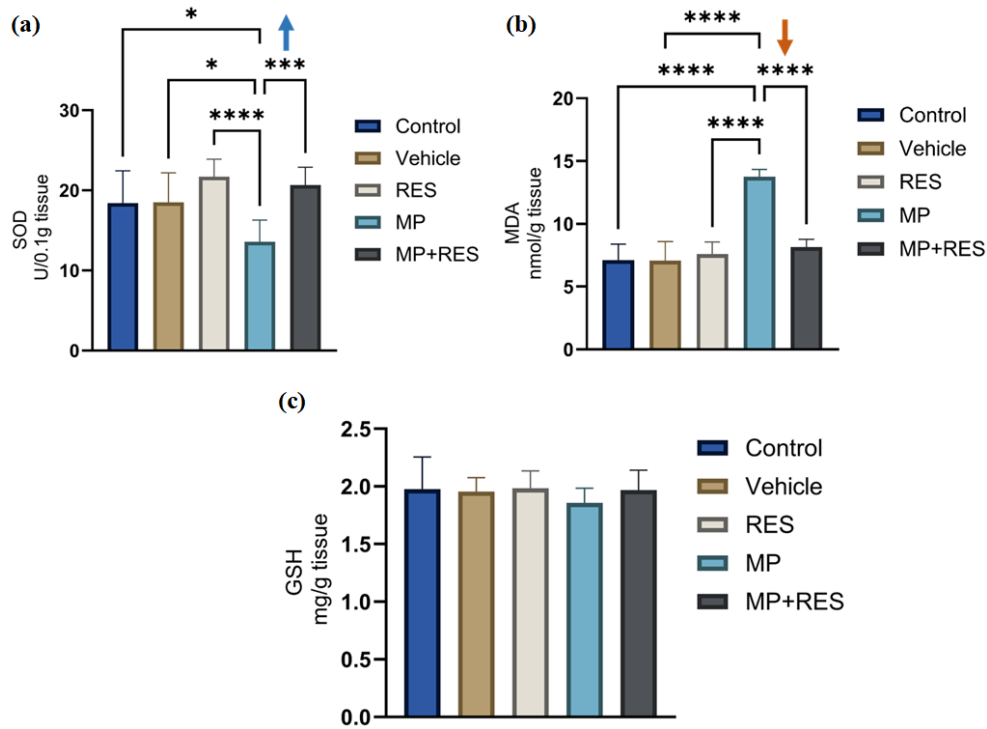


Fig. 3. RES mitigates oxidative damage generated by MP exposure. The combination of RES and MP treatment significantly (a) enhanced hepatic SOD activity and (b) reduced MDA level compared to the group receiving only MP. (c) GSH level remained statistically unchanged across all groups. Data are presented as mean \pm SD (n=8 per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test for all comparisons. *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001, and ****p \leq 0.0001. Significant increases and decreases are indicated by blue upward (\uparrow) and orange downward (\downarrow) arrows, respectively. RES: resveratrol, MP: methylparaben, SOD: superoxide dismutase, MDA: malondialdehyde, GSH: reduced glutathione.

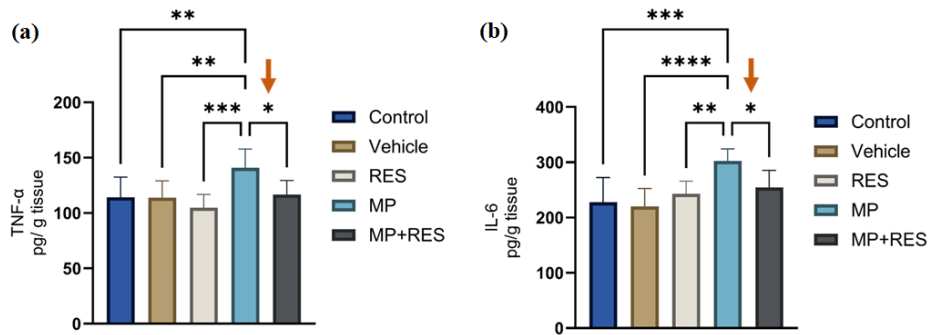


Fig. 4. RES suppresses proinflammatory markers elicited by MP exposure. The treatment with RES after exposure to MP significantly reduced hepatic (a) TNF- α and (b) IL-6 levels relative to the MP group. Data are presented as mean \pm SD (n=8 per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test for all comparisons. *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001, and ****p \leq 0.0001. Significant decreases are indicated by an orange downward arrow (\downarrow). RES: resveratrol, MP: methylparaben, TNF- α : tumor necrosis factor- α , IL-6: interleukin-6.

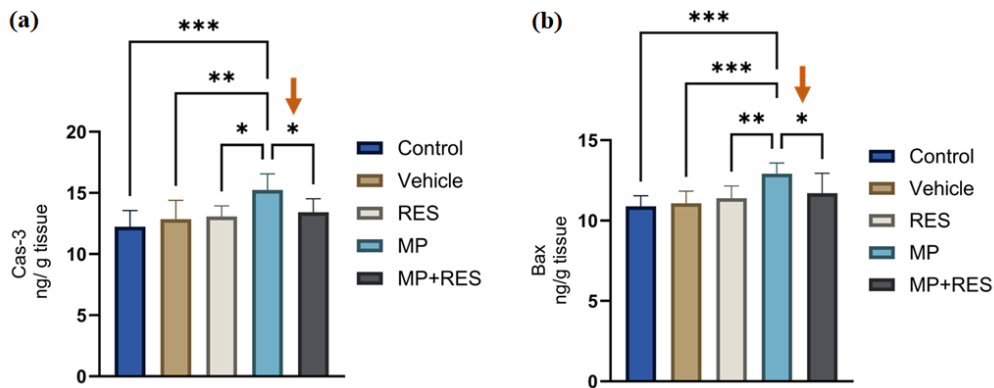


Fig. 5. RES inhibits apoptotic markers triggered by MP exposure. Treatment with RES after MP challenge significantly lowered hepatic (a) Cas-3 and (b) Bax levels compared to the MP-only group. Data are presented as mean \pm SD (n=8 per group). Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test for all comparisons. *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001, and ****p \leq 0.0001. Significant decreases are indicated by an orange downward arrow (\downarrow). RES: resveratrol, MP: methylparaben, Cas-3: caspase-3, Bax: the associated protein X.

(Du et al., 2024). Albumin and total protein are indicators of liver synthetic function, as the liver primarily manages protein synthesis; a decrease in serum albumin level is a significant indicator of liver disease (Lala et al., 2025). The reduction of the abundant protein albumin may explain the observed decrease in total protein concentration. These biochemical alterations correspond with histological findings of inflammatory infiltration, steatosis, and mild necrosis, supporting the hepatotoxic impact of MP as reported in rodent and fish models (Du et al., 2024; Calisir et al., 2025).

Co-treatment with RES attenuated these adverse effects, normalizing liver weight and biochemical markers and nearly restoring normal liver histology with reduced steatosis and inflammation. This outcome aligns with the findings of prior research regarding the relative weight of the liver, reduces serum or plasma ALT and AST levels, and increase albumin and total protein levels in rodents (Milton-Laskibar et al., 2022; Şehirli et al., 2008; Ahmad and Ahmad, 2014; Reda et al., 2022; Lee et al., 2010). This effect may be attributed to the lipid membrane protective action associated with RES (Fei et al., 2018), as it can integrate into lipid membranes and may enhance membrane stability (Abdu and Al-Bogami, 2019), thereby elucidating the reduction in serum AST and ALT activities in the MP+RES group. Given that RES protects against hepatic injury (Ahmad and Ahmad, 2014; Lee et al., 2010; Şehirli et al., 2008), an enhancement in liver synthetic function was observed, explaining the elevated albumin and total protein levels in the MP+RES group. It has been shown that RES reduces liver steatosis and inflammation in rats (Reda et al., 2022). Moreover, it has been demonstrated that RES have immunomodulatory effects by reducing inflammatory cell infiltration and fibrosis in the liver (Abdu and Al-Bogami, 2019). Additionally, RES decreased Kupffer cell activation in the liver (Chan et al., 2011), and suppressed neutrophil infiltration (Hassan-Khabbar et al., 2010).

Oxidative stress appears central to MP-induced liver injury, as shown by increased MDA levels and decreased SOD activity. These findings are supported by prior studies (Dubey et al., 2017; Pollack et al., 2020). The suggested mechanism is that lipid peroxidation products interact with SOD, causing targeted changes to histidine residues and promoting the formation of protein crosslinks, which ultimately results in decreased enzyme activity (Pollack et al., 2020). The suggested mechanism of oxidative stress elevation by MP involves the induction of free radical generation within the subcellular milieu (Chatterjee et al., 2024). In a state of oxidative stress, characterized by an excess of biological oxidants that disrupts the equilibrium between oxidation and antioxidant *in vivo*, cellular oxidants induce apoptosis via specific transcription factors or directly cause lipid peroxidation, damage to proteins and DNA, and dysregulation of enzyme expression (Zheng et al., 2023). The ROS play a pivotal role in the onset and advancement of several liver diseases, including those induced by hepatitis C, alcohol, drugs, or endotoxemia (Jaeschke et al., 2002). The primary and most thoroughly identified molecule generated from lipid peroxidation is MDA. It can covalently bond with proteins and nucleic acids, resulting in the creation of DNA-protein crosslinks and other adducts that harm biomolecules (Cordiano et al., 2023). The SOD functions as an antioxidant enzyme that neutralizes oxygen radicals. This enzyme turns harmful superoxide radicals into less reactive dioxygen and hydrogen peroxide (Zheng et al., 2023). The present data reinforce the central role of oxidative stress in MP-induced hepatic injury and underscore the necessity of antioxidant strategies in mitigating paraben toxicity.

The antioxidant capacity of RES was critical in mitigating oxidative damage, as indicated by the decreased level of MDA and the increased activity of SOD in the MP+RES group. Previous studies support these findings upon treatment with RES (Abdu and Al-Bogami, 2019; Chowdhury et al., 2022; Gocmez et al., 2019; Reda et al., 2022). Oxidative stress seems to diminish by RES through enhancing the expression of cellular antioxidant enzymes, such as SOD, and reducing lipid peroxidation (Bishayee et al., 2010). Our findings indicated that the advantageous effects of RES in mitigating MP-induced hepatic injury may be ascribed to its antioxidant properties, corroborating prior studies conducted in rodent models. These studies encompass hepatic ischemia-reperfusion, ethanol toxicity, carbon tetrachloride toxicity, and induced metabolic syndrome (Bishayee et al., 2010; Reda et al., 2022). Other suggested mechanisms of RES as an antioxidant are contingent upon the configuration of functional groups on the nuclear structure, thereby

functioning as a direct radical scavenger and chelating. Along with this, RES also acts as an indirect inducer of the cellular antioxidant system by influencing multiple antioxidant pathways, including the Kelch-like ECH-associated protein 1/nuclear factor erythroid 2-related factor 2 (Keap1/Nrf2) pathway (Chowdhury et al., 2022; Orrù et al., 2020). The activation of the Keap1/Nrf2 pathway, the primary cellular defense signal, is initiated to counteract the detrimental effects of electrophilic and oxidative stress, significantly contributing to cellular viability (Orrù et al., 2020).

Concurrent elevation of pro-inflammatory cytokines, TNF- α and IL-6, indicates an inflammatory response induced by MP treatment. Aung et al. identified a correlation between MP concentrations in urine samples and elevated plasma levels of IL-6 (Aung et al., 2019). The mechanism behind MP induction of inflammation involves triggering the mitogen-activated protein kinase (MAPK) and nuclear factor-kappaB (NF- κ B) signaling pathways (Du et al., 2024).

The anti-inflammatory action of RES involved downregulating TNF- α and IL-6. These results are consistent with other rodent studies reporting the effects of RES on these proinflammatory markers (Chan et al., 2011; Chowdhury et al., 2022; Hassan-Khabbar et al., 2010; Şehirli et al., 2008). The anti-inflammatory effect of RES is mostly attributed to the inhibition of the MAPK and NF- κ B signaling pathways (Reda et al., 2022).

Evidence of apoptosis was supported by increased Cas-3 and Bax levels in the MP group. This aligns with prior *in vivo* and *in vitro* studies showing MP-induced apoptosis (Dubey et al., 2017; Ateş et al., 2018). It has been reported that MP triggered mitochondrial and lysosomal membrane damage and ER stress. The mitochondrial-mediated apoptosis induced by MP is via Cas-3 upregulation (Dubey et al., 2017) and ER stress-mediated apoptosis through the inositol-requiring enzyme 1 α - X-box binding protein 1 pathway (Du et al., 2024). Mediators of ROS and pro-inflammation elevate Bax levels, leading to mitochondrial membrane destabilization and the release of cytochrome c. This release subsequently activates multiple caspases, including Cas-3, the primary effector (Sarawi et al., 2023).

The reduction in apoptotic markers Cas-3 and Bax upon RES administration is consistent with a previous study in rats (Zhang et al., 2019). The proposed mechanism involves activation of Sirtuin 1, which plays a key role in inhibiting ER stress pathway activation, suppressing BAX expression, and promoting mitochondrial biogenesis and function (Luo et al., 2023).

Together, these findings identify RES as a promising agent for protecting against MP-induced hepatotoxicity by targeting oxidative stress, inflammation, and apoptosis pathways. This study provides a thorough evaluation of the hepatoprotective effects of RES against MP-induced liver injury, incorporating a wide array of analyses, including biochemical markers, histopathological examination, oxidative stress parameters, inflammatory cytokines, and apoptosis indicators. The use of a well-established animal model with controlled dosing and administration routes allowed for a clear demonstration of the pharmacological potential of RES. Furthermore, the study's multifaceted approach provides robust evidence supporting the antioxidant, anti-inflammatory, and anti-apoptotic mechanisms of RES, thereby strengthening the scientific foundation for its potential therapeutic application. These aspects collectively underscore the rigor and depth of the investigation, setting a solid basis for further translational research. The doses of MP and RES in this study were intentionally set at higher experimental levels based on prior animal research to effectively unveil potential toxic and protective effects for a more comprehensive understanding (Oliveira et al., 2020; Gocmez et al., 2019). The administration route of RES was via intraperitoneal injection, unlike the oral route used in humans, where extensive first-pass metabolism reduces its bioavailability and efficacy (Walle, 2011). Thus, the observed hepatoprotective effects likely reflect pharmacological responses specific to these experimental conditions rather than typical human outcomes. Additionally, interspecies differences in metabolism limit direct clinical extrapolation (Maier-Salamon et al., 2011). Future studies should use oral RES doses reflective of human intake and include pharmacokinetic evaluations to better assess its therapeutic potential against paraben-induced liver injury.

5. Conclusions

This study is the first to demonstrate that RES effectively mitigates MP-induced hepatotoxicity by attenuating oxidative stress, inflammation, and apoptosis, restoring liver function and histological integrity. The higher doses used were based on previous animal studies, and RES was administered intraperitoneally, unlike the oral route typically used in humans. Given the impact of first-pass metabolism on oral bioavailability and interspecies metabolic differences, the observed hepatoprotective effects likely reflect pharmacological responses specific to the experimental model rather than typical human outcomes. Therefore, further research employing oral administration at clinically relevant doses, alongside pharmacokinetic evaluations and larger sample sizes, is essential to confirm these findings and advance the clinical translation of RES as a protective agent against paraben-induced liver injury.

CRedit authorship contribution statement

Latif Aljebali: Conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review & editing. **Gadah Albasher:** Conceptualization, methodology, project administration, resources, supervision, validation, writing – review & editing. **Nojood Altwaijry:** Methodology, resources, supervision, writing – review & editing. **Abir Alamro:** Conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

Data availability

The data presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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