

MitoQ alleviates malathion-induced hepatorenal toxicity via oxidative stress and inflammation modulation

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Abstract. Malathion, a commonly used organophosphate pesticide, induces severe hepatorenal toxicity, mitochondrial dysfunction and inflammatory responses primarily through oxidative stress and apoptosis. The present study investigated the protective effects of mitoquinol (MitoQ), a mitochondria-targeted antioxidant, against malathion-induced toxicity in male Wistar albino rats. A total of 50 rats were divided into the following five groups: i) Control; ii) malathion-only; iii) malathion + MitoQ; iv) MitoQ-only; and v) vehicle. Malathion exposure significantly elevated the levels of aspartate aminotransferase, alkaline phosphatase, creatinine, urea and uric acid and decreased total protein, albumin and globulin levels. At the mitochondrial level, malathion reduced antioxidant enzyme activity (superoxide dismutase, glutathione peroxidase and glutathione) and ATP production while increasing reactive oxygen species, leading to oxidative damage. Furthermore, malathion induced upregulation of pro-apoptotic markers such as Bax, and downregulation of the anti-apoptotic marker, Bcl-2. In addition, malathion increased TNF- α , NF- κ B, Toll-like receptor (TLR) 2 and TLR4 expression, and malathion toxicity induced severe hepatorenal damage, including vascular congestion, inflammatory infiltration and tubular degeneration. MitoQ co-administration revealed a trend towards mitigating altered hepatorenal markers, inflammatory markers and regulated apoptotic/antiapoptotic gene markers, with partial restoration in mitochondrial function and histopathological changes. In parallel, MitoQ normalized cellular changes induced by malathion in the liver and kidneys. In conclusion, malathion toxicity in the liver and kidneys is mediated by mitochondrial oxidative stress, apoptosis and inflammation. MitoQ exerts protective effects by restoring mitochondrial homeostasis, reducing inflammatory signaling and mitigating tissue

damage. Future research should explore longer treatment durations and potential synergistic effects with other antioxidants to optimize protection against pesticide-induced toxicity.

Introduction

Malathion is a widely used organophosphate insecticide applied in agriculture, public health programs and residential settings for pest control. It is classified as an acetylcholinesterase inhibitor, due to its phosphorothioate ($-P=S$) functional group (1). Animal studies have revealed that chronic exposure to malathion, even at low levels, causes severe liver and kidney issues (2,3). In addition, rat brains exposed to 25 mg/kg malathion resulted in acute oxidative stress (4). After 28 days of treatment with doses of 5 and 10 mg/kg malathion, brain oxidative damage becomes chronic (5), and after a short period of skin contact, malathion caused neurobehavioral problems and neuronal cell death (6). Malathion is a commonly used organophosphate insecticide that contributes to environmental contamination and increases exposure risks, particularly in developing countries (7,8). Specifically, occupational exposure, especially among factory workers, heightens the risk of malathion intoxication (9) as due to its lipophilic nature, it is rapidly absorbed and distributed across organs, leading to various toxic effects (10).

Malathion induces oxidative stress, increasing reactive oxygen species (ROS) and lipid peroxidation, which contributes to neurotoxicity and ranks it among the top neurotoxic agents (11,12). It also disrupts antioxidant activity in brain tissues, causing mitochondrial dysfunction, DNA damage, autophagy and apoptosis (13). Additionally, hepatorenal toxicity has been observed, with elevated liver enzymes, renal injury markers and increased risk of atherosclerosis (14).

As a mutagenic agent, malathion targets DNA and triggers hepatic glycogenolysis, leading to hyperglycemia and inflammatory toxicity (3). It also acts as an endocrine disruptor, causing reproductive toxicity and anemia (15). The World Health Organization classifies malathion as a slightly hazardous insecticide, recommending a dose of 2 g/m² with a residual effect of 60-90 days and an metered dose inhaler of 0.02 mg/kg/day (15). The Environmental Protection Agency reports that malathion is one of the most heavily used organophosphate pesticides worldwide, with ~30 million lbs applied annually, particularly in cultivating crops such as cotton, rice and vegetables (16,17). Its extensive use in vector control and

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agricultural practices, especially in low- and middle-income countries, has raised growing concerns regarding chronic exposure and bioaccumulation in humans and wildlife. Previous study has linked malathion exposure to oxidative stress-mediated damage in vital organs such as the liver, kidneys and brain, along with apoptotic and inflammatory complications (3). Occupational exposure, accidental ingestion and environmental contamination represent major routes of human contact, making malathion toxicity a pressing global health concern (9).

To address this, the importance of exploring targeted antioxidant therapies must be emphasized. Mitoquinol (MitoQ), a mitochondria-targeted derivative of coenzyme Q10, has emerged as a promising candidate for protecting against mitochondrial oxidative damage. Unlike conventional antioxidants, MitoQ accumulates selectively within mitochondria due to its lipophilic triphenylphosphonium moiety, allowing for more efficient ROS neutralization and preservation of mitochondrial function (18). While its protective effects have been demonstrated in various models of cardiovascular and neurodegenerative diseases, there is limited research on its use in mitigating pesticide-induced hepatorenal injury. The present study attempted to address this gap by evaluating the mechanistic and functional outcomes of MitoQ administration in a malathion toxicity model, with the aim of clearly defining the real world significance of malathion toxicity and providing a novel contribution in this area.

MitoQ is a mitochondria-targeted antioxidant designed to neutralize ROS and protect against oxidative damage (18). It is a modified form of coenzyme Q10 (ubiquinone), conjugated to a triphenylphosphonium cation, allowing it to accumulate selectively within mitochondria (19). This targeted delivery enhances its ability to mitigate mitochondrial dysfunction, reduce lipid peroxidation and prevent apoptosis, making it a promising therapeutic agent for neurodegenerative diseases, cardiovascular disorders, hepatorenal injury and metabolic dysfunction (18). MitoQ improves mitochondrial bioenergetics, stabilizes membrane potential and reduces inflammatory cytokine production (20), thereby protecting against oxidative stress-related diseases. It was hypothesized that MitoQ administration can attenuate malathion-induced hepatorenal toxicity by reducing oxidative stress, modulating apoptotic and inflammatory pathways, and preserving mitochondrial function.

Materials and methods

Chemicals. Malathion (98% active ingredient; O-dimethyl phosphorodithioate of diethyl mercaptosuccinate; Sigma-Aldrich; Merck KGaA) was soaked in 70% aqueous ethanol for 10 days at 25°C and filtered. The solution was evaporated in a vacuum and was semi-gelatinous. MitoQ was obtained from PitchBook. Unless otherwise specified, all other reagents and fine chemicals were of analytical grade and purchased from Sigma-Aldrich, Merck KGaA.

Experimental design and sampling. A total of 50 male Wistar albino rats (weight, 190±10 g; age, 8 weeks) were housed in stainless steel cages at 26±3°C with humidity 10±20%, in a well-ventilated room, maintaining a 12-h light/dark cycle with

unrestricted access to food and water. Prior to the experiment, the rats were given a 2-week acclimatization period to adjust to the laboratory conditions. Following acclimatization, the animals were randomly assigned to the following five experimental groups (n=10/group): i) The control group, which received normal saline intraperitoneally (i.p.); ii) malathion-only group, which were orally administered malathion once daily at a dose of 27 mg/kg body weight (1/50 of the oral LD₅₀), using corn oil as a vehicle via oral gavage (21); iii) malathion + MitoQ group, which were i.p. injected with MitoQ (2 mg/kg/day) (22), followed by oral malathion at a dose of 27 mg/kg body weight (1/50 of the oral LD₅₀); iv) MitoQ group, which were i.p. injected with MitoQ (2 mg/kg/day); and v) sham group, which were administered an oral standard corn oil (vehicle for malathion).

After the experiment, the final body weight of each rat was measured using a digital scale. Following deep anesthesia with i.p. 100 mg/kg pentobarbital sodium, 1 ml blood samples were collected via cardiac puncture prior to euthanasia by decapitation. Death was confirmed by the complete cessation of respiration and heartbeat following decapitation. The collected samples were placed in sterile, heparin-free centrifuge tubes and left to clot for 15 min at room temperature. Following centrifugation at 1,000 x g at 4°C for 10 min, the serum was carefully separated and stored at -20°C to analyze hepatic and renal function biomarkers. Following euthanasia, the liver and kidney tissues were excised, weighed and rinsed with ice-cold saline. The collected tissue samples were processed as follows: A portion of the liver and kidneys was fixed in 10% neutral-buffered formalin for histopathological evaluation. For analyzing various parameters, another sample of the liver and kidney tissues was minced and homogenized in a buffer containing 10 mM HEPES-KOH (pH 7.5), 1 mM EGTA, 0.25 mM sucrose and protease and phosphatase inhibitors. The homogenate was centrifuged at 20,000 x g for 10 min at 4°C using a Hettich Universal 32A cooling centrifuge. Following the experiment, the animal bodies were frozen until disposal by incineration. The remaining tissue samples were snap-frozen in liquid nitrogen (-80°C) and stored for gene expression analysis. The Ethics Committee of Taif University for Animal Use in Research reviewed and approved the present study (Taif, Saudi Arabia; approval no. TU-DSPP-2024-260), and all animal-related investigative procedures adhered to the National Institutes of Health requirements.

The humane endpoints used in the present study were in compliance with ethical guidelines and to minimize animal suffering, humane endpoints were established based on criteria such as a body weight loss >20%, severe clinical signs (such as, labored breathing, tremors, inability to move) or failure to eat or drink. Additionally, any signs of severe toxicity, including respiratory distress, would have triggered immediate euthanasia to prevent further suffering. All animals were closely monitored by veterinary staff and these endpoints were adhered to throughout the study. No animals reached these endpoints during the experiment.

Serum hepatorenal marker analysis. Serum activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined as previously described (23); alkaline phosphatase (ALP) activity was evaluated as

Table I. Primers for gene expression by reverse transcription-quantitative PCR.

Gene	Direction	Primer sequence (sense)	Primer sequence (antisense)	Accession number
BAX	Sense	GGCGAATTGGCGATGAACTG	ATGGTTCTGATCAGCTCGGG	NM_017059.2
BCL-2	Sense	GATTGTGGCCTTCTTTGAGT	ATAGTTCCACAAAGGCATCC	NM_016993.1
GAPDH	Sense	TCAAGAAGGTGGTGAAGCAG	AGGTGGAAGAATGGGAGTTG	NM_017008.4
TNF- α	Sense	GGG GCC ACC ACG CTC TTCTGT	GCA AAT CGGCTG ACG GTG TGG	NM_012675.3
TLR4	Sense	GGGAGGCACATCTTCTGGAG	TGAGGTTAGAAGCCTCGTGC	NM_019178.2
NF- κ B	Sense	GCTTTGCAAACCTGG GAATA	CAAGGTCAGAAT GCACCAGA	NM_001276711.2
TLR2	Sense	CAGATGGCCACAGGACTCAA	AAAGACCTGGAGCTGCCATC	NM_198769.2

TLR, toll-like receptor.

previously described (24) and total protein and albumin concentrations were assessed as previously described (25). Serum globulin levels were estimated by subtracting albumin from total protein to evaluate liver function. Serum creatinine and urea levels, used as indicators of kidney function, were analyzed as previously described (26,27). Serum uric acid was also measured following the protocol previously described (28). Serum total protein levels were determined following the method described by Gornall *et al* (29), while albumin concentration was measured according to the protocol by Doumas *et al* (25). Total globulin levels were calculated by subtracting albumin from total protein, as previously described (30).

Mitochondrial isolation. Testis homogenates were centrifuged at 2,000 x g for 10 min at 4°C in a 0.25 M sucrose solution. The pellet was discarded, and the supernatant was mixed with 0.75 M sucrose in HEPES buffer (Abcam), followed by centrifugation at 10,000 x g for 30 min at 4°C. The mitochondrial pellet obtained was resuspended in HEPES buffer after removing the supernatant. A further centrifugation at 10,000 x g for 10 min at 4°C was conducted, and the supernatant was discarded. The final mitochondrial pellet was resuspended in PBS. The isolated mitochondria were stored at -80°C for subsequent parameter analysis (31).

Oxidant and antioxidant mitochondrial markers. Mitochondrial superoxide dismutase (SOD) activity was evaluated by measuring its ability to inhibit the reduction of nitroblue tetrazolium dye induced by phenazine methosulfate. The absorbance was recorded at 560 nm, and the results were expressed as U/mg protein (32). Mitochondrial glutathione (GSH) levels were measured colorimetrically at 405 nm based on the reduction of 2,2'-Dithiobis(5-nitropyridine) by its sulfhydryl group, forming a yellow compound (33). The activity of GSH peroxidase (GPx) was measured as previously described (34). Reactive oxygen species (ROS) cat. no. LS-F9759; LS Bio) were measured according to the manufacturer's instructions.

Mitochondrial ATP measurement. Mitochondrial ATP levels were assessed using the ATP Assay Kit (Abnova Corporation, Catalog # KA1661), which is based on the phosphorylation of glycerol by ATP according to the manufacturer's instructions.

The resulting product was quantified colorimetrically at 570 nm, with results expressed as μ mol/mg protein.

Inflammatory marker analysis. Following the manufacturer's instructions, the assessed hepatorenal biomarkers included cytochrome *c* (cat. no. EAMT001; Merck KGaA), peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α ; cat. no. SEH337Ra; CLOUD-CLONE CORP, CCC), mitochondrial transcription factor A (TFAM; cat. no. E1616Ra; Shanghai Korain Biotech Co., Ltd.) and TNF- α (cat. no. abx050220; Abbexa).

Gene expression analysis. Total RNA was extracted from renal and hepatic tissue using TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions. cDNA was synthesized immediately using the MultiScribe RT enzyme kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and used in triplicate for quantitative (q)PCR analysis. qPCR was carried out using Power SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) on a 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The relative mRNA expression levels of target genes were compared with the control group, with β -actin as the internal reference gene (35). Primer sequences and gene accession numbers are provided in Table I.

Histopathological examination. Liver and kidney tissue samples were processed using standard paraffin embedding techniques (36). Sections (5 μ m-thick) were cut from the paraffin blocks, stained with hematoxylin and eosin (H&E) at room temperature for 30 min for hematoxylin and 1 min and examined under a light microscope.

Samples from kidney and liver were immersed in neutral formaldehyde solution (10%, Sigma-Aldrich, USA) for 24 h. As described by Bancroft and Layton (37), using a paraffin integration device hepatic and renal tissues were fixed. Histopathological damage was scored using a semi-quantitative scale as follows, 0: Normal histology; 1: Mild degeneration or infiltration; 2: Moderate necrosis, congestion or inflammation; 3: Severe damage with widespread cell death and infiltration. The scores for liver and kidney damage were recorded and analyzed for statistical differences across treatment groups. These scores reflect the extent of tissue damage

Table II. Effect of MitoQ and/or malathion on liver and kidney functions markers.

Variable	Control	Malathion	Malathion + MitoQ	MitoQ	Vehicle
Final body weight (g)	242.6±3.5 ^a	198.2±2.8 ^c	223.9±3.1 ^b	244.3±3.2 ^a	241.7±3.7 ^a
ALT (U/l)	6.45±0.11 ^c	32.81±0.12 ^a	12.28±0.13 ^b	6.16±0.18 ^c	5.88±0.14 ^c
ALP (U/l)	357.716.76 ^d	627.42±6.28 ^a	394.40±7.13 ^b	364.47±10.71 ^{c,d}	347.84±8.42 ^d
AST (U/l)	68.41±1.21 ^c	120.66±3.44 ^a	75.85±0.79 ^b	70.09±2.06 ^c	66.89±5.62 ^c
Total protein (g/dl)	6.69±0.12 ^a	3.75±0.07 ^c	5.58±0.10 ^b	6.72±0.11 ^a	6.71±0.12 ^a
Albumin (g/dl)	3.14±0.06 ^a	1.76±0.03 ^c	2.62±0.05 ^b	3.10±0.05 ^a	3.15±0.06 ^a
Globulin, g/dl)	3.55±0.06 ^a	1.99±0.04 ^c	2.96±0.05 ^b	3.62±0.06 ^a	3.56±0.06 ^a
Creatinine (mg/dl)	0.89±0.02 ^c	4.53±0.02 ^a	1.69±0.02 ^b	0.85±0.02 ^c	0.81±0.02 ^c
Urea (mg/dl)	19.99±0.35 ^c	69.93±1.25 ^a	26.17±0.27 ^b	19.09±1.56 ^c	18.22±0.44 ^c
Uric acid (mg/dl)	24.78±0.44 ^c	86.71±2.32 ^a	42.51±1.34 ^b	23.67±0.70 ^c	22.59±0.55 ^c

Data are presented as mean ± SE (n=10 per group). One-way ANOVA followed by Duncan's multiple range test was used to compare all groups (Control, Malathion, Malathion + MitoQ, MitoQ and Vehicle). Bars with different letters are significant in between at P<0.05. Groups sharing the same letter are not significantly different. MitoQ, mitoquinol; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

caused by malathion exposure and the protective effects of MitoQ.P.

Statistical analysis. Data were analyzed using one-way analysis of variance (ANOVA) to determine statistical differences. Prior to ANOVA, the data were assessed for normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. When significant differences were observed, Duncan's multiple range test was applied as a post hoc analysis to identify specific group differences. The liver and kidney damage scores were analyzed using the Kruskal-Wallis test, followed by Dunn's post hoc test, as these are ordinal categorical data. These results are presented as median (interquartile range) All other results are presented as the mean ± standard error, and P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were carried out using SPSS software (version 25; IBM Corp.).

Results

Effect of malathion and MitoQ on body weight. Body weight of rats in all groups was recorded weekly throughout the 30-day experimental period. Growth of rats exposed to malathion was checked compared with other groups, with a significantly lower final body weight compared with the control (Fig. S1). By contrast, the group co-treated with MitoQ and malathion demonstrated improved growth, with partial recovery toward control values over time. Notably, no significant changes were observed in the MitoQ-only and the vehicle group when compared with control throughout the experimental period.

Effect of MitoQ and/or malathion on liver and kidney function markers. Data presented in Table II were derived using standard biochemical assays carried out on serum samples collected from all experimental groups. The administration of malathion significantly increased liver and kidney function markers compared with the control and vehicle groups. AST and ALP levels were markedly elevated in the malathion-only group (32.81±0.12 U/l and 627.42±6.28 IU/l,

respectively) compared with the control (6.45±0.11 U/l and 357.72±6.76 IU/l, respectively). However, co-administration of MitoQ with malathion significantly decreased these elevations (AST, 12.28±0.13 U/l; ALP 394.40±7.13 IU/l), indicating a protective effect. Similarly, total protein, albumin and globulin levels were significantly decreased in the malathion group (3.75±0.07 g/dl, 1.76±0.03 g/dl and 1.99±0.04 g/dl, respectively), but MitoQ co-treatment partially restored these levels (5.58±0.10 g/dl, 2.62±0.05 g/dl and 2.96±0.05 g/dl, respectively). Kidney function markers, including creatinine, urea and uric acid, were significantly elevated following malathion exposure compared with other groups (4.53±0.02 mg/dl, 69.93±1.25 mg/dl and 86.71±2.32 mg/dl, for control, malathion and MitoQ plus malathion, respectively). By comparison, MitoQ co-administration mitigated these increases (1.69±0.02 mg/dl, 26.17±0.27 mg/dl and 42.5±1.34 mg/dl, respectively). MitoQ alone did not significantly alter these parameters compared with the control and vehicle groups. These findings suggest that malathion induces significant hepatotoxicity and nephrotoxicity, while MitoQ protects against these toxic alterations (Table II).

MitoQ restores mitochondrial antioxidant defenses and energy production while reducing malathion-induced oxidative stress. Analysis revealed significant changes in mitochondrial antioxidant defenses, ATP production and oxidative stress across different experimental groups. Malathion exposure led to a marked reduction in mitochondrial SOD, GPx, GSH and ATP levels, while significantly increasing mitochondrial ROS levels compared with the control group. Co-treatment with MitoQ (malathion + MitoQ group) significantly restored SOD, GPx, GSH and ATP levels, although they remained lower than the control, indicating partial recovery of mitochondrial function. Notably, the MitoQ-only and vehicle groups revealed no significant differences compared with the control group, suggesting that MitoQ does not induce oxidative stress or alter mitochondrial function under normal conditions (Fig. 1). These findings confirmed that MitoQ mitigates malathion-induced

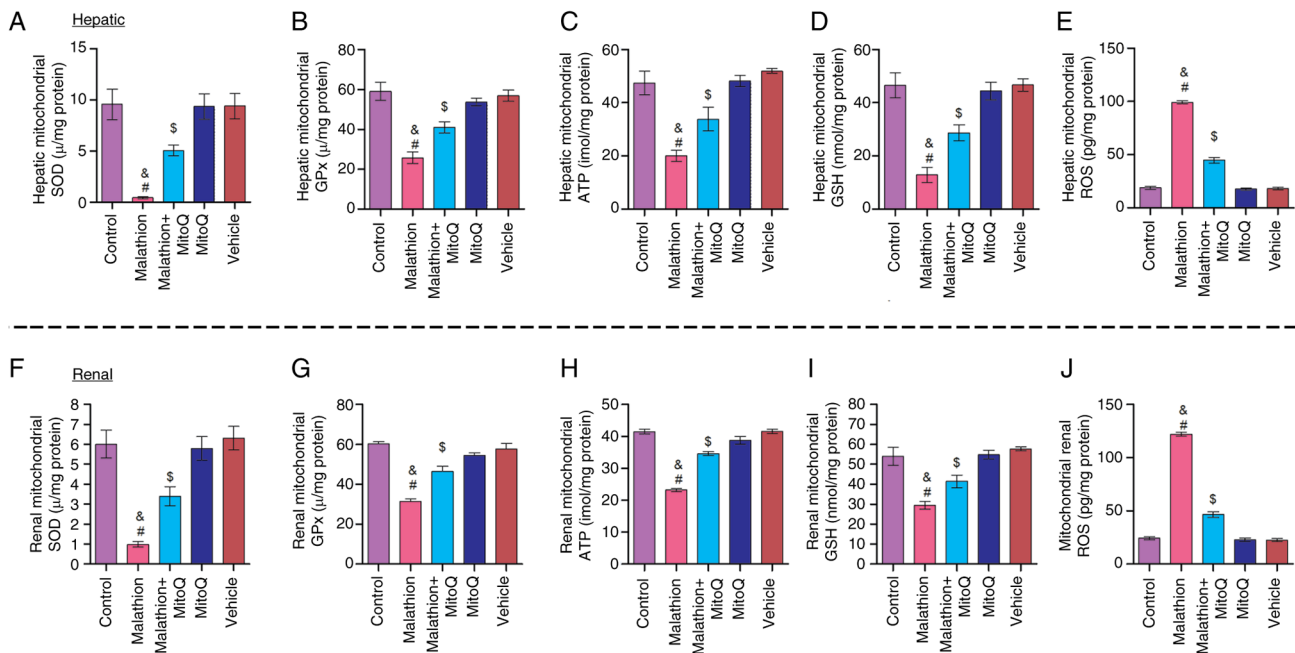


Figure 1. Effect of MitoQ on hepatic and renal mitochondrial function in malathion-treated rats. (A) Mitochondrial SOD activity, (B) GPx activity, (C) ATP levels, (D) GSH levels and (E) ROS levels in hepatic tissue. renal tissues (F) Mitochondrial SOD activity, (G) GPx activity, (H) ATP levels, (I) GSH levels and (J) ROS levels (bottom panel). Data are expressed as the mean $\pm$ standard error (n=10). *P<0.05 vs. malathion + MitoQ, #P<0.05 vs. Control, MitoQ, and vehicle. \$P<0.05 vs. Control, MitoQ, and vehicle. MitoQ, mitoquinol; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSH, glutathione; ROS, reactive oxygen species.

mitochondrial dysfunction and oxidative stress, enhancing mitochondrial antioxidant defenses and energy production.

Protective effects of MitoQ against malathion-induced changes in cytochrome *c*, TNF- α , PGC-1 α and TFAM levels. As seen in Fig. 2, significant mitochondrial function and inflammation alterations were observed across different experimental groups. Cytochrome *c* levels were significantly increased in the malathion-only group compared with the control, indicating enhanced mitochondrial damage and apoptosis. The malathion + MitoQ group revealed a significant reduction in cytochrome *c* levels compared with the malathion-only group, although levels remained higher than the control group. Notably, the MitoQ-only and vehicle groups exhibited no significant difference compared with the control group, suggesting that MitoQ does not independently induce mitochondrial damage.

Similarly, TNF- α levels were significantly elevated in the malathion group, indicating induction of an inflammatory response. Co-treatment with MitoQ in the malathion + MitoQ group significantly decreased TNF- α levels compared with the malathion-only group, although values remained higher compared with the control. No significant difference was observed between the control, MitoQ-only and vehicle groups, confirming that MitoQ alone does not induce inflammation.

Regarding mitochondrial biogenesis markers, PGC-1 α levels were significantly decreased in the malathion-only group compared with the control, suggesting impaired mitochondrial function. The malathion + MitoQ group exhibited a significant increase in PGC-1 α levels compared with the malathion-only group, indicating partial recovery. However, PGC-1 α levels in the malathion + MitoQ group remained significantly decreased

compared with the control. By contrast, the control, MitoQ-only and vehicle groups had no significant difference, suggesting normal mitochondrial function. Similarly, TFAM levels were significantly decreased in the malathion-only group compared with the control, suggesting mitochondrial biogenesis impairment. The malathion + MitoQ group revealed a significant increase in TFAM levels compared with the malathion-only group, although this was still significantly reduced compared with the control. The MitoQ-only and vehicle groups exhibited TFAM levels comparable with the control, indicating that MitoQ alone may maintain mitochondrial biogenesis (Fig. 2).

Overall, these findings highlighted the considerable detrimental effects of malathion on mitochondrial function and inflammation, as evidenced by increased cytochrome *c* and TNF- α , alongside decreased PGC-1 α and TFAM expression levels. The co-administration of MitoQ revealed a trend towards mitigation of these effects (P<0.05), suggesting its potential protective role. However, the recovery was only partial, indicating that additional interventions may be required to restore mitochondrial and inflammatory homeostasis completely.

MitoQ mitigates malathion-induced dysregulation of apoptotic markers (*Bax* and *Bcl-2*) in hepatorenal tissues. Significant alterations in the expression of pro-apoptotic and anti-apoptotic genes were observed in both renal and hepatic tissues following malathion exposure. As seen in Fig. 3, the malathion-only group exhibited a significant increase in Bax mRNA expression in renal and hepatic tissues compared with the control, indicating enhanced pro-apoptotic activity (Fig. 3). Co-treatment with MitoQ (malathion + MitoQ group) significantly reduced Bax expression compared with the malathion-only group, although levels remained higher compared

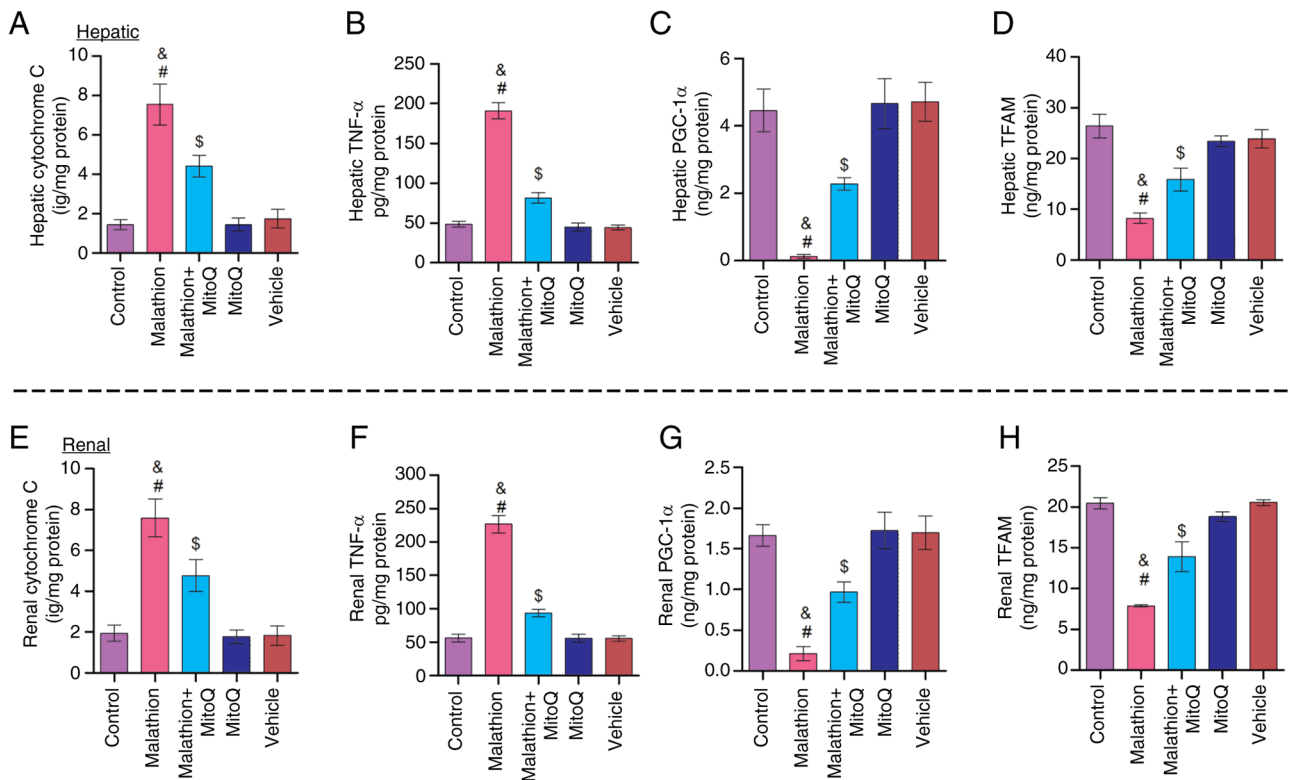


Figure 2. Effect of MitoQ protection on hepatic and renal mitochondrial biomarkers in malathion-treated rats. Hepatic (top panel) and renal (bottom panel) levels of (A and E) cytochrome *c*, (B and F) TNF- α , (C and G) PGC-1 α and (D and H) TFAM. Data are expressed as the mean $\pm$ standard error (n=10). Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple-range post hoc test [#]P<0.05 vs. malathion + MitoQ, [&]P<0.05 vs. Control, MitoQ, and vehicle. ^{\$}P<0.05 vs. Control, MitoQ, and vehicle. MitoQ, Mitoquinol; PGC-1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; TFAM, mitochondrial transcription factor A.

with the control. By contrast, the MitoQ-only and vehicle groups showed no significant differences from the control, suggesting that MitoQ alone does not upregulate pro-apoptotic pathways (Fig. 3).

Conversely, the malathion-only group revealed a significant decrease in Bcl-2 mRNA expression in renal and hepatic tissues compared with the control, indicating suppressed anti-apoptotic mechanisms (Fig. 3B and D). The malathion + MitoQ group exhibited a significant (increase in Bcl-2 expression relative to the malathion-only group, although it remained decreased compared with the control. Notably, the MitoQ-only and vehicle groups had the highest Bcl-2 expression levels, particularly in hepatic tissues, suggesting that MitoQ may have an additional protective role in enhancing anti-apoptotic gene expression (Fig. 3).

Protective role of MitoQ against malathion-induced upregulation of TNF- α and NF- κ B in hepatorenal tissues. Significant changes were observed in the mRNA expression levels of TNF- α and NF- κ B in renal and hepatic tissues following malathion exposure. The malathion-only group exhibited a significant increase in TNF- α mRNA expression in both renal (Fig. 4A) and hepatic (Fig. 4C) tissues compared with the control, suggesting induction of an inflammatory response. Co-treatment with MitoQ (malathion + MitoQ group) significantly decreased TNF- α expression levels in both tissues compared with the malathion-only group, although levels remained higher compared with the

control. No significant differences were observed between the control, MitoQ-only and vehicle groups, indicating that MitoQ does not independently influence TNF- α expression levels (Fig. 4).

A similar trend was observed for NF- κ B expression levels in both renal (Fig. 4B) and hepatic (Fig. 4D) tissue, where the malathion-only group exhibited a significant increase compared with the control, indicating activation of inflammatory pathways. The malathion + MitoQ group demonstrated a significant decrease in NF- κ B expression compared with the malathion-only group, but expression remained higher compared with the control. No significant differences were observed between the control, MitoQ-only and vehicle groups, confirming that MitoQ does not induce inflammatory signaling when administered independently.

MitoQ attenuates malathion-induced upregulation of Toll-like receptor (TLR) 2 and TLR4 in hepatorenal tissues. Significant changes were observed in TLR2 and TLR4 mRNA expression in renal and hepatic tissues following malathion exposure. The malathion-only group exhibited a significant increase in TLR2 expression in both renal (Fig. 5A) and hepatic (Fig. 5C) tissues compared with the control, suggesting an upregulation of innate immune response pathways. Co-treatment with MitoQ (malathion + MitoQ group) significantly decreased TLR2 expression compared with the malathion-only group, although levels remained higher than the control. No significant differences

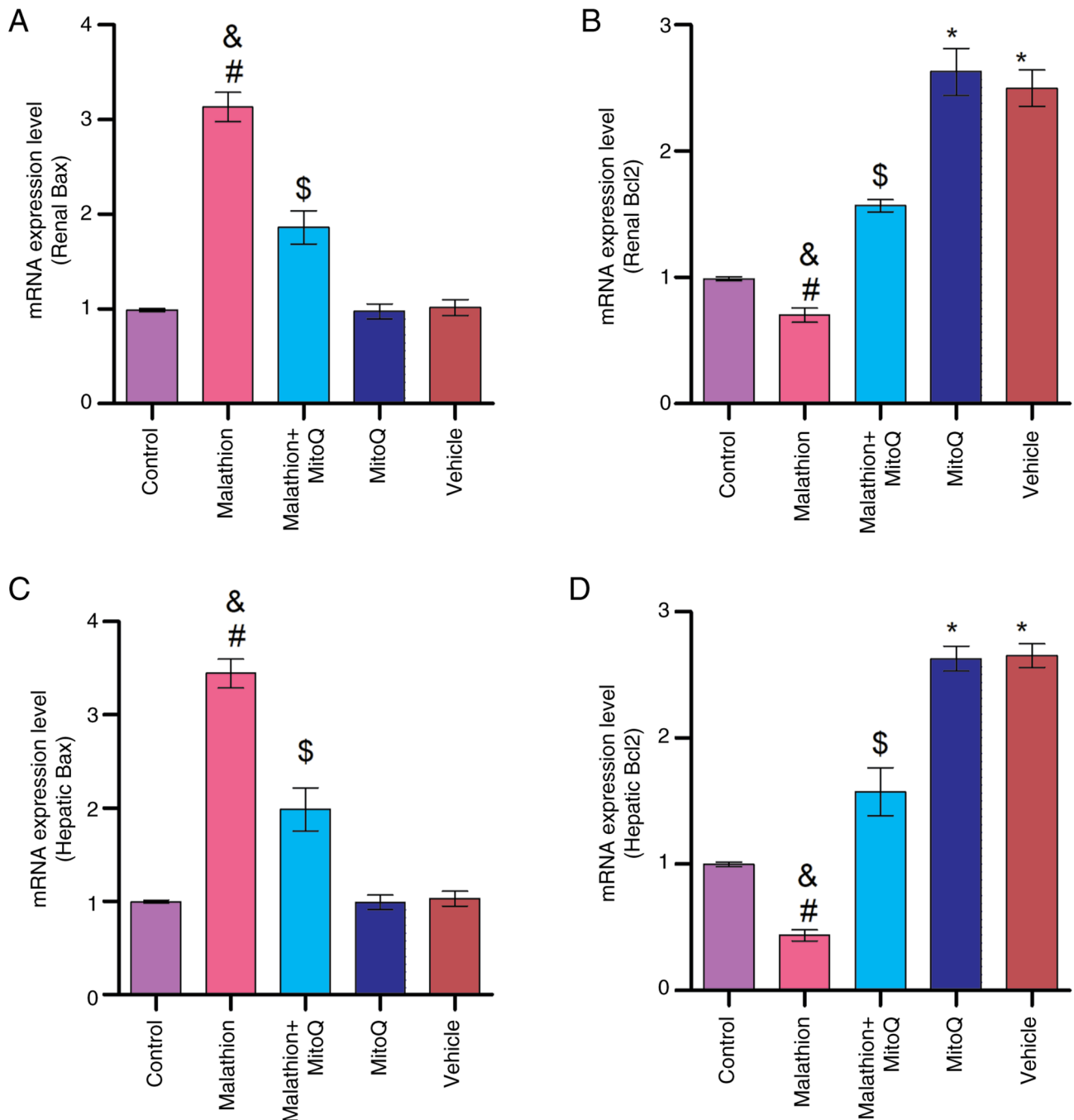


Figure 3. Effect of MitoQ protection on hepatic and renal mRNA expression levels in malathion-treated rats. mRNA expression levels of Bax in (A) renal and (C) hepatic tissues. mRNA expression levels of Bcl-2 in (B) renal and (D) hepatic tissues. Data are expressed as the mean $\pm$ standard error (n=10). [#]P<0.05 vs. malathion + MitoQ. [&]P<0.05 vs. Control, MitoQ, and vehicle. ^{\$}P<0.05 vs. Control, MitoQ, and vehicle. ^{*}P<0.05 vs. Control.

were observed between the control, MitoQ-only and vehicle groups, indicating that MitoQ alone may not alter TLR2 expression. Similarly, TLR4 expression in both renal (Fig. 5B) and hepatic (Fig. 5D) tissues was significantly elevated in the malathion-only group compared with the control, further supporting the activation of inflammatory pathways. The malathion + MitoQ group revealed a significant decrease in TLR4 expression levels compared with the malathion-only group, although it remained increased compared with the control. Notably, no significant differences in TLR4 expression were observed between the control, MitoQ-only and vehicle groups in renal tissues,

while in hepatic tissues, the malathion + MitoQ group demonstrated a partial reduction which remained increased compared with the control (Fig. 5).

Protective effect of MitoQ against malathion-induced hepatorenal histopathological alterations. H&E-stained liver sections revealed hepatic alterations across different treatment groups (Fig. 6). The control groups (Fig. 6A and E) exhibited normal liver architecture with well-defined hepatocytes and hepatic arteries. By contrast, the malathion-only group (Fig. 6B) revealed hepatic damage, including vascular congestion, hepatocyte swelling, macrosteatosis, nuclear pyknosis

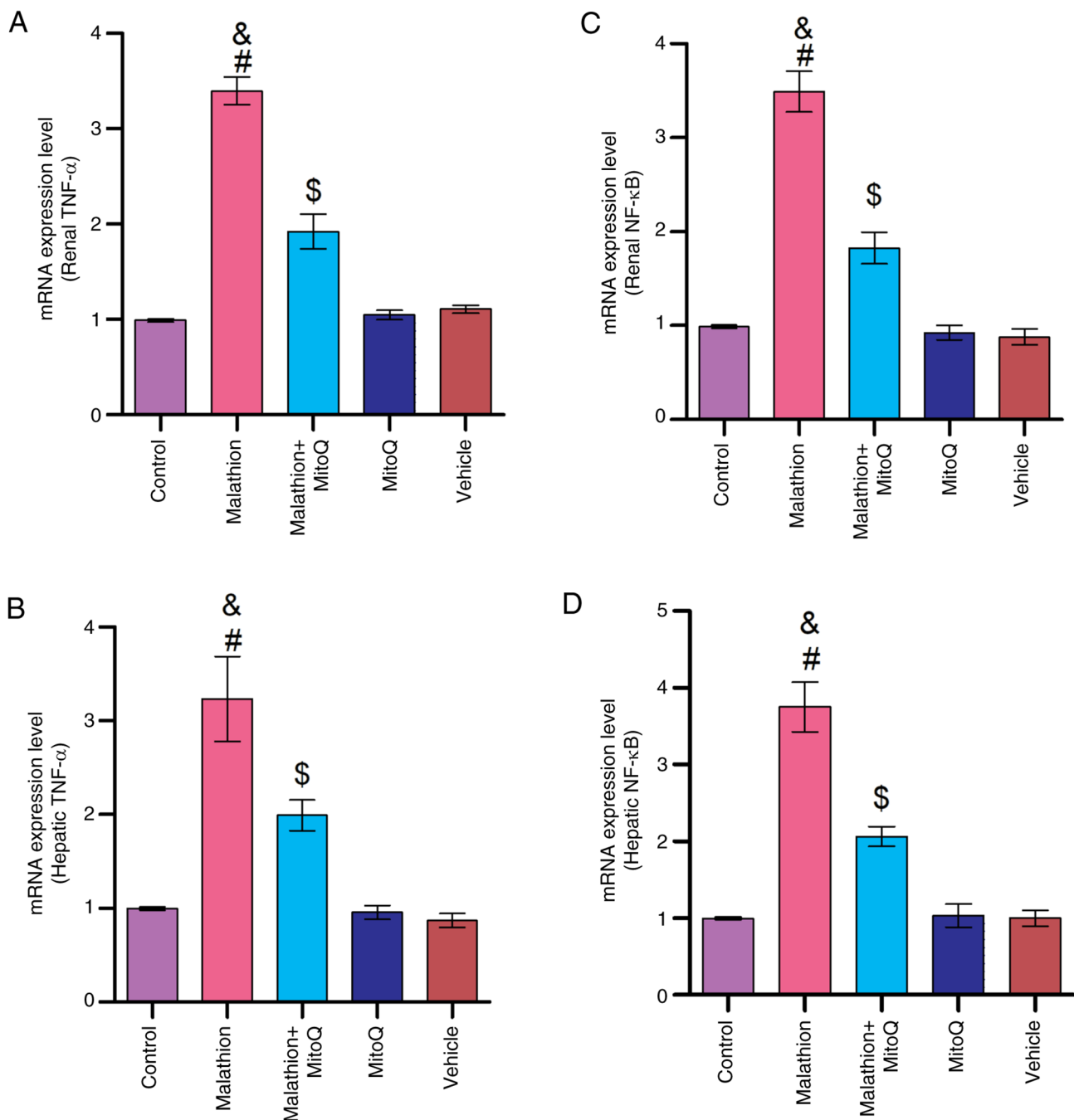


Figure 4. Effect of MitoQ protection on hepatic and renal mRNA expression levels of inflammatory markers in malathion-treated rats. mRNA expression levels of TNF- α in (A) renal and (C) hepatic tissues. mRNA expression levels of NF- κ B in (B) renal and (D) hepatic tissues. Data are expressed as the mean $\pm$ standard error (n=10). [#]P<0.05 vs. malathion + MitoQ, [&]P<0.05 vs. Control, MitoQ, and vehicle. ^{\$}P<0.05 vs. Control, MitoQ, and vehicle.

and peri-portal inflammatory infiltration, indicating toxicity. Co-treatment with MitoQ (malathion + MitoQ group; Fig. 6-C) partially mitigated these effects, demonstrating reduced hepatocyte damage and mild fibrosis in the portal area. The MitoQ-only group (Fig. 6D) revealed mild congestion and hepatocyte steatosis, suggesting minimal liver alterations. Overall, malathion induced hepatic injury, while MitoQ provided partial protection, reducing, but not completely preventing liver damage.

The H&E-stained renal cortex histomicrographs (Fig. 7) revealed structural changes across treatment groups. The control group (Fig. 7A) demonstrated normal glomeruli

and renal tubules, indicating intact kidney architecture. By contrast, the malathion-only group (Fig. 7B) demonstrated nephrotoxicity, characterized by glomerular basement membrane thickening, mesangial expansion, vacuolar degeneration of renal tubules and vascular congestion. The malathion + MitoQ group (Fig. 7C) exhibited mild glomerular thickening and tubular degeneration, suggesting that MitoQ partially protects against malathion-induced renal damage. The MitoQ-only and vehicle groups (Fig. 7D and E) revealed largely intact glomeruli but with mild renal tubule alterations, including vacuolar degeneration and proteinaceous accumulation. These findings suggest

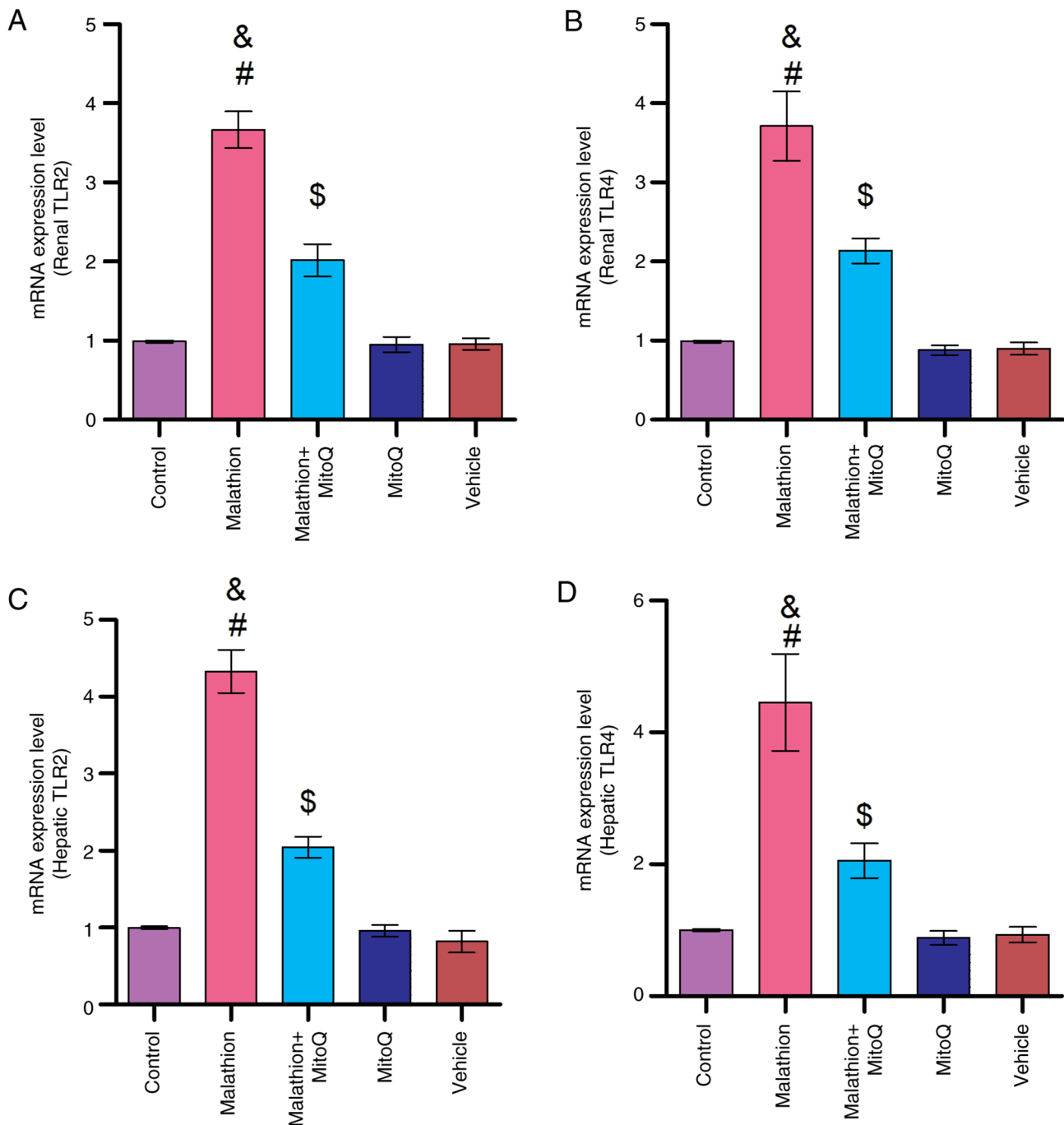


Figure 5. Effect of MitoQ protection on hepatic and renal mRNA expression levels of TLR2 and TLR4 in malathion-treated rats. mRNA expression levels of TLR2 in (A) renal and (C) hepatic tissues. mRNA expression levels of TLR4 in (B) renal and (D) hepatic tissues. Data are expressed as the mean $\pm$ standard error (n=10). [#]P<0.05 vs. malathion + MitoQ, [&]P<0.05 vs. Control, MitoQ, and vehicle. ^{\$}P<0.05 vs. Control, MitoQ, and vehicle. MitoQ, Mitoquinol; TLR, toll-like receptor.

that malathion may induce renal damage, while MitoQ may mitigate these effects, offering partial nephroprotection. The collective damage score for liver and kidney changes are shown in Table III.

Discussion

The application of the organophosphate insecticide malathion, in agriculture and veterinary practices, particularly in developing countries, has led to notable environmental contamination and poses serious health risks to humans and animals (8). The

hepato-renal toxicity associated with malathion exposure is primarily associated with oxidative stress, activation of inflammatory cytokines, disruption of metabolic processes, induction of apoptosis and alterations in gene expression (3).

The findings of the present study highlighted the hepatotoxic and nephrotoxic effects of malathion and the protective role of MitoQ in mitigating these toxic alterations. Malathion exposure resulted in a significant increase in liver and kidney function markers, including AST and ALP levels, which were markedly elevated compared with the control group. This increase in hepatic enzyme levels indicated

Table III. Histopathological scores.

Group	Mean liver damage score	Mean kidney damage score
Control	0.20±0.13 ^c	0.10±0.10 ^c
Malathion	2.80±0.20 ^a	2.60±0.22 ^a
Malathion + MitoQ	1.10±0.15 ^b	1.20±0.18 ^b
MitoQ	0.30±0.10 ^c	0.20±0.12 ^c
Vehicle	0.30±0.11 ^c	0.10±0.10 ^c

n=10 per group. Superscript letters within the same column indicate statistically significant differences at $P<0.05$. Groups sharing the same letter are not significantly different. 0=normal histology, 1=mild degeneration/infiltration, 2=moderate necrosis/congestion/inflammation, 3=severe damage with widespread cell death and infiltration. MitoQ, mitoquinol.

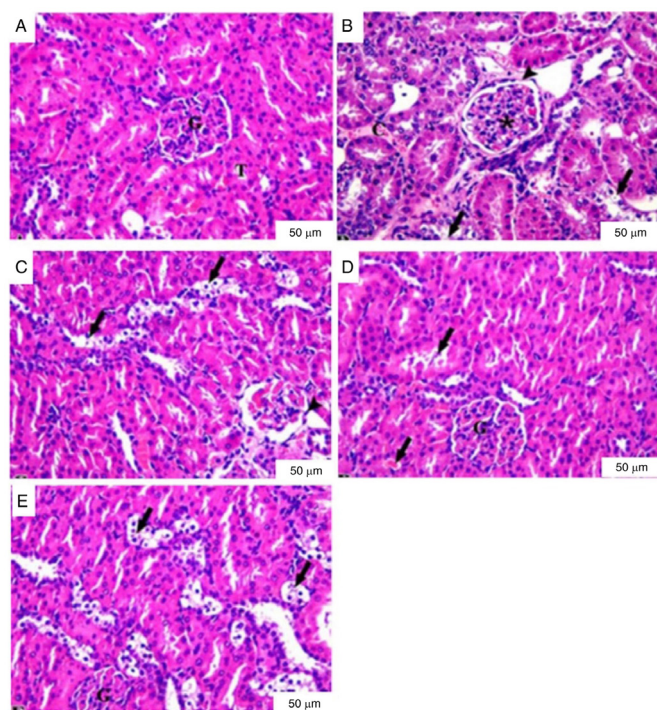


Figure 6. Hematoxylin and eosin-stained renal cortex of different treatment groups. (A) Control group revealed normal intact glomeruli (indicated with the letter G) and convoluted tubules (indicated with the letter T). (B) Malathion group revealed thickening of the glomerular basement membrane (arrowhead), mesangial expansion (*), vacuolar degeneration of renal tubules (arrows) and congestion of renal blood vessels (indicated with the letter C). (C) Malathion + MitoQ group revealed mild thickening of glomerular basement membrane (arrowhead) and vacuolar degeneration of renal tubules (arrows). (D) MitoQ group revealed intact renal glomeruli (indicated with the letter G) and an accumulation of a proteinaceous substance inside the lumen of some renal tubules (arrows). (E) Vehicle group revealed intact renal glomeruli (indicated with the letter G) and vacuolar degeneration of renal tubules (arrows). MitoQ, mitoquinol.

hepatocellular damage, potentially due to oxidative stress and inflammatory responses induced by malathion exposure (38). Co-administration with MitoQ significantly reduced these elevations, suggesting its ability to protect hepatic cells by counteracting oxidative damage and preserving liver function.

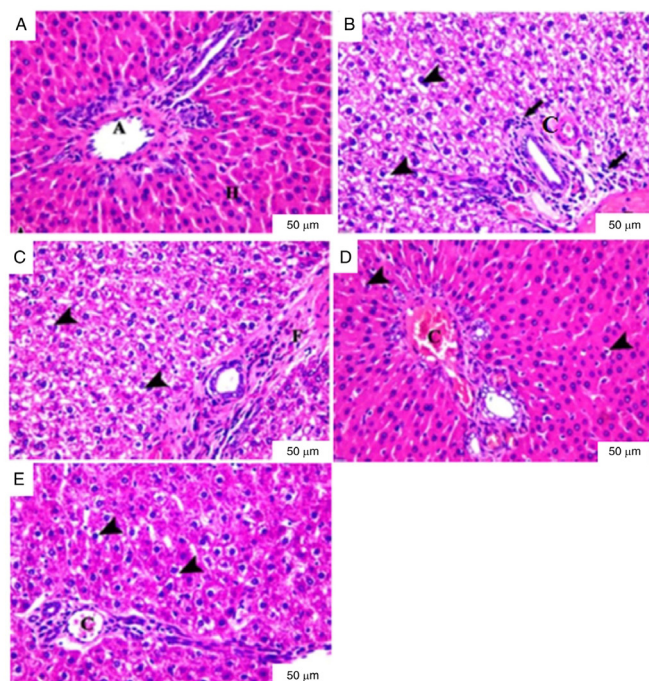


Figure 7. Hematoxylin and eosin-stained portal areas of livers of different treatment groups. (A) Control group demonstrated normal hepatic architecture with polyhedral-shaped hepatocyte and centrally located nuclei (indicated with the letter H) and hepatic arteries (indicated with the letter A). (B) Malathion group revealed severe congestion of hepatic blood vessels (indicated with the letter C), swelling and macro steatosis of hepatocytes with nuclear pyknosis (arrowheads) and peri-portal infiltration of inflammatory cells (arrows). (C) Malathion + MitoQ group revealed swelling and macro steatosis of hepatocytes with nuclear pyknosis (arrowheads), and mild increase of fibrous tissue (indicated with the letter F) in the portal area. (D) MitoQ group revealed congestion of hepatic blood vessels (indicated with the letter C) and mild steatosis of some hepatocytes (arrowheads). (E) Vehicle group revealed congestion of hepatic blood vessels (indicated with the letter C) and steatosis of hepatocytes (arrowheads). MitoQ, mitoquinol.

Similarly, malathion exposure significantly reduced total protein, albumin and globulin levels, which are key indicators of hepatic synthetic capacity. The observed decline in these parameters suggests liver dysfunction and impaired protein metabolism, consistent with a previous study (39). However, MitoQ co-administration partially restored levels of these factors, indicating its potential role in preserving hepatic integrity and function. Moreover, malathion significantly elevated kidney function markers, including creatinine, urea and uric acid levels, suggesting renal impairment and dysfunction. The increase in these markers reflects glomerular and tubular damage, probably due to oxidative stress, inflammation and apoptosis induced by malathion (40). MitoQ co-administration significantly mitigated these increases, restoring renal function parameters closer to normal levels. This protective effect can be attributed to the antioxidant properties of MitoQ, which reduce mitochondrial oxidative stress and prevent nephrotoxicity. Notably, MitoQ alone did not induce significant changes in liver and kidney function markers, indicating that it does not negatively affect hepatic or renal physiology in normal conditions. These results align with previous findings demonstrating the therapeutic potential of MitoQ in counteracting oxidative stress-induced organ damage without exerting toxic effects (18).

MitoQ, a mitochondria-targeted antioxidant, has demonstrated protective effects on liver and kidney function by reducing oxidative stress and inflammation (18). It lowers hepatic markers such as ALT and AST, indicating reduced liver damage and improves renal function by decreasing creatinine and blood urea nitrogen levels (41). Notably, Cengiz *et al* (42) demonstrated that ellagic acid effectively mitigated D-galactosamine-induced hepatic injury in rats, as evidenced by reduced serum ALT, AST and ALP levels and improved histological features, including reduced expression of the pro-apoptotic markers Bax and caspase-3. Similar to the findings of the present study, the authors' study highlights the importance of targeting biochemical markers and apoptotic signaling pathways to assess hepatoprotective effects. The histopathological improvements observed with MitoQ in the present study align with those reported for ellagic acid, reinforcing the value of integrating cellular, molecular and tissue-level evaluations in toxicological models.

The observed mitochondrial dysfunction and oxidative stress following malathion exposure are consistent with prior research associating organophosphate toxicity with mitochondrial damage and ROS overproduction. Malathion significantly decreases mitochondrial antioxidant enzyme levels (SOD, GPx and GSH) and ATP production while elevating mitochondrial ROS, indicating oxidative stress and energy depletion. These findings align with previous studies demonstrating that pesticides induce mitochondrial dysfunction by disrupting the electron transport chain, reducing ATP synthesis and increasing ROS levels, ultimately leading to oxidative damage and apoptosis (38,43).

The present study highlighted the mitochondria-targeted antioxidant MitoQ as a protective agent against malathion-induced oxidative stress, apoptosis and inflammation, as indicated by changes in cytochrome *c*, TNF- α , PGC-1 α and TFAM levels. Malathion induces mitochondrial dysfunction and inflammatory responses, leading to cellular damage (41). In the present study, increased cytochrome *c* levels in the malathion-only group suggest enhanced mitochondrial membrane permeabilization and apoptosis, consistent with a previous study which revealed that malathion triggers oxidative stress-induced mitochondrial apoptosis (44). However, MitoQ administration significantly reduced cytochrome *c* release, indicating its ability to stabilize mitochondrial membranes and limit apoptotic signaling, as seen in its neuroprotective effects following ischemia/reperfusion injury (45).

Similarly, TNF- α levels were significantly elevated in malathion-treated rats confirming an inflammatory response. TNF- α is a key pro-inflammatory cytokine that activates NF- κ B pathways, promoting further oxidative stress and apoptosis (46). The ability of MitoQ to suppress TNF- α levels suggests that it attenuates inflammation by inhibiting mitochondrial ROS production and NF- κ B activation, a mechanism consistent with its observed effects in brain ischemia and neuroinflammation (22). However, despite significant reductions in TNF- α observed in the present study, levels remained increased compared with control values, suggesting only partial suppression of the inflammatory cascade. This indicates that MitoQ alone may not fully prevent inflammation and requires combinatorial therapeutic approaches. While the present study primarily focused on *in vivo* assessment of

the protective effects of MitoQ, incorporating *in vitro* models can provide valuable mechanistic insights. Teksoy *et al* (47) demonstrate the utility of *in vitro* neurotoxicity models in evaluating oxidative and apoptotic responses, using immunohistochemical markers such as Bax, TNF- α and TUNEL in brain tissue affected by hepatic encephalopathy. The authors' findings underscore the role of mitochondrial pathways and inflammation in tissue damage and the importance of targeting these via antioxidant therapies such as silymarin (35). These observations are mechanistically aligned with the findings of the present study, where MitoQ mitigated malathion-induced oxidative stress and apoptosis in hepatic and renal tissues. Thus, future integration of complementary *in vitro* assays would strengthen the mechanistic interpretation of the protective roles of MitoQ, especially through detailed analysis of the Bax/Bcl-2 balance, caspase activation and NF- κ B signaling under controlled cellular conditions.

Regarding mitochondrial biogenesis, the significant reduction in PGC-1 α and TFAM levels in malathion-treated mice indicated impaired mitochondrial function and biogenesis suppression (48). PGC-1 α is a master regulator of mitochondrial biogenesis, working in coordination with TFAM, a key factor in mitochondrial DNA (mtDNA) maintenance and replication. Decreased levels of these markers are associated with reduced mitochondrial efficiency and increased oxidative damage (49). The ability of MitoQ to restore PGC-1 α and TFAM expression levels suggests that it promotes mitochondrial biogenesis and protects against mtDNA damage, a mechanism previously observed in a study on mitochondrial dysfunction in ischemic brain injury (45). These findings suggest that malathion-induced mitochondrial damage and inflammation demonstrate a trend towards mitigation by MitoQ, reinforcing its potential as a therapeutic agent.

Malathion exposure significantly increased Bax expression, enhancing apoptotic signaling while simultaneously reducing Bcl-2 levels, impairing cell survival mechanisms. These findings align with previous reports demonstrating that malathion induces mitochondrial oxidative stress, leading to cytochrome *c* release and activation of the intrinsic apoptotic pathway (50,51).

The significant reduction in Bax expression in the malathion + MitoQ group suggests that MitoQ effectively attenuates mitochondrial-mediated apoptosis, potentially by reducing oxidative stress and stabilizing mitochondrial membranes, a mechanism previously reported in ischemia-reperfusion injury models (52). Conversely, the observed increase in Bcl-2 expression following MitoQ co-treatment suggests that MitoQ enhances cell survival by modulating anti-apoptotic pathways. Bcl-2 is crucial in maintaining mitochondrial integrity, preventing cytochrome *c* release and inhibiting apoptosis (53). Notably, in the present study the highest Bcl-2 levels were revealed in the MitoQ-only and vehicle groups, particularly in hepatic tissues, suggesting that MitoQ may independently upregulate protective anti-apoptotic pathways in the liver. This is consistent with a previous report demonstrating that MitoQ upregulates cellular survival markers in models of oxidative stress-related liver damage (54). These findings confirm that MitoQ may provide notable protection against malathion-induced apoptosis in renal and hepatic tissues, although partial apoptotic dysregulation persists.

The significant increase in TNF- α and NF- κ B expression in renal and hepatic tissues following malathion exposure suggests an inflammatory response, consistent with a prior study associating organophosphate toxicity to oxidative stress and inflammation (3). TNF- α , a key pro-inflammatory cytokine, performs a key role in hepatorenal damage by activating downstream apoptotic and inflammatory cascades. Increased NF- κ B expression further suggests the activation of inflammatory pathways, as NF- κ B is a notable transcription factor regulating cytokine production and oxidative stress responses (55). In the present study, the observed reduction in TNF- α and NF- κ B levels in the malathion + MitoQ group highlighted the ability of MitoQ to mitigate inflammatory damage, possibly by reducing mitochondrial oxidative stress and preventing excessive NF- κ B activation. This aligns with previous findings that mitochondrial-targeted antioxidants suppress NF- κ B signaling and reduce pro-inflammatory cytokines in oxidative stress models (56).

In the present study, the significant upregulation of TLR2 and TLR4 expression in renal and hepatic tissues following malathion exposure suggested an activated innate immune response, consistent with previous findings linking organophosphate toxicity to inflammatory pathway activation (57). TLR2 and TLR4 are key pattern recognition receptors that detect pathogen-associated molecular patterns and damage-associated molecular patterns (DAMPs), leading to NF- κ B activation and cytokine release (58).

The increased expression of TLRs in the malathion-only group aligns with a previous study demonstrating that pesticide-induced oxidative stress enhances TLR signaling, exacerbating inflammation and tissue damage (59). The significant reduction in TLR2 and TLR4 expression in the malathion + MitoQ group suggests that MitoQ effectively downregulates inflammatory signaling by reducing mitochondrial ROS production and preventing NF- κ B activation. This is consistent with prior research demonstrating that mitochondrial-targeted antioxidants can suppress TLR-mediated inflammation in oxidative stress models (60).

The observed oxidative stress, inflammation and apoptosis in malathion-induced hepatorenal injury may be associated with mechanisms involving key transcription factors such as NF- κ B and proinflammatory cytokines. A recent study by Cengiz *et al* (61) demonstrated that co-exposure to cadmium and arsenic considerably elevated oxidative stress, increased MDA levels and activated NF- κ B and IL-1 β expression in rat liver tissue, which were ameliorated by curcumin administration. Their findings align with the results of the present study, revealing that malathion exposure partially promotes oxidative and inflammatory cascades via NF- κ B and TLR pathways and that MitoQ modulates these signals. Moreover, both studies highlight the importance of antioxidant defense mechanisms, such as SOD activity, as a protective axis. These converging findings further support the therapeutic potential of mitochondria-targeted antioxidants in restoring redox balance and regulating inflammatory gene expression under toxicant stress.

The NF- κ B pathway coordinates cellular responses to oxidative stress and inflammation (62). Upon exposure to malathion, elevated levels of ROS serve as upstream activators of the NF- κ B pathway, leading to the translocation of

NF- κ B to the nucleus, where it promotes the transcription of pro-inflammatory genes such as TNF- α , IL-1 β and IL-6. This inflammatory amplification loop exacerbates tissue injury and primes cells for mitochondrial apoptosis. Mechanistically, NF- κ B signaling has been demonstrated to regulate the balance between pro-apoptotic and anti-apoptotic Bcl-2 family proteins. Increased NF- κ B activity can upregulate Bax and downregulate Bcl-2, leading to mitochondrial outer membrane permeabilization, cytochrome *c* release and caspase-9/-3 activation (53). In the present study, malathion-induced activation of NF- κ B was associated with elevated Bax expression and reduced Bcl-2 expression, consistent with enhanced apoptotic signaling. Notably, MitoQ treatment suppressed NF- κ B activation and modulated the Bax/Bcl-2 ratio favorably, suggesting that its protective effects are partly mediated by inhibiting NF- κ B-driven mitochondrial apoptosis. This aligns with a study reporting the capacity of the antioxidant to disrupt redox-sensitive transcription factors and restore mitochondrial homeostasis (63).

Notably, the absence of significant differences in TLR expression among the control, MitoQ-only and vehicle groups suggested that MitoQ may not inherently modulate TLR pathways under physiological conditions, reinforcing its safety as a therapeutic agent. Notably, while TLR4 expression in renal tissues was fully restored with MitoQ treatment, hepatic tissues retained a slight elevation, suggesting that the liver may be more susceptible to persistent inflammatory signaling following pesticide exposure. This aligns with previous findings indicating that hepatic TLR4 activation is central to systemic inflammatory responses to toxicants (57).

The findings of the present study collectively demonstrated that malathion exposure induces oxidative stress, inflammatory activation and structural damage in renal and hepatic tissues, while MitoQ provided partial protection. Malathion significantly reduced mitochondrial antioxidant enzyme levels (including SOD, GPx and GSH), decreased ATP production and increased mitochondrial ROS levels, indicating severe oxidative stress and mitochondrial dysfunction. This oxidative imbalance is associated with the upregulation of TLR2 and TLR4 expression in renal and hepatic tissues, suggesting that malathion-induced mitochondrial dysfunction contributes to innate immune activation via TLR-mediated inflammatory pathways. The protective effects of MitoQ against malathion-induced toxicity appears to be associated with its ability to modulate redox-sensitive inflammatory signaling pathways (64). One proposed mechanism is that MitoQ inhibits mitochondrial ROS production, which serves as a key upstream activator of the TNF- α /NF- κ B pathway. Under oxidative stress conditions, elevated mitochondrial ROS activates I κ B kinase, leading to the phosphorylation and degradation of I κ B α , thereby promoting the nuclear translocation of NF- κ B and the transcription of pro-inflammatory cytokines such as TNF- α and IL-6 (65). By scavenging mitochondrial ROS, MitoQ prevents this activation cascade and dampens NF- κ B-driven inflammation (66).

In addition, TLRs, particularly TLR2 and TLR4, perform a central role in initiating innate immune responses to cellular damage, including oxidative damage caused by pesticides such as malathion. Upon activation, TLRs recruit adaptor proteins such as MyD88, leading to downstream

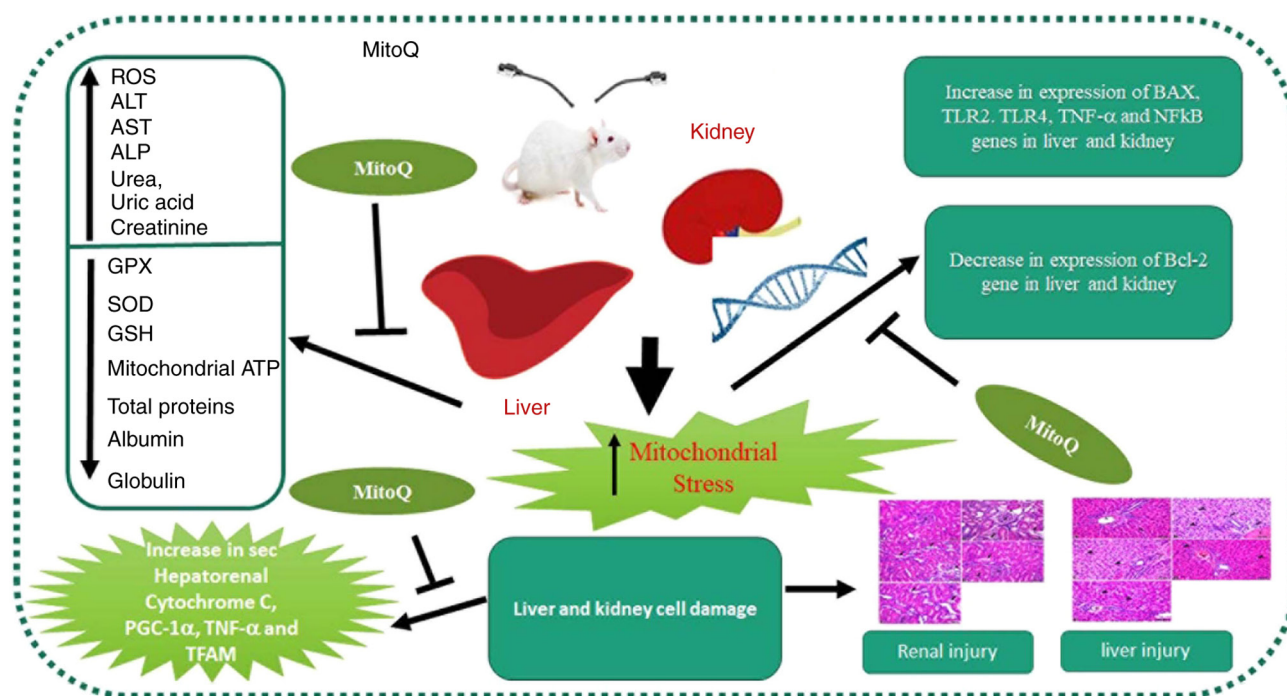


Figure 8. Collective protective impacts of MitoQ against malathion-induced hepatorenal toxicity. MitoQ, mitoquinol; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSH, glutathione; ROS, reactive oxygen species; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; PGC-1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; TFAM, mitochondrial transcription factor A.

activation of NF- κ B and enhanced expression of inflammatory mediators (67). Mitochondrial dysfunction and the associated ROS production can serve as endogenous danger signals (such as DAMPs), triggering TLR pathways (68). MitoQ, by preserving mitochondrial integrity and reducing ROS accumulation, possibly limits the release of these DAMPs, thereby preventing excessive TLR stimulation and downstream inflammatory signaling (69).

MitoQ is a lipophilic cation that selectively accumulates in mitochondria due to its triphenylphosphonium moiety, allowing it to neutralize mitochondrial ROS, where they are predominantly and directly produced (18). By reducing mitochondrial superoxide and hydrogen peroxide levels, MitoQ prevents oxidative damage to mtDNA, lipids and proteins. This suppression of mitochondrial oxidative stress interrupts the activation of redox-sensitive transcription factors such as NF- κ B, reducing the transcription of pro-inflammatory cytokines such as TNF- α and IL-6. The preservation of mitochondrial function also contributes to cellular energy maintenance and attenuates apoptosis via Bax/Bcl-2 signaling regulation.

MitoQ effectively restored mitochondrial antioxidant defenses and ATP production while reducing mitochondrial ROS levels, aligning with the suppression of TLR2 and TLR4 expression, thereby mitigating malathion-induced inflammatory responses. These findings support the hypothesis that oxidative stress and inflammation are associated with malathion toxicity and that MitoQ acts primarily through mitochondrial protection to suppress downstream inflammatory signaling. The toxicity of malathion disrupts mitochondrial function by elevating ROS production and impairing oxidative phosphorylation. This imbalance causes

a drop in ATP levels and promotes mitochondrial membrane depolarization. As a result, cytochrome *c* is released into the cytoplasm, activating the intrinsic apoptotic pathway through caspase-9 and downstream caspase-3 (3). The results of the present study support this mechanism, demonstrating reduced mitochondrial SOD, ATP depletion and increased Bax expression in malathion-treated rats. Conversely, co-treatment with MitoQ restored mitochondrial function and preserved redox homeostasis. This was accompanied by an upregulation of Bcl-2 and a decrease in Bax, suggesting that MitoQ exerts anti-apoptotic effects via stabilization of the mitochondrial membrane and inhibiting cytochrome *c* release.

Histopathological analysis further confirmed the hepatorenal damage induced by malathion, as evidenced by vascular congestion, hepatocyte swelling, nuclear pyknosis and inflammatory infiltration in the liver, along with glomerular thickening, renal tubular degeneration and vascular congestion in the kidney (51). Co-treatment with MitoQ partially alleviated these pathological alterations, reducing tissue damage and fibrosis. However, some residual structural abnormalities persisted, consistent with the incomplete recovery observed in mitochondrial and inflammatory markers. While the present study provided comprehensive *in vivo* evidence demonstrating the protective effects of MitoQ against malathion-induced oxidative stress, inflammation and apoptosis in hepatic and renal tissues, the absence of *in vitro* validation must be acknowledged as a limitation. Although molecular markers such as TNF- α , NF- κ B, Bax, Bcl-2, TLR2 and TLR4 were assessed via qPCR and supported by histopathological and mitochondrial analyses, future studies incorporating *in vitro* models (such as hepatocytes and renal epithelial cells) will be essential to confirm the direct cellular mechanisms of MitoQ

action. These models can further dissect the signaling pathways involved in regulating specific markers under controlled experimental conditions. Although the present study evaluated the protective effects of MitoQ over a 30-day *in vivo* treatment period, it did not include cytotoxicity assays to assess dose-related or time-dependent interactions at the cellular level. Future studies will incorporate *in vitro* cytotoxicity assays to validate the safety profile of MitoQ and its temporal modulation of malathion-induced cellular toxicity.

While the present study confirmed the short-term protective effects of MitoQ against malathion-induced hepatic and renal toxicity, the long-term efficacy and safety of such treatments remains to be fully elucidated. Future research should investigate dose-response relationships, chronic exposure scenarios and varied treatment timepoints to improve defining of the therapeutic window. Additionally, chronic exposure models and post-treatment follow-ups would offer valuable insights into whether MitoQ provides sustained protection and modulates adaptive stress responses over time.

Alternative delivery platforms such as nanocarriers, liposomal encapsulation or targeted prodrug formulations may improve the bioavailability, tissue specificity and pharmacokinetic profile of MitoQ to enhance its clinical potential further. These approaches could allow for lower dosing, reduced systemic toxicity and optimized outcomes, particularly under long-term or repeated environmental toxin exposure conditions. Moreover, combinatory therapies using MitoQ alongside other well-established antioxidants such as N-acetylcysteine, resveratrol or silymarin may offer synergistic protective effects by concurrently targeting redox imbalance, inflammation and mitochondrial dysfunction. Such regimens could prove especially effective in mitigating complex, multi-organ toxicological injuries and warrant further exploration in future preclinical and translational studies.

In conclusion, the present study demonstrated that MitoQ offered protective effects against malathion-induced hepatic and renal toxicity by attenuating oxidative stress, modulating inflammatory responses and regulating apoptotic signaling pathways. Beyond these findings, the present study underscored the therapeutic potential of mitochondria-targeted antioxidants in managing organophosphate-induced toxicity. Given the growing concern regarding environmental pesticide exposure and its systemic health consequences, MitoQ may serve as a promising adjunct or standalone intervention in mitigating such effects. However, to fully validate its clinical relevance, future research should investigate dose-response relationships, long-term safety and the potential synergistic effects of MitoQ when combined with other antioxidant or anti-inflammatory agents. Additionally, studies across different models and human cell lines will be essential to translate these findings into practical therapeutic strategies. The collective protective effects of MitoQ are illustrated in Fig. 8.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contribution

SAA conceptualized, analyzed data and wrote the present study. The author has read and approved the final manuscript. SAA confirms the authenticity of all the raw data.

Ethics approval and consent to participate

All animal procedures were conducted in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011). The Ethics Committee of Taif University for Animal Use in Research reviewed and approved the study protocol under project (TU-DSPP-2024-260). Efforts were made to minimize animal suffering and reduce the number of animals used.

Patient consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

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