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*Basic Study*

# Effects of fullerenol C60 on hepatic ischemia-reperfusion injury in desflurane-anesthetized rats



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**Abstract**

BACKGROUND

Hepatic ischemia-reperfusion injury (IRI) occurs in various clinical settings, including liver surgery, transplantation, trauma, hemorrhagic shock, and temporary vascular occlusion. It is associated with oxidative stress, inflammation, and hepatocellular injury. Desflurane is an inhalational anesthetic, and fullerenol C60 is a carbon-derived compound investigated for possible tissue-protective effects.

AIM

To evaluate their effects on hepatic IRI in rats.

METHODS

Thirty rats were randomly divided into five groups ($n$ = 6 each): Control, IRI, IRI + fullerenol C60 (IRI-F), IRI + desflurane, and IRI-F + desflurane (IRI-F-D). Hepatic ischemia was induced for 120 minutes, followed by 120 minutes of reperfusion. Fullerenol C60 (100 mg/kg, intraperitoneally) was administered 30 minutes before ischemia, and desflurane (6%) was administered during the ischemia-reperfusion period. Malondialdehyde (MDA) levels and catalase (CAT), glutathione-S-transferase (GST), and arylesterase (ARE) activities were measured. Histopathological examination included assessment of hepatocyte degeneration, sinusoidal dilatation, pyknotic nuclei, necrotic cells, and parenchymal mononuclear cell infiltration.

RESULTS

MDA levels were significantly higher in the IRI group than in the control group (0.25 ± 0.01 nmol/mg protein *vs* 0.10 ± 0.01 nmol/mg protein, $P < 0.0001$), whereas all treatment groups showed significantly lower MDA levels than the IRI group. CAT, GST, and ARE activities were significantly reduced in the IRI group but improved in the treatment groups ($P < 0.0001$ for all). Histopathological analysis demonstrated significant intergroup differences in hepatocyte degeneration ($P = 0.003$), sinusoidal dilatation ($P =$

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0.018), pyknotic nuclei ($P$ = 0.031), and mononuclear cell infiltration ($P$ = 0.003). The most severe injury was observed in the IRI group, whereas fullerenol-treated groups showed marked improvement. Necrotic cell counts did not differ significantly among groups ($P$ = 0.113).

CONCLUSION

Fullerenol C60 attenuated hepatic IRI by reducing lipid peroxidation, preserving antioxidant enzyme activity, and improving histopathological findings. Desflurane also exerted protective effects. However, direct pairwise comparisons between the IRI-F and IRI-F-D groups showed no statistically significant differences in any biochemical or histopathological parameter, indicating that combined treatment did not provide a significant additional benefit over fullerenol C60 alone (all $P$ > 0.05). Fullerenol C60 may represent a promising adjunctive approach for reducing hepatic injury associated with ischemia-reperfusion.

**Key Words:** Hepatic ischemia-reperfusion; Fullerenol C60; Desflurane; Oxidative stress; Liver injury

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**Core Tip:** Hepatic ischemia-reperfusion injury (IRI) remains a major challenge during liver surgery and transplantation because oxidative stress and inflammation can cause significant tissue damage. This experimental study demonstrated that fullerenol C60 significantly reduced lipid peroxidation, preserved endogenous antioxidant enzyme activity, and improved histopathological liver injury in desflurane-anesthetized rats.

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Desflurane also provided partial protection, although the combined treatment showed no clear superiority over fullerenol alone. These findings suggest that fullerenol C60 may be a promising adjunctive strategy for limiting hepatic IRI and preserving liver function. Further translational and clinical studies are required to confirm optimal dosing, mechanisms, safety, and therapeutic applicability in humans.

## INTRODUCTION

Hepatic ischemia-reperfusion injury (IRI) is a major cause of postoperative hepatic dysfunction and liver failure. It occurs in various clinical settings, including liver surgery, transplantation, trauma, and hemorrhagic shock[1-3]. Although restoration of blood flow is essential for tissue survival, reperfusion paradoxically aggravates liver injury through oxidative stress, inflammatory activation, microvascular dysfunction, and hepatocellular damage. Studies have shown that the systemic impact of IRI results from the activation of neutrophils, the complement cascade, and the release of proinflammatory and vasoactive mediators, including eicosanoids, NO, cytokines, and reactive oxygen species[4-6]. Despite extensive investigation of ischemic conditioning, antioxidant and anti-inflammatory agents, anesthetic-related interventions, and mitochondria-targeted therapies, no consistently effective clinical treatment has yet been established.

Hepatic injury develops during both ischemia and reperfusion phases. Ischemia leads to ATP depletion, intracellular acidosis, calcium overload, and mitochondrial dysfunction, whereas reperfusion triggers excessive reactive oxygen species generation and inflammatory signaling, resulting in lipid peroxidation, endothelial injury, and progressive hepatocellular damage[7-9].

Desflurane is a low-solubility volatile anesthetic that has been investigated as a potential conditioning agent in hepatic IRI. Its possible protective effects have been linked to modulation of oxidative stress, inflammatory responses, and cellular injury pathways, although current evidence remains limited and heterogeneous[10]. Fullerenol C60, a water-soluble polyhydroxylated fullerene derivative, has also attracted interest because of its potential to reduce oxidative tissue injury and preserve antioxidant defense

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mechanisms. Experimental studies have suggested that fullerenol C60 may attenuate biochemical and histopathological liver damage in oxidative stress-related conditions[11]. Although the hepatoprotective effects of fullerenol C60 have previously been investigated in hepatic ischemia-reperfusion injury under sevoflurane anesthesia, it remains unclear whether similar protection can be achieved in the presence of desflurane. Volatile anesthetics may differ in their pharmacological characteristics and cellular protective profiles, potentially influencing oxidative stress responses and tissue injury. Therefore, evaluating fullerenol C60 in conjunction with desflurane may provide additional insight into the interaction between antioxidant therapy and anesthetic-mediated organ protection. Therefore, this experimental study was designed to investigate the effects of fullerenol C60 and desflurane on hepatic IRI in rats by evaluating oxidative stress markers, antioxidant enzyme activities, and histopathological changes in liver tissue.

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## MATERIALS AND METHODS

### *Animals and ethical approval*

The experimental protocol was reviewed and approved by the Gazi University Experimental Animals Ethics Committee prior to the study (Approval No. G.Ü.ET-22.021; Approval date: 24 February 2022). All animal care and experimental procedures were performed in accordance with institutional guidelines and internationally accepted principles for the care and use of laboratory animals. Thirty male Wistar albino rats aged 4 months and weighing 250-350 g was housed at the Gazi University Laboratory Animals Breeding and Experimental Research Center under controlled environmental conditions (20-21 °C; 12-hour light/12-hour dark cycle) with free access to standard chow and water until 2 hours before anesthesia. All procedures were performed in accordance with established principles for the care and use of laboratory animals[12]. The animals were randomly allocated into five groups ($n$ = 6 each): Control, IRI, IRI + fullerenol C60 (IRI-F), IRI + desflurane (IRI-D), and IRI-F + desflurane (IRI-F-D).

*Ischemia-reperfusion model*

A standardized partial hepatic ischemia-reperfusion model was used. After induction of anesthesia, the rats were placed in the supine position, and body temperature was maintained by thermal insulation throughout the procedure. Following abdominal skin disinfection with povidone-iodine, a midline laparotomy was performed to expose the liver and hepatoduodenal ligament. The portal triad structures supplying the median and left lateral lobes were identified, and partial hepatic inflow occlusion was achieved by applying an atraumatic microvascular clamp to the porta hepatis (Figure 1). This interrupted arterial and portal venous inflow to approximately 70% of the liver while preserving venous drainage of the non-ischemic lobes, corresponding to an experimental partial Pringle maneuver. Successful induction of ischemia was confirmed by pallor of the affected lobes. After 120 minutes of ischemia, the clamp was removed and reperfusion was allowed for 120 minutes, as confirmed by restoration of normal liver color. Sham-operated animals underwent the same surgical exposure without vascular clamping. At the end of the reperfusion period, all rats were sacrificed under deep anesthesia by blood collection from the heart, and liver tissue samples were harvested for biochemical and histopathological analyses.

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*Anesthesia*

All rats were anesthetized with ketamine (50 mg/kg, intraperitoneal injection; Ketalar®, Pfizer, Türkiye) and xylazine hydrochloride (10 mg/kg, intraperitoneal injection; 2%, Alfazyne, Ege Vet, İzmir, Türkiye) before surgery. Anesthetic depth was assessed by monitoring responses to surgical stimulation, and additional ketamine was administered when necessary. In the desflurane-treated groups (IRI-D and IRI-F-D), desflurane was administered at 6% in a transparent closed chamber throughout the ischemia and reperfusion periods. The chamber was equipped with separate inlets for oxygen and desflurane delivery and outlet openings for waste gas evacuation. Oxygen was used as the carrier gas during desflurane administration. Rats in the remaining groups maintained spontaneous respiration under injectable anesthesia alone.

***Histopathological analysis***

Histopathological evaluation of liver tissues was performed at the Department of Histology and Embryology, Kirikkale University. At the end of the experimental period, liver specimens were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Sections of 5 μm thickness were cut from paraffin blocks using a microtome (Leica RM2245, Germany) and stained with hematoxylin and eosin for evaluation of general tissue architecture and ischemia-reperfusion-related histomorphological alterations. Sections were examined under a light microscope (Leica DM 4000B, Germany) connected to a computer system, and representative photomicrographs were captured using Leica LAS V4.9 software. Histopathological examination focused on hepatocellular degeneration, sinusoidal dilatation, prenecrotic and necrotic cellular changes, pyknotic nuclear alterations, and parenchymal mononuclear inflammatory cell infiltration. Tissue injury was graded semiquantitatively according to the histological scoring method described by Abdel-Wahhab *et al*[13], where (-), (+), (++), and (+++) indicated absent, mild, moderate, and severe injury, respectively. All histological assessments were performed by the same pathologist under blinded conditions.

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***Biochemical analysis***

Biochemical analyses were performed at the Department of Medical Biochemistry, Gazi University. Lipid peroxidation and oxidative stress in liver tissue were evaluated by measuring malondialdehyde (MDA) levels together with catalase (CAT), glutathione-S-transferase (GST), and arylesterase (ARE) activities. MDA levels were determined by the thiobarbituric acid method, based on the reaction between MDA and thiobarbituric acid under acidic conditions to form a pink chromogen with maximal absorbance at 532 nm. 1,1,3,3-tetraethoxypropane was used as the standard for MDA quantification. CAT activity was measured according to the method described by Aebi[14], based on the decomposition of hydrogen peroxide and the corresponding decrease in absorbance at

240 nm. GST activity was determined by the method of Habig *et al*[15], which is based on the GST-catalyzed conjugation reaction monitored spectrophotometrically as an increase in absorbance at 340 nm. ARE activity was measured according to the spectrophotometric method described by Furlong *et al*[16], based on the hydrolysis of phenylacetate and the corresponding change in absorbance at 270 nm at 25 °C.

### *Statistical analysis*

Statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, United States). Data are reported as mean ± SE of the mean. The normality of data distribution was assessed using the Shapiro-Wilk test. Comparisons among the five groups were performed using one-way analysis of variance (ANOVA) for normally distributed variables, followed by Tukey's *post hoc* test. Variables that did not meet the assumption of normality were analyzed using the Kruskal-Wallis test followed by appropriate pairwise comparisons. A $P$ value of $< 0.05$ was considered statistically significant.

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## RESULTS

### *Histopathological results*

Histopathological examination revealed significant intergroup differences in hepatocyte degeneration, sinusoidal dilatation, pyknotic nuclei, and parenchymal mononuclear cell infiltration, whereas the number of cells undergoing necrosis did not differ significantly among groups. Hepatocyte degeneration was significantly greater in the IRI group than in the control group (1.67 ± 0.21 *vs* 0.33 ± 0.21, $P = 0.003$). Lower degeneration scores were observed in the IRI-F (0.67 ± 0.21), IRI-D (1.00 ± 0.26), and IRI-F-D (0.67 ± 0.21) groups. A similar pattern was observed for sinusoidal dilatation ($P = 0.018$). The highest score was found in the IRI group (1.83 ± 0.31), whereas lower scores were recorded in the IRI-F (0.83 ± 0.17), IRI-D (1.17 ± 0.31), and IRI-F-D (1.00 ± 0.26) groups. Pyknotic nuclei were significantly more common in the IRI group compared with the control group (1.33 ± 0.21 *vs* 0.33 ± 0.21, $P = 0.031$). Lower values were found in the IRI-F (0.50 ± 0.22), IRI-D (0.67 ±

0.21), and IRI-F-D (0.67 ± 0.21) groups. Parenchymal mononuclear cell infiltration was highest in the IRI group (1.67 ± 0.33) and lowest in the control group (0.17 ± 0.17), with a significant overall difference among groups ($P$ = 0.003). Lower infiltration scores were observed in the IRI-F (0.67 ± 0.21), IRI-D (1.00 ± 0.26), and IRI-F-D (0.67 ± 0.21) groups. Although the number of cells undergoing necrosis was higher in the IRI group than in the control group (1.00 ± 0.00 *vs* 0.33 ± 0.21), the difference was not statistically significant ($P$ = 0.113).

Direct pairwise comparisons between the IRI-F and IRI-F-D groups demonstrated no statistically significant differences in hepatocyte degeneration, sinusoidal dilatation, pyknotic nuclei, necrotic cell counts, or parenchymal mononuclear cell infiltration ($P$ = 1.000, $P$ = 0.652, $P$ = 0.586, $P$ = 0.524, $P$ = 1.000, respectively).

Overall, the most pronounced histopathological injury was observed in the IRI group. Lower injury scores were observed in the treatment groups, particularly in the fullerenol-containing groups (Figure 2 and Table 1).

***Biochemical markers***

Biochemical analysis revealed significant intergroup differences in MDA, CAT, GST, and ARE levels ($P$ < 0.0001 for all parameters). MDA levels were significantly higher in the IRI group than in the control group (0.25 ± 0.01 nmol/mg protein *vs* 0.10 ± 0.01 nmol/mg protein, $P$ < 0.0001). All treatment groups showed lower MDA levels than the IRI group, with values of 0.14 ± 0.01 nmol/mg protein in the IRI-F group, 0.21 ± 0.01 nmol/mg protein in the IRI-D group, and 0.16 ± 0.01 nmol/mg protein in the IRI-F-D group. CAT activity was significantly lower in the IRI group than in the control group (170.93 ± 11.78 IU/mg protein *vs* 385.75 ± 32.07 IU/mg protein, $P$ < 0.0001). CAT activity was higher in the IRI-F group (327.82 ± 13.84 IU/mg protein), IRI-F-D group (282.25 ± 23.59 IU/mg protein), and IRI-D group (204.95 ± 19.30 IU/mg protein) than in the IRI group. A similar pattern was observed for GST activity ($P$ < 0.0001). GST activity decreased from 1.10 ± 0.07 IU/mg protein in the control group to 0.58 ± 0.04 IU/mg protein in the IRI group and increased to 0.95 ± 0.04 IU/mg protein, 0.78 ± 0.05 IU/mg protein, and 0.82 ± 0.11

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IU/mg protein in the IRI-F, IRI-D, and IRI-F-D groups, respectively. ARE activity also differed significantly among groups ($P < 0.0001$). ARE activity was 1.24 ± 0.07 IU/mg protein in the control group and 0.78 ± 0.03 IU/mg protein in the IRI group, whereas values of 1.15 ± 0.05 IU/mg protein, 1.01 ± 0.07 IU/mg protein, and 1.08 ± 0.06 IU/mg protein were observed in the IRI-F, IRI-D, and IRI-F-D groups, respectively.

Similarly, direct pairwise comparisons between the IRI-F and IRI-F-D groups revealed no statistically significant differences in MDA levels or CAT, GST, and ARE activities ($P = 0.077$, $P = 0.145$, $P = 0.210$, $P = 0.406$, respectively).

Overall, the IRI group showed the highest MDA levels and the lowest CAT, GST, and ARE activities, whereas more favorable biochemical values were observed in the treatment groups, particularly in the fullerenol-treated groups (Table 2).

## DISCUSSION

The present study demonstrated that hepatic ischemia-reperfusion injury was associated with significant oxidative stress and histopathological damage. Both fullerenol C60 and desflurane attenuated these alterations, although the protective effect was more pronounced in the fullerenol-treated groups. These findings support the potential hepatoprotective role of fullerenol C60 in experimental hepatic ischemia-reperfusion injury.

Hepatocellular injury progresses through apoptosis, necrosis, and regulated cell death pathways[17]. The severity of hepatic IRI is influenced by the duration of both ischemia and reperfusion[18]. In the present study, 120 minutes of ischemia followed by 120 minutes of reperfusion was sufficient to induce significant biochemical and histopathological alterations, supporting the successful establishment of acute hepatic IRI[19]. Although necrotic cell scores were numerically higher in the IRI group, the difference did not reach statistical significance. This may be related to the relatively short reperfusion interval, during which oxidative and inflammatory changes become more evident than advanced necrotic tissue destruction. Similar protocols have been widely

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used in experimental studies investigating early-phase hepatic IRI and potential protective interventions.

The histopathological findings observed in the present study are consistent with previous reports describing hepatocyte degeneration, sinusoidal dilatation, inflammatory cell infiltration, and nuclear alterations as characteristic features of hepatic ischemia-reperfusion injury[20,21]. Fullerenol C60 markedly attenuated these changes, supporting earlier studies that demonstrated its protective effects against oxidative tissue injury in different experimental models. The lower histopathological scores observed after fullerenol administration are in line with studies reporting reduced cellular degeneration, inflammatory infiltration, and tissue damage in experimental oxidative injury models[21,22]. Although necrotic cell counts were higher in the IRI group, their lack of statistical significance may reflect the relatively early assessment period. Overall, the histopathological findings were consistent with the biochemical results and support the hepatoprotective effects of the investigated interventions.

Biochemical findings further confirmed the development of hepatic IRI. The IRI group showed a marked increase in MDA levels and significant reductions in CAT, GST, and ARE activities, indicating enhanced lipid peroxidation and impairment of endogenous antioxidant defenses[23]. Similar biochemical alterations in experimental hepatic IRI models have been consistently reported. Treatment with fullerenol C60 significantly lowered MDA levels and improved antioxidant enzyme activities, suggesting attenuation of oxidative injury and preservation of redox balance[24]. The present findings should also be considered in relation to our previous study evaluating fullerenol C60 in a hepatic ischemia-reperfusion model under sevoflurane anesthesia[24]. In that study, fullerenol administration similarly reduced oxidative stress and attenuated histopathological injury. The current findings extend our previous observations by demonstrating that the hepatoprotective effects of fullerenol C60 are preserved under desflurane anesthesia, suggesting that these protective effects are not limited to a specific volatile anesthetic environment. Together, these findings suggest that the hepatoprotective effects of fullerenol C60 are not restricted to a specific volatile anesthetic

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environment and are primarily mediated through antioxidant mechanisms. Fullerenol C60 appears to act mainly by limiting oxidative damage and preserving antioxidant defense systems, whereas desflurane may modulate oxidative stress and cellular injury pathways[25,26]. The protective effects of fullerenol C60 are likely multifactorial. Fullerenol possesses a unique polyhydroxylated carbon cage structure that enables efficient scavenging of reactive oxygen species generated during reperfusion. By reducing oxidative stress, fullerenol may limit lipid peroxidation, preserve mitochondrial integrity, and prevent subsequent activation of inflammatory and cell death pathways. Previous studies have also suggested that fullerenol can enhance endogenous antioxidant defenses and attenuate tissue injury under conditions associated with oxidative stress[23,24]. In the present study, the marked reduction in MDA levels together with preservation of CAT, GST, and ARE activities supports the hypothesis that suppression of oxidative stress represents a major mechanism underlying the observed hepatoprotective effects. The observed protective effects of desflurane are consistent with previous reports suggesting that volatile anesthetics may attenuate ischemia-reperfusion injury through modulation of oxidative stress, inflammatory responses, and intracellular survival pathways[25,26]. Although the present study did not investigate molecular signaling pathways, our biochemical and histopathological findings support the concept that desflurane exerts a partial hepatoprotective effect in acute hepatic ischemia-reperfusion injury. Desflurane also exerted favorable biochemical effects, although the magnitude of improvement was less pronounced than that observed in the fullerenol-treated groups. The findings suggest that the protective effects of the investigated interventions are closely related to the suppression of oxidative stress and preservation of endogenous antioxidant capacity.

Volatile agents are an essential part of perioperative medicine and present in almost every patient undergoing general anesthesia[27]. Several anesthetic agents (such as sevoflurane, desflurane, isoflurane, halothane, enflurane, and xanthine oxidase) have been shown in various organs of animal models to decrease oxidative damage and inflammation, as well as protect against IR injury[10,28,29]. In the present study, both

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interventions were associated with biochemical and histopathological improvement compared with the IRI group. However, combined treatment did not provide a statistically significant additional benefit over fullerenol C60 alone. In particular, some antioxidant enzyme activities were better preserved in the fullerenol-only group, suggesting that the addition of desflurane did not confer a consistent additive benefit under the present experimental conditions. Therefore, while both agents demonstrated hepatoprotective potential, their combined use did not produce a clearly synergistic effect. Importantly, this observation was supported by direct pairwise statistical comparisons between the IRI-F and IRI-F-D groups, which demonstrated no statistically significant differences in either biochemical or histopathological outcomes. Therefore, the conclusion regarding the absence of an additive effect is based on formal statistical testing rather than descriptive comparison alone. One possible explanation is that both interventions exert their protective effects predominantly through modulation of oxidative stress pathways. If these mechanisms partially overlap, the potential for additive protection may be limited. Alternatively, fullerenol C60 alone may have achieved near-maximal attenuation of oxidative injury in this experimental model, leaving limited room for further improvement with the addition of desflurane.

Several limitations of the present study should be acknowledged. First, this was an experimental animal study, and the findings cannot be directly extrapolated to clinical practice. Second, the sample size in each group was relatively small. Third, the evaluation period was limited to the acute phase of reperfusion; longer observation periods might have provided additional information regarding delayed necrosis, regeneration, and long-term functional recovery. Fourth, although histopathological and oxidative stress markers were assessed, inflammatory cytokines, apoptotic mediators, and molecular signaling pathways were not specifically analyzed. In addition, immunohistochemical analyzes of apoptosis- and hypoxia-related markers such as Caspase-3 and HIF-1α were not performed. Future studies incorporating these markers may provide further mechanistic insights into the protective effects of fullerenol C60. Furthermore, the present study was not specifically designed to compare the relative hepatoprotective efficacy of

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different volatile anesthetic agents. Therefore, direct conclusions regarding the superiority of desflurane or sevoflurane cannot be drawn from the available data. In addition, desflurane was administered only in the designated treatment groups, whereas the remaining groups received injectable anesthesia alone. Although all animals received the same baseline ketamine-xylazine anesthesia, the additional administration of desflurane may represent a potential confounding factor when interpreting the independent contribution of anesthetic-related protection.

## CONCLUSION

In conclusion, hepatic ischemia-reperfusion caused significant biochemical and histopathological liver injury in this experimental model. Fullerenol C60 markedly attenuated oxidative stress and tissue damage and showed the most consistent protective effect among the investigated interventions. Desflurane also exerted partial protective effects, although its benefit was less pronounced. Combined treatment did not provide a statistically significant additional benefit over fullerenol C60 alone. These findings suggest that fullerenol C60 may represent a promising adjunctive approach against hepatic IRI; however, further experimental and clinical studies are needed to clarify its mechanisms and translational relevance.

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**Footnotes**

**Institutional animal care and use committee statement:** The experimental protocol was reviewed and approved by the Gazi University Experimental Animals Ethics Committee prior to the study (Approval No. G.Ü.ET-22.021; Approval date: 24 February 2022).

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest regarding the publication of this paper.

**Data sharing statement:** The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**ARRIVE guidelines statement:** The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

**AI contribution statement:** No artificial intelligence tools were used in the preparation, writing, analysis, or editing of this manuscript.

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## Figures and Tables

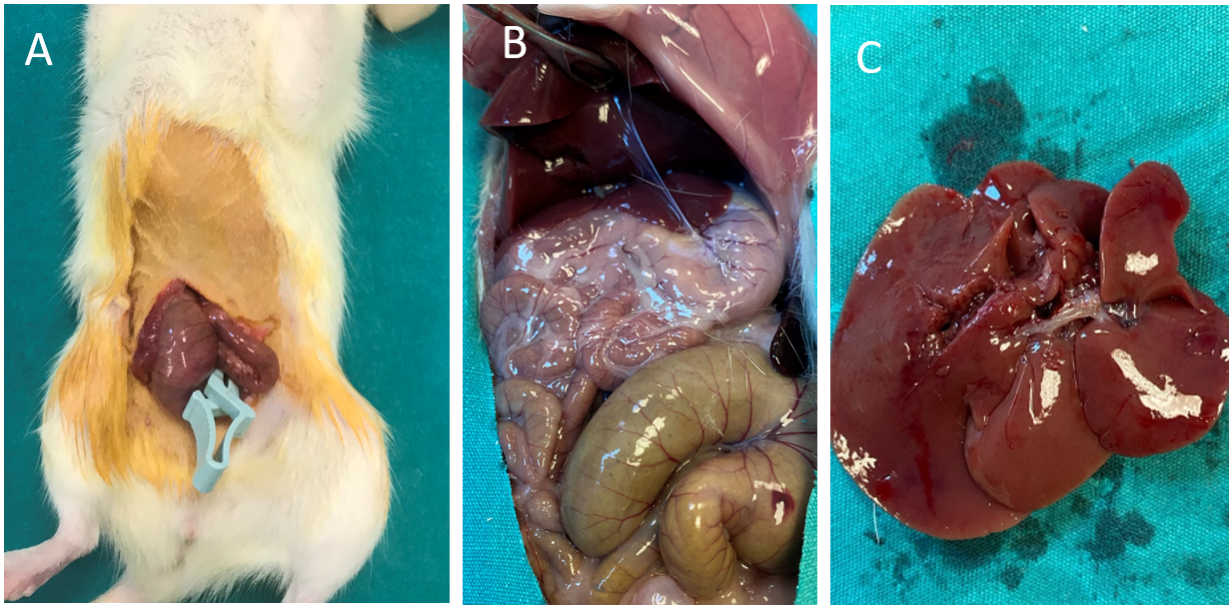



**Figure 1 Experimental procedure and gross appearance of the liver during partial hepatic ischemia and tissue collection.** A: Partial hepatic ischemia induced by atraumatic vascular clamping of the portal triad supplying the median and left lateral liver lobes; B: Macroscopic appearance of the liver during the ischemic period, demonstrating discoloration of the ischemic lobes; C: Gross appearance of the excised liver tissue.

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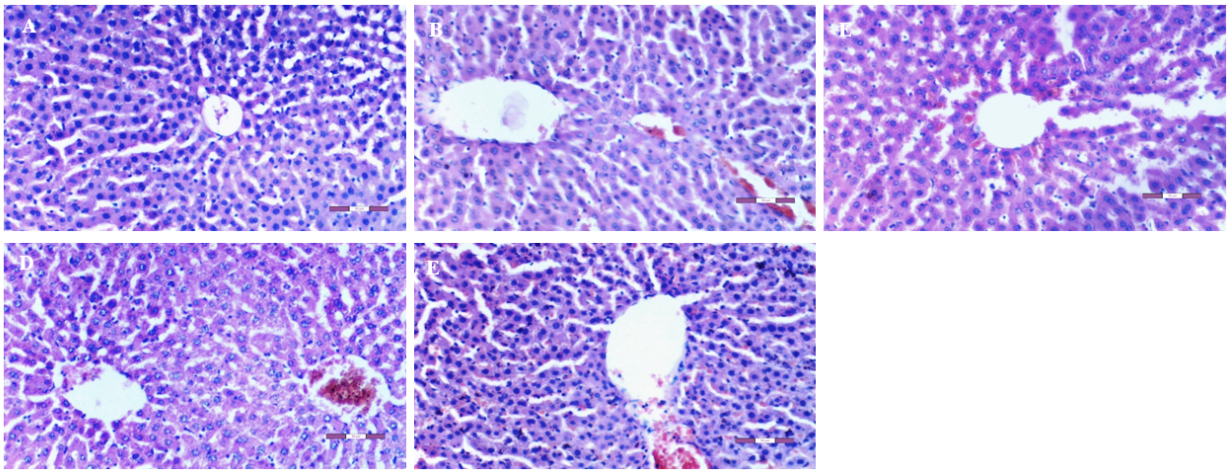


**Figure 2 Representative hematoxylin and eosin-stained liver section.** A: Control group (group C): Normal hepatic architecture is preserved, with well-organized hepatocyte

cords, intact sinusoidal spaces, and normal nuclear morphology. No evidence of hepatocellular degeneration, inflammatory cell infiltration, or ischemia-reperfusion injury (IRI)-related tissue injury is observed (× 200); B: IRI group: Marked hepatic injury is evident, characterized by hepatocellular degeneration, prominent sinusoidal dilatation, pyknotic nuclei, and parenchymal mononuclear inflammatory cell infiltration, consistent with severe IRI-induced tissue damage (× 200); C: Desflurane-treated IRI group: Histopathological injury is less pronounced than in the untreated IRI group, with reduced hepatocellular degeneration and sinusoidal dilatation accompanied by mild inflammatory cell infiltration, indicating partial preservation of hepatic tissue architecture (× 200); D: Fullerenol C60-treated IRI group: Liver architecture is largely preserved, with only mild hepatocellular degeneration, limited sinusoidal dilatation, and minimal inflammatory cell infiltration, demonstrating attenuation of IRI-induced histopathological injury (× 200); E: Combined fullerenol C60 and desflurane-treated IRI group: Mild to moderate histopathological alterations, including limited hepatocellular degeneration, sinusoidal dilatation, and scattered inflammatory cell infiltration, are observed. Overall tissue injury is markedly reduced compared with the untreated IRI group and is comparable to that observed in the fullerenol-treated group (× 200).

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**Table 1 Rat liver tissue histopathological findings, mean ± SEM**

| | Group C (*n* = 6) | Group IRI (*n* = 6) | Group IRI-F (*n* = 6) | Group IRI-D (*n* = 6) | Group IRI-F-D (*n* = 6) | *P* value[1] |
|---|---|---|---|---|---|---|
| Hepatocyte degeneration | 0.33 ± 0.21 | 1.67 ± 0.21[a] | 0.67 ± 0.21[b] | 1.00 ± 0.26[a,b] | 0.67 ± 0.21[b] | 0.003 |
| Sinusoidal dilation | 0.50 ± 0.22 | 1.83 ± 0.31[a] | 0.83 ± 0.17[b] | 1.17 ± 0.31 | 1.00 ± 0.26[b] | 0.018 |
| Pyknotic nuclei | 0.33 ± 0.21 | 1.33 ± 0.21[a] | 0.50 ± 0.22[b] | 0.67 ± 0.21[b] | 0.67 ± 0.21[b] | 0.031 |
| Cells undergoing necrosis | 0.33 ± 0.21 | 1.00 ± 0.00 | 0.50 ± 0.22 | 0.83 ± 0.31 | 0.67 ± 0.21 | 0.113 |
| Parenchymal mononuclear cell infiltration | 0.17 ± 0.17 | 1.67 ± 0.33[a] | 0.67 ± 0.21[b] | 1.00 ± 0.26[a] | 0.67 ± 0.21[b] | 0.003 |

[a]$P < 0.05$ *vs* control group.

[b]$P < 0.05$ *vs* ischemia-reperfusion injury group.

[1]Determined by Kruskal-Wallis test.

C: Control; IRI: Ischemia-reperfusion injury; IRI-F: Ischemia-reperfusion injury + fullerenol C60; IRI-D: Ischemia-reperfusion injury + desflurane; IRI-F-D: Ischemia-reperfusion injury + fullerenol C60 + desflurane.

**Table 2 Rat liver tissue oxidative status parameters, mean ± SEM**

| | Group C (*n* = 6) | Group IRI (*n* = 6) | Group IRI-F (*n* = 6) | Group IRI-D (*n* = 6) | Group IRI-F-D (*n* = 6) | *P* value[1] |
|---|---|---|---|---|---|---|
| MDA (nmol/mg protein) | 0.10 ± 0.01 | 0.25 ± 0.01[a] | 0.14 ± 0.01[a,b] | 0.21 ± 0.01[a,b] | 0.16 ± 0.01[a,b] | < 0.0001 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| CAT (IU/mg protein) | 385.75 ± 32.07 | 170.93 ± 11.78[a] | 327.82 ± 13.84[b] | 204.95 ± 19.30[a] | 282.25 ± 23.59[a,b] | < 0.0001 |
| GST (IU/mg protein) | 1.10 ± 0.07 | 0.58 ± 0.04[a] | 0.95 ± 0.04[b] | 0.78 ± 0.05[a] | 0.82 ± 0.11[a,b] | < 0.0001 |
| ARE (IU/mg protein) | 1.24 ± 0.07 | 0.78 ± 0.03[a] | 1.15 ± 0.05[b] | 1.01 ± 0.07[a,b] | 1.08 ± 0.06[b] | < 0.0001 |

[a]$P < 0.05$ *vs* control group.

[b]$P < 0.05$ *vs* ischemia-reperfusion injury group.

[1]Determined by Kruskal-Wallis test.

C: Control; IRI: Ischemia-reperfusion injury; IRI-F: Ischemia-reperfusion injury + fullerenol C60; IRI-D: Ischemia-reperfusion injury + desflurane; IRI-F-D: Ischemia-reperfusion injury + fullerenol C60 + desflurane; MDA: Malondialdehyde; CAT: Catalase; GST: Glutathione-S-transferase; ARE: Arylesterase.

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