Use of Cannabidiol as a prophylactic treatment in acute mesenteric ischemia

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Abstract

Background

Intestinal ischemia-reperfusion injury (i-IRI) is a critical clinical condition resulting from a transient interruption and subsequent restoration of blood flow to the intestine. This process generates a highly inflammatory and oxidative environment, causing severe local damage to the intestinal mucosa and triggering systemic effects that compromise distant organs, such as the liver. Cannabidiol (CBD), a non-psychoactive phytocannabinoid, has demonstrated anti-inflammatory, antioxidant, and antiapoptotic properties. Therefore, the purpose of this study was to evaluate the prophylactic impact of cannabidiol treatment in an experimental rat model of intestinal ischemia-reperfusion injury, analyzing molecular, functional, and structural parameters in both the intestine and the liver.

Results

We evaluated the gene expression of inflammatory, antioxidant, and apoptotic markers alongside malondialdehyde (MDA), glutathione metabolites, histological damage, intestinal absorptive capacity, and serum biochemical profiles in young (4-month) and middle-aged (12-month) rats. The results demonstrated that i-IRI induced significant local and systemic damage across both age groups, evidenced by severe histological injury (grade close to 5), elevated intestinal MDA ($p < 0.0001$), and impaired D-xylose absorption ($p < 0.0001$). Prophylactic treatment with CBD partially reversed these pathological alterations. Specifically, CBD significantly normalized the gene expression of proinflammatory and proapoptotic markers—such as reducing intestinal iNOS ($p = 0.0419$) and BAX ($p = 0.0022$) in 12-month-old rats —and attenuated lipid peroxidation (intestinal MDA reduction, $p = 0.0015$ in older rats). Furthermore, CBD reduced histological damage and significantly improved intestinal absorptive capacity, particularly in the 12-month cohort ($p < 0.0001$). Serum biochemical analysis confirmed CBD's capacity to preserve hepatic membrane integrity by reducing AST and ALT leakage in both 4-month ($p = 0.0002$ and $p = 0.0442$, respectively) and 12-month-old rats ($p = 0.0173$ and $p = 0.0242$), while also maintaining systemic metabolic homeostasis.

Conclusions

These findings suggest that cannabidiol exerts a significant protective effect against the local and systemic damage induced by intestinal ischemia-reperfusion injury. By preserving intestinal architecture and function while mitigating secondary hepatic injury, cannabidiol positions itself as a promising potential therapeutic candidate to minimize the high morbidity and mortality associated with this severe pathology.

Background

Acute mesenteric ischemia (AMI) is a severe medical condition characterized by an abrupt reduction or interruption of intestinal blood flow, leading to an inadequate supply of oxygen and nutrients that ultimately results in intestinal infarction [1]. Paradoxically, the restoration of blood flow, while essential for tissue survival, exacerbates the initial ischemic damage. During the hypoxic phase, xanthine dehydrogenase is converted into xanthine oxidase, an enzyme with a high capacity for generating reactive oxygen species (ROS) [2]. Upon reperfusion, these ROS are massively released into the bloodstream, triggering intestinal ischemia-reperfusion injury (i-IRI). This pathophysiological event causes profound structural damage to the intestinal mucosa; the ischemic phase compromises oxygen delivery, leading to a loss of epithelial integrity, interstitial edema, and intestinal barrier imbalance[3]. The subsequent intense local inflammatory response and ROS generation during reperfusion further degrade the mucosa, resulting in submucosal edema, villous shortening, and epithelial necrosis. These structural alterations directly impair the functional absorptive capacity of the intestine[4].Consequently, serum concentrations of D-xylose—a monosaccharide actively absorbed by the intestinal epithelium without hepatic metabolism—serve as a reliable functional marker to evaluate intestinal absorptive capacity and indirect epithelial injury [5].

The pathophysiology of i-IRI is heavily dependent on massive ROS production, which induces an intense state of oxidative stress with both local and systemic repercussions [6]. This oxidative imbalance overwhelms endogenous antioxidant defense systems. Key cellular defenses include glutathione peroxidase (GPx) and glutathione S-transferase (GST), which neutralize peroxides and lipid oxidation products by conjugating them with reduced glutathione; glutathione reductase (GR), which regenerates glutathione; and gamma-glutamylcysteine synthetase (yGCS), the rate-limiting enzyme in its synthesis [7]. Similarly, heme oxygenase-1 (HO-1), an enzyme inducible by oxidative stress, degrades heme into biliverdin, free iron, and carbon monoxide, providing cytoprotective and antioxidant effects [8]. When these defenses are breached, lipid peroxidation occurs, which can be accurately quantified by using malondialdehyde (MDA) as a highly sensitive biochemical marker of oxidative damage [9].

In parallel, i-IRI activates a robust inflammatory cascade and programmed cell death mechanisms [10]. Interleukin-1 beta (IL-1β) acts as a central proinflammatory cytokine that amplifies innate immunity, promotes leukocyte adhesion to the endothelium, and stimulates the release of further inflammatory mediators [11]. Additionally, endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) play dual roles; while eNOS is typically protective, its overexpression under stress contributes to endothelial dysfunction [12]. Conversely, iNOS produces sustained, large quantities of nitric oxide that react with superoxide to form peroxynitrite, a highly toxic reactive species [13]. Ultimately, this hostile environment triggers apoptosis, upregulating proapoptotic genes such as BAX and BAD, which promote mitochondrial outer membrane permeabilization, cytochrome c release, and subsequent caspase activation [14].

Crucially, massive ROS production during reperfusion severely compromises mitochondria. To counteract this dysfunction, mitochondrial biogenesis—coordinated by the master regulator PGC-1α—is essential to restore healthy mitochondria and antioxidant defenses [15]. However, the severe i-IRI insult

impairs PGC-1α signaling, causing damaged mitochondria to accumulate. The subsequent leakage of mitochondrial contents (e.g., ROS and mitochondrial DNA) into the cytosol acts as a potent danger signal that directly triggers the local inflammatory cascade [16].

The deleterious effects of i-IRI extend beyond the intestine, causing generalized damage to other organs, particularly those with a high proportion of endothelial cells, such as the liver. Although the liver is not directly subjected to mesenteric occlusion, functional and molecular studies have demonstrated that it undergoes significant alterations following an i-IRI episode [17, 18]. During reperfusion, the sudden return of blood flow to the intestine releases large amounts of ROS, proinflammatory cytokines, bacterial lipopolysaccharides, and nitric oxide into the portal circulation, which directly reaches the liver [19]. Continuous exposure to these signals from the injured intestine compromises hepatic functional integrity, triggering secondary inflammatory and oxidative responses [18]. This systemic dissemination is a major contributor to the high mortality associated with intestinal ischemia, frequently progressing to endotoxemia, systemic inflammatory response syndrome (SIRS), and multiple organ dysfunction syndrome (MODS) [19].

Currently, clinical practice guidelines for AMI primarily focus on the rapid restoration of mesenteric blood flow and the surgical resection of infarcted intestinal segments [20]. However, there is a distinct lack of specific pharmacological therapies addressing the reperfusion injury itself. In this context, plant-derived compounds have shown significant potential in experimental therapies for ischemia-reperfusion syndromes[21].Cannabidiol (CBD), a non-psychotropic phytocannabinoid, has demonstrated beneficial effects in various pathological conditions driven by oxidative stress and inflammation [9]. Its molecular structure, which is rich in phenolic hydroxyl groups, enables it to act as a direct antioxidant that neutralizes free radicals and interrupts lipid peroxidation chain reactions [22]. Furthermore, CBD can chelate metal ions such as iron and copper, preventing their participation in the Fenton reaction and thereby inhibiting the formation of highly cytotoxic hydroxyl radicals [23].

Clinically, acute mesenteric ischemia predominantly affects older populations [24]. Aging is widely associated with a progressive decline in endogenous antioxidant defense mechanisms and an increase in chronic, low-grade inflammation, which can significantly exacerbate the deleterious effects of ischemia-reperfusion injury and limit the efficacy of pharmacological interventions[25]. While traditional experimental i-IRI models have predominantly utilized young animals, incorporating older subjects can provide valuable insights that more closely reflect the clinical demographic. To establish a more clinically translatable model, our experimental design incorporates both young adult (4-month-old) and middle-aged (12-month-old) rats. This comparative approach allows us to evaluate whether the baseline physiological decline associated with aging influences the severity of injury and, crucially, to determine whether the prophylactic efficacy of CBD is preserved across different age groups.

Despite these theoretical advantages, the specific prophylactic efficacy of CBD on the intricate molecular pathways of i-IRI and its secondary hepatic complications remains to be fully elucidated. Therefore, the present study aimed to evaluate the prophylactic protective effect of CBD against the molecular,

morphological, and functional alterations triggered by i-IRI in the intestine, and to assess its capacity to limit secondary systemic repercussions in the liver.

Methods

Study Design and Ethics Statement

The primary aim of this experimental study was to evaluate the prophylactic effects of CBD on molecular, morphological, and functional alterations in a rat model of intestinal ischemia-reperfusion injury. All procedures involving animals were conducted in accordance with current legislation regarding the use of animals for experimental purposes and were formally approved by the Animal Experimentation Ethics Committee of the University of the Basque Country (approval number M20/2024/165).

Animals and Housing

Male WAG/RijHsd rats aged 4 and 12 months were used in this study. The animals were housed under a controlled 12-hour light-dark cycle at a constant temperature of 24°C and handled in accordance with Spanish and European regulations governing animal experimentation. They were provided with ad libitum access to water and standard rodent chow (Panlab A-04).

Experimental Groups and Surgical Protocol

Experimental Groups and Surgical Protocol
The animals were divided into three experimental groups (Fig. 1): (1) a healthy control group not subjected to i-IRI; (2) an untreated i-IRI group (IRI); and (3) an i-IRI group treated with 10 mg/kg IV CBD. Within each group, half of the rats were designated for histological and biochemical analyses, while the remaining half underwent intestinal absorptive function testing.

Surgical interventions for the IRI and CBD groups involved a midline laparotomy to identify the superior mesenteric artery (Fig. 2). Blood flow was reversibly interrupted using a microvascular clamp (Yasargil), and proper placement was confirmed by the absence of distal pulses. Post-operatively, animals received subcutaneous meloxicam (2 mg/kg) for analgesia. In the treatment group, CBD was administered intravenously at a dose of 10 mg/kg exactly thirty minutes before the end of the ischemic period. For its preparation, pure CBD extract (Phexia, Madrid, Spain) was used to prepare a 0.3 g/mL CBD stock solution in dimethyl sulfoxide (DMSO), which was subsequently diluted in a refined olive oil and soybean oil emulsion (ClinOleic®, 3% DMSO v/v) to achieve a final working concentration of 10 mg/mL.

Following the reperfusion period, a second laparotomy was performed for sample collection. Blood was drawn from the abdominal aorta to obtain serum. A 6-cm segment of the terminal ileum was immediately excised and fixed in 4% formaldehyde for histological evaluation. Additionally, fresh

samples of intestine and liver were washed in cold phosphate-buffered saline (PBS) and snap-frozen in liquid nitrogen for molecular assays.

Serum Biochemical Analysis

To obtain serum, the total circulating blood volume was collected from the abdominal aorta using a 21G needle. The blood was then centrifuged for 10 min at 3000 rpm in SST™ II Advance tubes (BD Vacutainer®, New Jersey, USA). The serum was aliquoted and stored at -80°C until analysis, which was carried out using a Cobas® 8000 c702 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The following biochemical markers were measured to further assess hepatic function, renal function, and metabolic status: albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urate, urea, lactate dehydrogenase (LDH), creatinine, creatine kinase (CK), and glucose. All reagents were sourced from Roche Diagnostics GmbH.

Histological Assessment

Intestinal tissue samples fixed in 4% formaldehyde for 24 hours were embedded in paraffin and stained with hematoxylin and eosin using standard protocols. For each animal, six serial sections separated by 1 cm were analyzed (Fig. 3). The degree of histological injury in the intestinal mucosa was evaluated using Chiu's scale, which assigns a score from 0 (normal) to 5 (severe damage). The evaluation was performed by a blinded observer using a light microscope at 10× magnification.

Intestinal Absorptive Function

The functional capacity of the intestine was evaluated using the D-xylose absorption test. A 1 mL aqueous solution of D-xylose (0.615 mg/mL) was administered directly via an 18G esophageal cannula. One hour after administration, blood samples were collected to obtain serum. Absorbance was measured at 554 nm, and serum D-xylose concentrations were calculated and expressed in µg/mL.

Molecular and Biochemical Analyses

To assess lipid peroxidation, malondialdehyde (MDA) levels were quantified in tissue samples using the thiobarbituric acid reactive substances (TBARS) assay.

Tissue glutathione levels, including total glutathione (GSHT), reduced glutathione (GSH), and oxidized glutathione (GSSG), were quantified in intestinal and hepatic homogenates using a commercial assay kit (ELK Biotechnology BC030/31) according to the manufacturer’s instructions. The GSSG/GSH ratio was calculated as a marker of the tissue redox state, and all values were normalized to the total protein concentration of the samples.

For gene expression analysis, mRNA was isolated from frozen tissues. RNA concentration and purity (260/280 nm ratio) were determined using a spectrophotometer (BioDrop, Fisher Scientific, Waltham, MA, USA), and RNA integrity was verified by 1% agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized from 2 µg of mRNA utilizing a reverse transcription kit (StaRT Reverse Transcription Kit, AnyGenes, Paris, France). Quantitative real-time polymerase chain reaction (RT-qPCR) was performed using a thermal cycler (7500 Fast Real-Time PCR System, Applied Biosystems, Cambridge, MA, USA). The amplification of 18S mRNA was used as an endogenous control for each sample. Relative changes in mRNA expression were calculated in duplicate using the 2^(-ΔΔCt) method.

Statistical Analysis

Data processing and statistical analyses were performed using Prism v8 software (GraphPad Software, Inc., San Diego, CA, USA). The normality of the data distribution was first evaluated. For non-normally distributed data, the Kruskal-Wallis test was applied, followed by Dunn's multiple comparisons test. For data exhibiting a normal distribution, a one-way analysis of variance (ANOVA) was utilized, followed by Tukey's multiple comparisons test. A confidence level exceeding 95% ($p < 0.05$) was established as the criterion for statistical significance.

Results

Morphological and Functional Intestinal Assessment

Histological analysis (Fig. 4) of the intestine revealed a significant increase in the histological injury grade (HIG) in animals subjected to i-IRI, encompassing the global assessment as well as the mesenteric and antimesenteric borders, compared with the control group. In all cases, the mean HIG in the IRI group was close to 5 on the Chiu scale, reflecting severe structural damage (Fig. 5A-C). CBD treatment notably reduced the HIG, particularly in the global analysis and at the mesenteric border, while the improvement was less pronounced at the antimesenteric border.

These morphological findings were consistent with the absorptive assessment. The D-xylose intestinal absorption test (Fig. 5D) demonstrated a significant reduction in plasma levels in the IRI group compared to the control across both age groups ($p < 0.0001$), indicating impaired absorptive function following injury. CBD treatment significantly increased these levels, especially in the 12-month-old rats ($p < 0.0001$), yielding values closer to those of the control group.

Intestinal Gene Expression Profile (RT-qPCR)

Intestinal gene expression analysis (Fig. 6) showed significant increases in IL-1β, iNOS, and eNOS in the IRI group compared to the control, particularly in the 12-month-old rats. Specifically, iNOS expression in 12-month-old rats displayed significant differences between the control and IRI groups ($p = 0.0029$), and a subsequent reduction following CBD treatment compared to the IRI group ($p = 0.0419$). Overall, the

CBD-treated group experienced a reduction in these levels, with significant differences compared to the IRI group in most cases.

Regarding oxidative stress, the genes yGCS, GPx, GR, HO-1, and GST exhibited increased expression in the IRI group, whereas PGC-1α showed a decrease compared to the control in 4-month-old rats ($p = 0.0084$). In 12-month-old rats, GPx presented a significant increase in the IRI group compared to the control ($p = 0.0085$), with a trend toward reduction after CBD treatment ($p = 0.0114$). Similarly, HO-1 expression in these older rats was markedly higher in the IRI group versus the control ($p < 0.0001$) and was significantly reduced with the prophylactic treatment ($p = 0.0004$). CBD administration reduced the expression of the elevated antioxidant genes and increased PGC-1α ($p = 0.0007$) in 4-month-old rats, partially normalizing the oxidative profile.

For apoptotic genes, an increase in BAX and BAD expression was detected following i-IRI, which was more pronounced in the 12-month-old animals. In these animals, BAX showed significant differences between the control and IRI groups ($p = 0.0048$), as well as between the IRI and CBD groups ($p = 0.0022$). CBD treatment successfully reduced the levels of both genes in both age groups, returning them closer to control group values.

Intestinal and Hepatic Lipid Peroxidation (MDA)

In the intestinal tissue, MDA levels (Fig. 7) increased significantly in the IRI group compared to the control ($p < 0.0001$) at both ages. The experimental treatment induced a significant decrease in intestinal MDA levels, with a greater reduction observed in the older rats ($p = 0.0015$).

Similar results were observed in the hepatic tissue. MDA levels also increased in the IRI group relative to the control, although with less intensity than in the intestine, reaching significance only in the 4-month-old animals ($p = 0.0241$). CBD treatment yielded a reduction in these levels, achieving higher significance in the 4-month-old cohort ($p = 0.0011$).

Hepatic Gene Expression Profile (RT-qPCR)

In the hepatic gene expression analysis (Fig. 8), significant increases in TNFα, iNOS, and eNOS were observed in the IRI group compared to the control group, suggesting an activation of the inflammatory response in the liver following i-IRI; these increases were more marked in the 12-month-old animals. In 12-month-old rats, a significant increment of iNOS was detected in the IRI group versus the control ($p = 0.0005$), which was reduced following CBD treatment ($p = 0.0225$). Furthermore, in 4-month-old rats, TNFα showed a notable increase in the IRI group compared to the control ($p < 0.0001$) and was significantly reduced with CBD ($p = 0.0015$). The treated group showed a generalized decrease in these proinflammatory genes, achieving statistically significant differences compared to the IRI group in some instances.

In contrast to the pattern observed in the intestine, genes related to the antioxidant system (yGCS, GPx, GR, and GST) showed a significant decrease in the IRI group compared to the control group in the liver. In

4-month-old rats, GPx expression was significantly reduced in the IRI group versus the control ($p = 0.0001$) and presented a trend toward recovery after treatment ($p = 0.0432$). CBD reversed this decrease, elevating expression to levels comparable with the control group, especially in the 12-month-old animals.

Regarding apoptotic markers, BAX expression increased in the IRI group, and this increase was reversed following CBD administration, bringing values closer to baseline levels. In 4-month-old rats, BAX showed a significant increase versus the control ($p = 0.0008$) and a clear decrease with CBD ($p = 0.0009$), reflecting a pronounced protective effect at the hepatic level in young animals.

Intestinal and Hepatic Glutathione System Metabolites

In the intestinal glutathione metabolite analysis (Fig. 9), the IRI group exhibited a marked oxidative response, characterized by significant increases in total glutathione (GSHT) and oxidized glutathione (GSSG) compared to controls in 4-month-old animals. Prophylactic CBD treatment in this cohort showed a clear trend toward reducing these levels ($p = 0.053$ for GSHT and $p = 0.1$ for GSSG). Furthermore, in 12-month-old individuals, a significant increment of GSHT was detected following i-IRI, which was significantly attenuated by CBD treatment. Overall, the treated groups showed a generalized decrease in the oxidized glutathione pool and the GSSG/GSH ratio, achieving values closer to baseline levels and minimizing the oxidized tissue environment.

In contrast to the pattern observed in the intestine, metabolites related to the hepatic glutathione system (GSHT, GSSG, GSH, and the GSSG/GSH ratio) did not show significant alterations. In both 4-month-old and 12-month-old rats, these hepatic markers remained statistically stable following the ischemic insult, and CBD administration did not induce further modifications. This lack of variation reflects a different temporal or systemic dynamic in the liver at the evaluated 2-hour reperfusion time point.

Serum Biochemical Profile

Serum biochemical analysis showed significant increases in hepatic and systemic injury enzymes following intestinal ischemia-reperfusion (Fig. 10). Specifically, AST and ALT were significantly elevated in the IRI group compared to the control group across both age groups ($p < 0.0001$). Prophylactic treatment with CBD significantly reduced these levels in both 4-month-old ($p = 0.0002$ for AST; $p = 0.0442$ for ALT) and 12-month-old rats ($p = 0.0173$ for AST; $p = 0.0242$ for ALT). Similarly, LDH and CK levels were markedly increased in the IRI group compared to the control ($p < 0.0001$), and CBD administration induced a significant decrease in these markers at both ages ($p < 0.0001$).

Regarding renal and metabolic markers, serum urea and urate exhibited significant increases in the IRI group compared to the control. CBD treatment significantly reduced urea levels in both young ($p < 0.0001$) and older animals ($p = 0.0055$). For creatinine and urate, the CBD-induced reduction reached statistical significance primarily in the 4-month-old cohort ($p = 0.0191$ and $p = 0.0033$, respectively).

Conversely, markers associated with hepatic synthetic function and homeostasis were negatively affected by i-IRI. Serum albumin was significantly reduced in the IRI group compared to the control. CBD

administration successfully reversed this decrease, elevating concentrations in both 4-month-old ($p = 0.0008$) and 12-month-old rats ($p = 0.0413$) to levels closer to the control group. Finally, alkaline phosphatase (ALP) and glucose levels showed a significant decrease following i-IRI, with prophylactic CBD significantly recovering ALP levels in the 4-month-old animals ($p = 0.0285$).

Discussion

In our model, i-IRI-induced structural damage to the intestinal mucosa directly compromised absorptive capacity. The D-xylose test demonstrated a significant decrease in the IRI group compared to controls. This functional loss corresponded with characteristic ischemia-reperfusion histological damage, including submucosal edema, villous destruction, and epithelial discontinuity, which reduce the functional absorptive surface [5]. Consistent with García-Alonso et al. [26], CBD treatment partially reversed these alterations, improving D-xylose absorption and preserving mucosal architecture, demonstrating dual functional and structural protection. This aligns with Huertos et al., who reported CBD-mediated mucosal protection in neonatal necrotizing enterocolitis, a condition sharing epithelial injury patterns with i-IRI [27].

Furthermore, i-IRI profoundly activated intestinal oxidative stress, inflammation, and apoptosis pathways. Within the oxidative axis, antioxidant enzymes (GPx, GST, GR, yGCS, and HO-1) were notably overexpressed [28], likely as a compensatory response against excessive ROS accumulation during reperfusion [29]. CBD significantly reversed this overexpression, reflecting reduced initial stimulus rather than impaired defense. Its phenolic hydroxyl groups enable CBD to act as a direct antioxidant, neutralizing free radicals and inhibiting lipid peroxidation [23]. By mitigating the ROS load, CBD prevents the need for massive antioxidant gene activation, an effect similar to hyperbaric oxygenation in comparable models [30].

Additionally, i-IRI decreased PGC-1α expression, especially in young animals, reflecting compromised mitochondrial metabolism secondary to oxidative and inflammatory damage. Since PGC-1α can mediate nuclear factor kappa B (NF-κB) activation to decrease oxidative stress [31], its normalization by CBD suggests preserved functional mitochondrial balance and stabilization of critical metabolic pathways.

Parallel to oxidative stress, i-IRI induced a robust local immune response, increasing IL-1β, iNOS, and eNOS expression. Their overexpression suggests a pathological imbalance driving microcirculatory deterioration and epithelial damage [12, 13]. CBD significantly decreased these markers, indicating effective inflammatory modulation linked to reduced redox stimuli. This confirms CBD acts by neutralizing ROS and interfering with inflammatory cascades, consistent with the protective effects of NO pathway inhibitors in intestinal reperfusion [32].

This hostile microenvironment ultimately triggered apoptosis, confirmed by increased BAX and BAD expression in the intestinal epithelium [14]. CBD significantly reduced these proapoptotic genes, preserving cellular viability and mucosal integrity. This antiapoptotic effect acts synergistically with its

antioxidant and anti-inflammatory properties, paralleling CBD-mediated BAX/TNFα reduction in trauma models [33].

The redox imbalance was biochemically corroborated by glutathione metabolite quantification. The significant accumulation of total and oxidized glutathione following i-IRI confirms intense oxidative stress and massive expansion of the glutathione pool to counteract the reperfusion ROS burst [34, 35]. Prophylactic CBD mitigated this response, reducing GSSG and significantly lowering GSHT levels. By acting as a direct antioxidant, CBD neutralizes free radicals before they exhaust cellular defenses, preventing overactivation of the glutathione system [36].

Concordantly, MDA levels—a lipid peroxidation marker—significantly increased in both intestine and liver following i-IRI, reflecting local structural damage [37] and systemic repercussion. Hepatic oxidative stress likely results from the portal transit of inflammatory mediators, endotoxins, and ROS from the injured intestine[14]. CBD effectively limited this lipid peroxidation in both tissues, confirming its direct antioxidant action and aligning with previous reports of cannabinoid-mediated MDA reduction in ischemia models [38, 39].

The systemic propagation of i-IRI deeply impacted the liver. Molecularly, i-IRI significantly increased hepatic IL-1β, TNFα, eNOS, iNOS, and BAX expression, indicating severe secondary inflammatory and apoptotic activation [17]. CBD partially reversed these changes, demonstrating systemic efficacy [9]. This hepatoprotection was functionally corroborated by serum analysis. The portal influx of ROS and inflammatory mediators compromised hepatocyte membrane integrity, dramatically elevating circulating AST, ALT, and LDH. Prophylactic CBD significantly blunted this enzymatic leakage. This translates molecular findings (downregulation of TNFα, iNOS, and BAX) into functional preservation of hepatic viability, agreeing with CBD's hepatoprotective profile in other ischemia-reperfusion models [40].

Interestingly, hepatic antioxidant gene expression sharply contrasted with intestinal findings. While the intestine overexpressed glutathione system genes, the liver exhibited a significant decrease. This inverted pattern reflects the delayed, systemic nature of hepatic injury. Portal inflammatory signals and ROS likely saturate the liver's antioxidant capacity, causing mitochondrial dysfunction and functional inhibition of nuclear factor erythroid 2-related factor 2 (Nrf2) regulated pathways [18, 41]. CBD restored hepatic antioxidant gene expression to control levels. This likely results from CBD’s direct antioxidant action, preservation of mitochondrial integrity, and potential Nrf2 reactivation [42], reversing the systemic transcriptional inhibition [9].

Beyond hepatocellular injury, i-IRI compromised hepatic synthetic capacity (reduced albumin, ALP, glucose) [17, 43] and induced broader systemic damage reflecting renal hypoperfusion (elevated urea, urate, creatinine, CK)[44, 45]. CBD treatment prevented this metabolic decline and attenuated systemic markers, reinforcing its pleiotropic capacity to maintain global liver function and limit progression toward multiple organ dysfunction syndrome (MODS) [46, 47].

Conversely, the physical hepatic glutathione pool (GSHT, GSH, GSSG) remained stable at the 2-hour reperfusion window. This reinforces the delayed nature of hepatic injury: while portal ROS rapidly cause membrane leakage and downregulate antioxidant genes, depleting the hepatic glutathione reserve requires longer temporal progression[48]. Thus, CBD's hepatoprotection is primarily driven by dampening the initial intestinal oxidative burst, preventing the secondary systemic hit before it irreversibly depletes hepatic antioxidant stores [49].

Regarding age, contrary to our hypothesis, the acute mesenteric ischemic insult was so overwhelming that it bypassed the higher baseline defenses of young (4-month) rats, resulting in uniformly severe structural and functional collapse across both age groups. Despite this lack of baseline differentiation, CBD effectively reduced damage across the lifespan with specific nuances. In 4-month-old rats, CBD prominently buffered systemic-metabolic shock (normalizing renal/metabolic markers) and attenuated secondary hepatic MDA. In contrast, 12-month-old rats faced a harsher local molecular environment but exhibited a more robust functional rescue in intestinal absorption and reduction of local lipid peroxidation following treatment. This versatility underscores CBD's robust protective profile across different biological stages.

A key limitation of this study is its reliance on mRNA expression for tissue-level evaluations. While strongly indicative of pathway activation, mRNA levels do not account for post-transcriptional or translational modifications. Future preclinical studies should incorporate direct protein expression techniques (e.g., Western blot or immunohistochemistry) to fully validate the precise translational mechanisms underlying CBD's multiorgan protective effects.

Conclusions

In conclusion, prophylactic CBD reduces local and systemic damage in a rat model of i-IRI by preserving intestinal structure and absorptive function while downregulating proinflammatory and proapoptotic genes and restoring antioxidant defenses in both the intestine and liver. Clinically, the management of acute mesenteric ischemia relies mainly on surgery, lacking pharmacological strategies to prevent reperfusion injury. By simultaneously targeting redox, immune, and apoptotic pathways, CBD emerges as a promising multiorgan protective candidate to limit the morbidity, mortality, and multiorgan dysfunction syndrome associated with i-IRI.

Abbreviations

• **AMI**
Acute mesenteric ischemia
• **ANOVA**
Analysis of variance
• **BAD**
Bcl-2-associated death promoter

• **BAX**
Bcl-2-associated X protein
• **CBD**
Cannabidiol
• **Bcl-2**
B-cell lymphoma 2
• **mRNA**
Messenger ribonucleic acid
• **NF-κB**
Nuclear factor kappa B
• **cDNA**
Complementary DNA
• **SEM**
Standard error of the mean
• **DMSO**
Dimethyl sulfoxide
• **eNOS**
Endothelial nitric oxide synthase
• **HIG**
Histological injury grade
• **GPx**
Glutathione peroxidase
• **GR**
Glutathione reductase
• **GST**
Glutathione S-transferase
• **HO-1**
Heme oxygenase 1
• **i-IRI**
Intestinal ischemia-reperfusion injury
• **IL-1β**
Interleukin 1 beta
• **iNOS**
Inducible nitric oxide synthase
• **MDA**
Malondialdehyde
• **MODS**
Multiple organ dysfunction syndrome
• **NO**
Nitric oxide

• **Nrf2**
Nuclear factor erythroid 2-related factor 2
• **PBS**
Phosphate-buffered saline
• **PGC-1α**
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
• **ROS**
Reactive oxygen species
• **RT-qPCR**
Quantitative real-time polymerase chain reaction
• **SIRS**
Systemic inflammatory response syndrome
• **TBARS**
Thiobarbituric acid reactive substances
• **TNFα**
Tumor necrosis factor alpha
• **yGCS**
Gamma-glutamylcysteine synthetase
• **ALP**
Alkaline phosphatase
• **ALT**
Alanine aminotransferase
• **AST**
Aspartate aminotransferase
• **CK**
Creatine kinase
• **LDH**
Lactate dehydrogenase
• **GSH**
Reduced glutathione
• **GSHT**
Total glutathione
• **GSSG**
Oxidized glutathione

Declarations

Ethics approval and consent to participate

All procedures involving animals were conducted in accordance with current legislation regarding the use of animals for experimental purposes and were formally approved by the Animal Experimentation

Ethics Committee of the University of the Basque Country (approval number M20/2024/165).

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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Authors' contributions

SJMP and **LIC** contributed equally to this work, performing molecular assays, data analysis, and drafting the manuscript. **BHP** and **LR** designed the study, analyzed data, supervised the project, and critically revised the text. **IGA** and **BHP** conducted the surgical procedures and animal modeling. **MOC** contributed to tissue processing and molecular analyses. **SIC** performed the serum biochemical analyses. **SDP** provided supervision, resources, and critical review. All authors read and approved the final manuscript.

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Figures

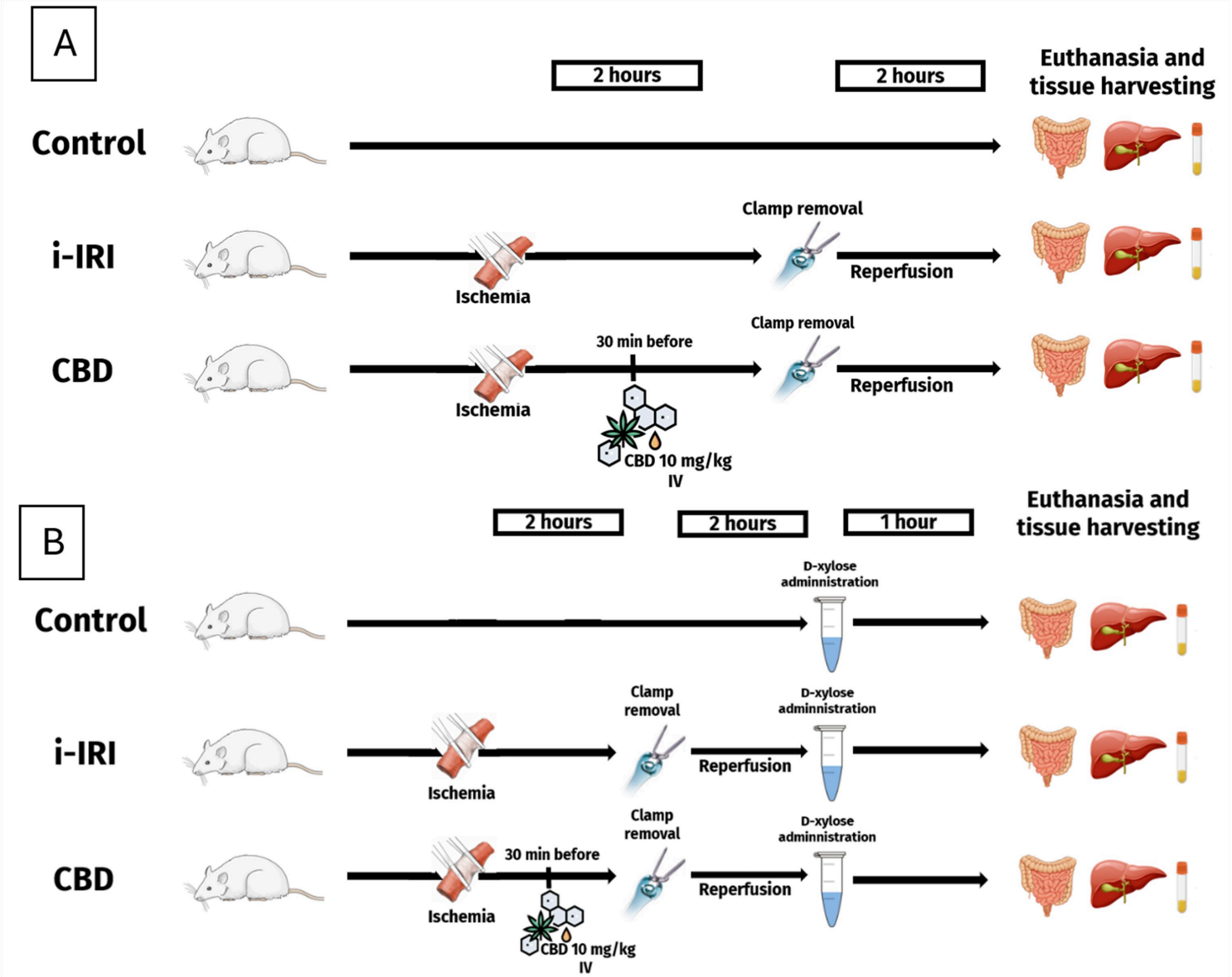

Figure 1

Schematic representation of the experimental design. Each row represents the groups used for histological, molecular, and serum studies (A), and for the intestinal absorption assessment using the D-xylose test (B).

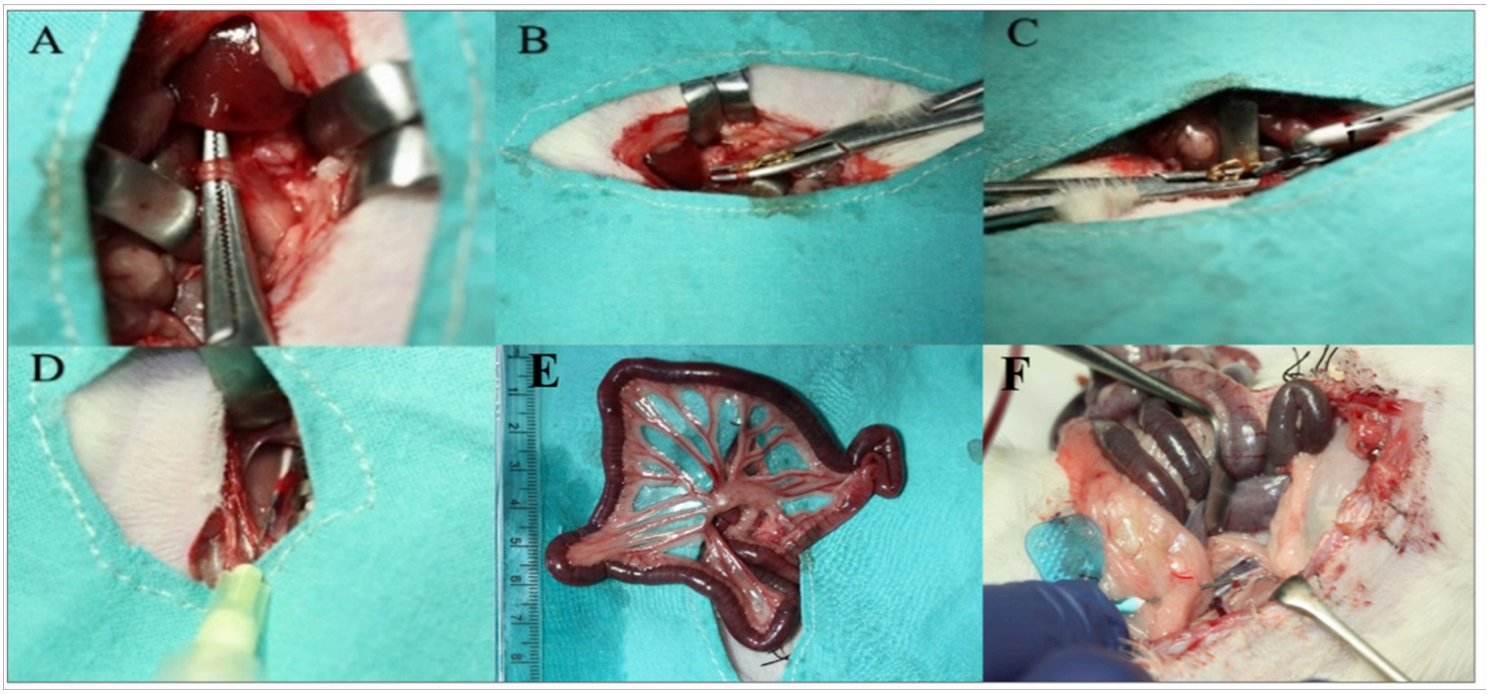

Figure 2

Photographs of different stages during the surgical procedure. Superior mesenteric artery (SMA) localization (A), proximal micro-cross-clamping of the SMA (B, C), intravenous administration of CBD (10 mg/kg) via the femoral vein (D), appearance of the small intestine of a CBD-treated rat after 2 hours of ischemia-reperfusion (E), and blood collection from the abdominal aorta (F).

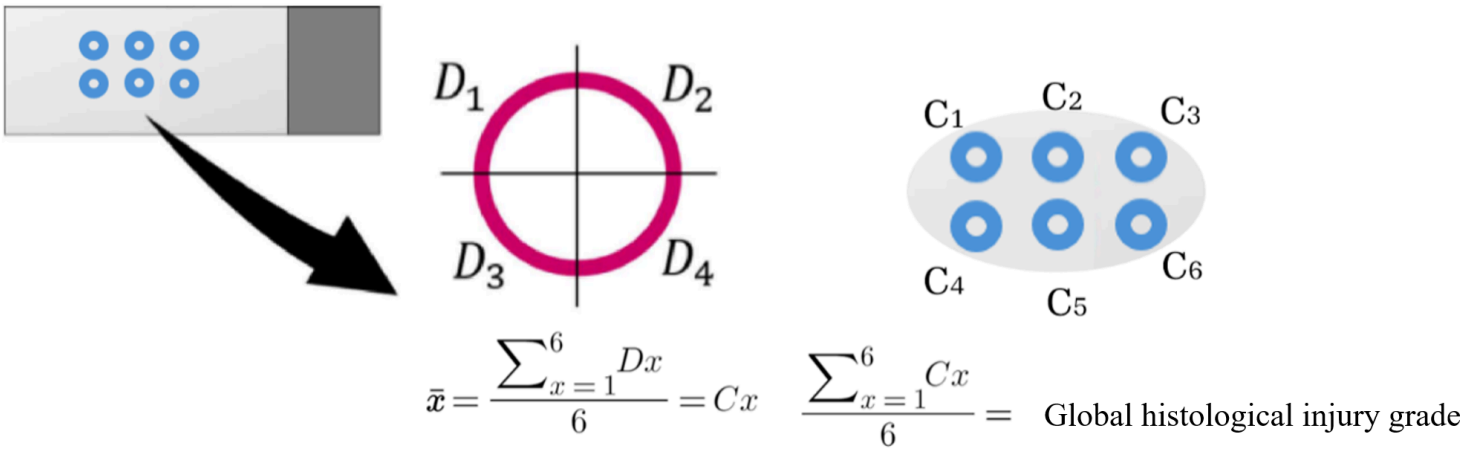

Figure 3

Calculation method for the numerical determination of histological damage. Six transverse intestinal sections were taken per animal. Each section was divided into four quadrants, and damage was measured using the arithmetic mean of the quadrants. Finally, the arithmetic mean of the sections was calculated to obtain the global histological injury grade for each animal.

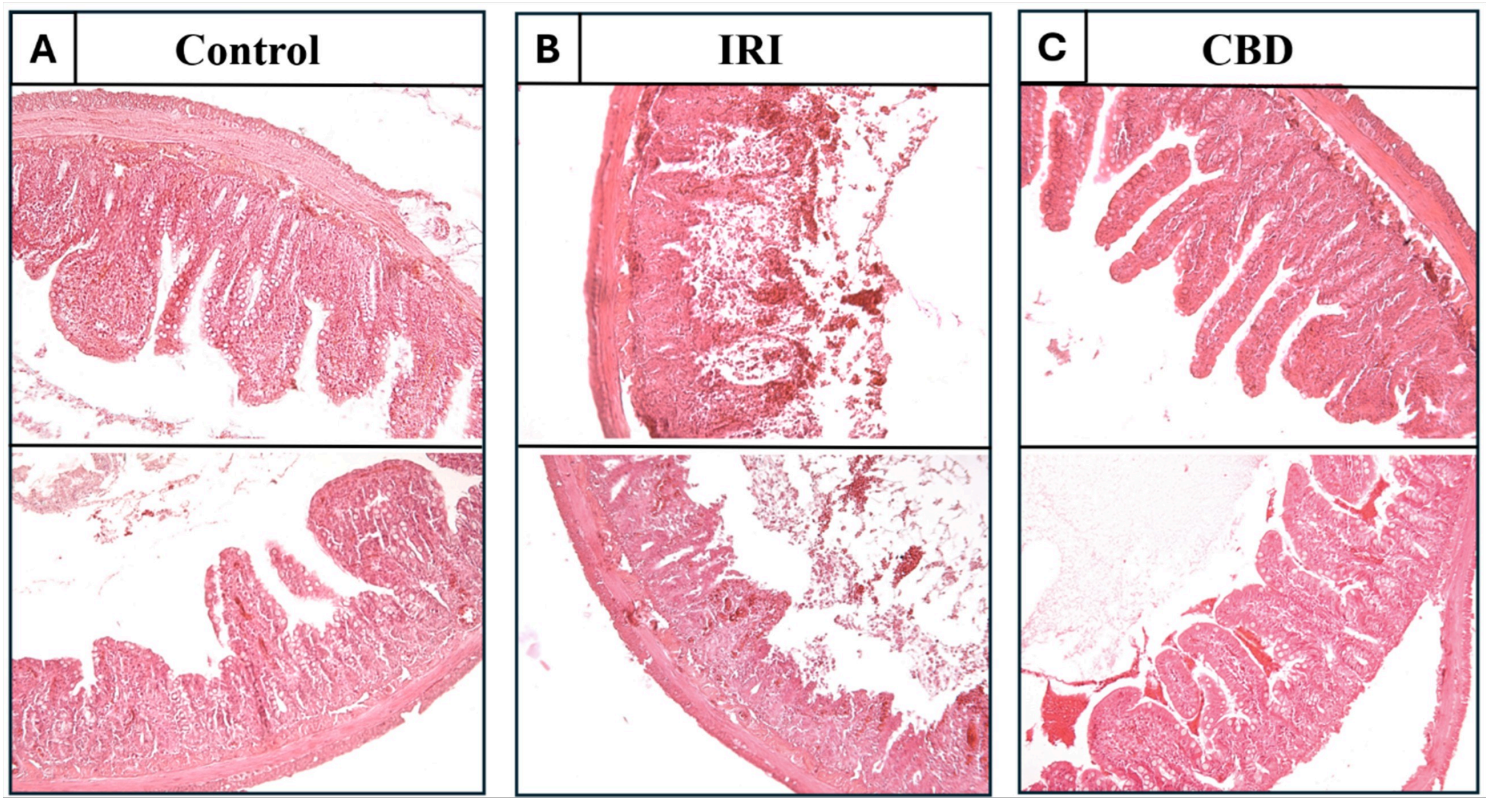

Figure 4

Representative histological images of small intestine sections. Sections were stained with hematoxylin and eosin. The control group (A) shows a preserved mucosal architecture with well-defined villi and no structural damage. The IRI group (B) displays severe lesions characterized by intraluminal hemorrhage, loss of epithelial integrity, and morphological destruction of villi. In the CBD-treated group (C), the overall villous structure is conserved, though mild edema is present, suggesting a protective effect of the treatment against i-IRI-induced tissue damage (10x magnification).

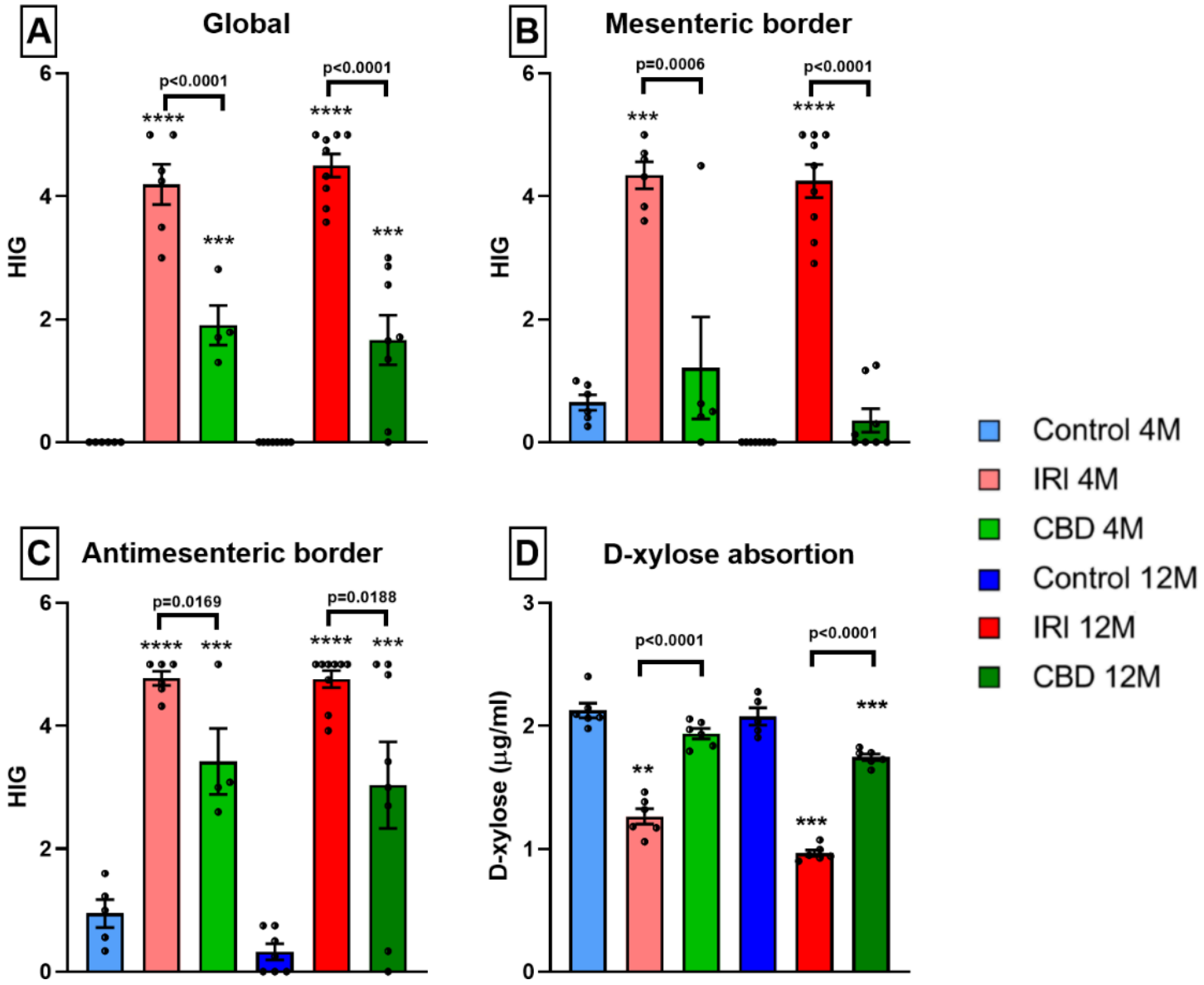

Figure 5

Evaluation of intestinal ischemia-reperfusion injury and CBD treatment effect. Assessment in young (4 months) and old (12 months) rats. (A-C) Histological injury grade evaluated globally (A), at the mesenteric border (B), and at the antimesenteric border (C). (D) Serum D-xylose concentration as a functional marker of intestinal absorption. Data are presented as mean ± SEM (n=6-8 per group). Statistical differences were analyzed using ANOVA followed by Tukey's post-hoc test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 vs. control group of the same age. Horizontal lines indicate comparisons between groups with the corresponding p-value.

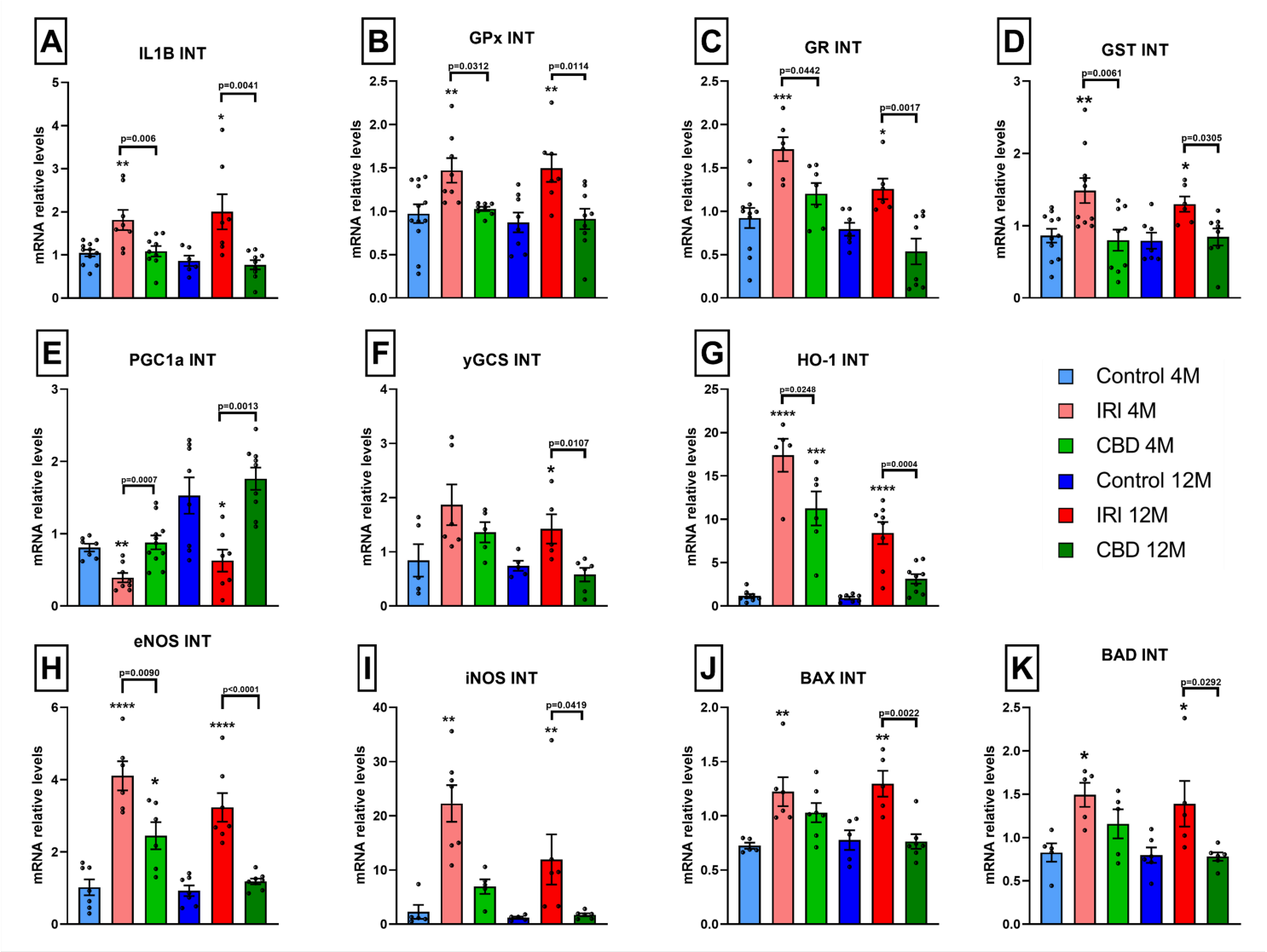

Figure 6

Relative intestinal gene expression levels in young and old rats. Analysis of mRNA expression in young (4M) and old (12M) rats after intestinal ischemia-reperfusion (IRI), with and without CBD treatment. Analyzed genes include proinflammatory (A: IL-1β), antioxidant (B: GPx, C: GR, D: GST, F: yGCS, G: HO-1), mitochondrial biogenesis regulators (E: PGC1α), nitric oxide-dependent enzymes (H: eNOS, I: iNOS), and proapoptotic markers (J: BAX, K: BAD). Data are presented as mean ± SEM (n=6-8 per group). Statistical differences were analyzed using ANOVA followed by Tukey's post-hoc test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 vs. control group of the same age.

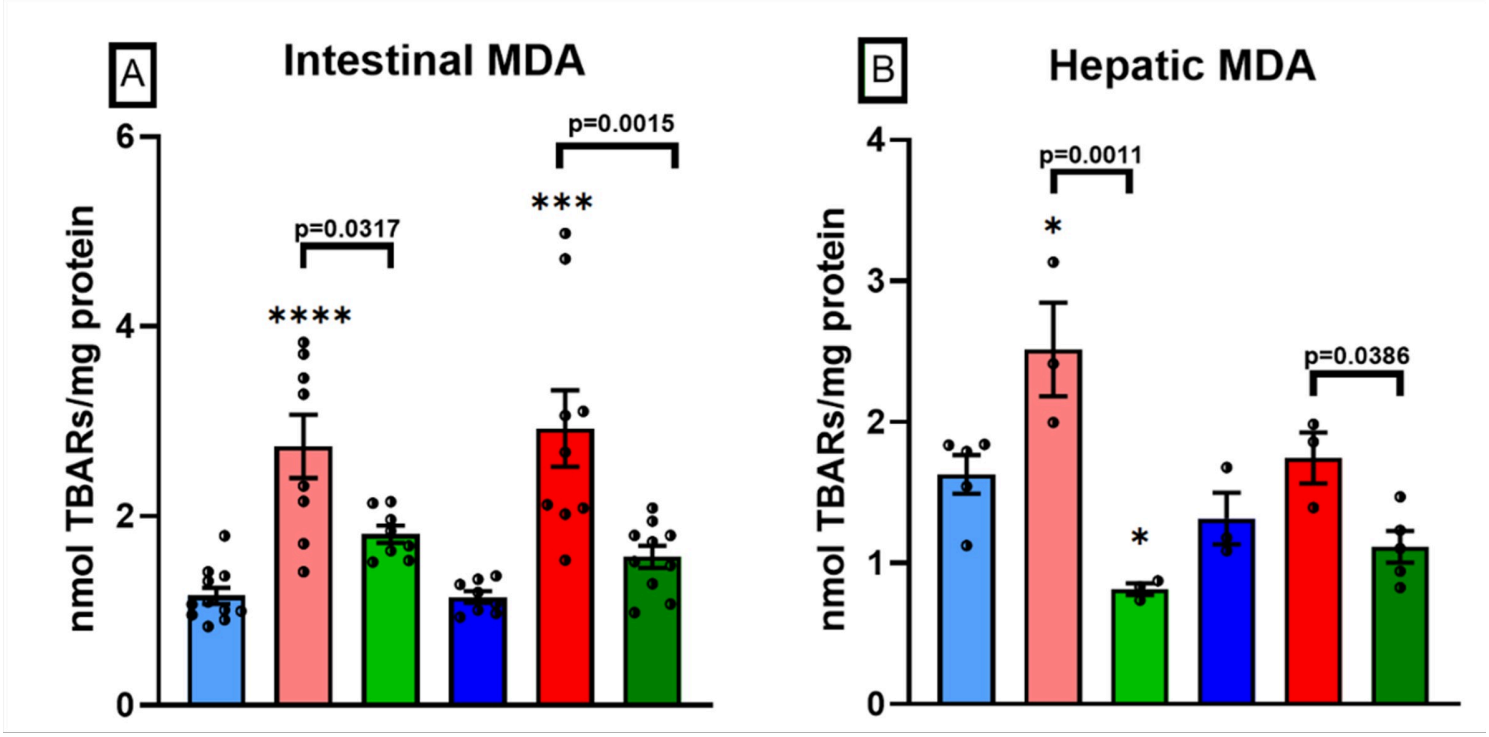

Figure 7

Malondialdehyde (MDA) quantification in intestinal and hepatic tissue. MDA levels in the intestine (A) and liver (B) of young (4M) and old (12M) rats after intestinal ischemia-reperfusion (i-IRI), with or without CBD treatment. MDA, a marker of lipid peroxidation, is expressed as nmol TBARS/mg of protein. Data are presented as mean ± SEM (n=4 for intestine and n=8-9 for liver per group). Statistical differences were analyzed using ANOVA followed by Tukey's post-hoc test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 vs. control group of the same age.

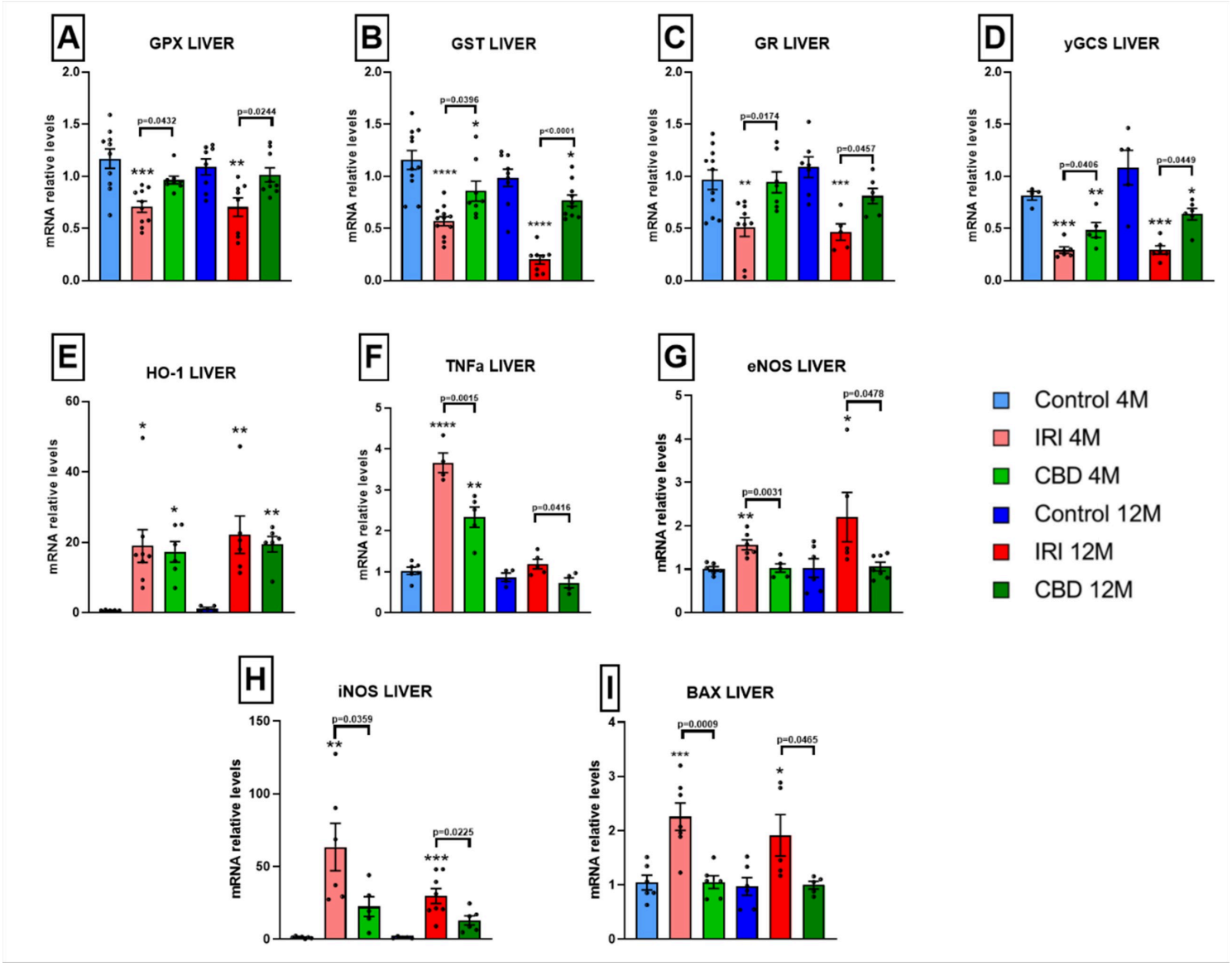

Figure 8

Relative hepatic gene expression levels in young and old rats. Analysis of mRNA expression in hepatic tissue of young (4M) and old (12M) rats after intestinal ischemia-reperfusion (IRI), with and without CBD treatment. Analyzed genes include antioxidant (A: GPx, B: GST, C: GR, D: yGCS, E: HO-1), inflammatory (F: TNFα), nitric oxide-dependent enzymes (G: eNOS, H: iNOS), and proapoptotic markers (I: BAX). Data are presented as mean ± SEM (n=6-8 per group). Statistical differences were analyzed using ANOVA followed by Tukey's post-hoc test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 vs. control group of the same age.

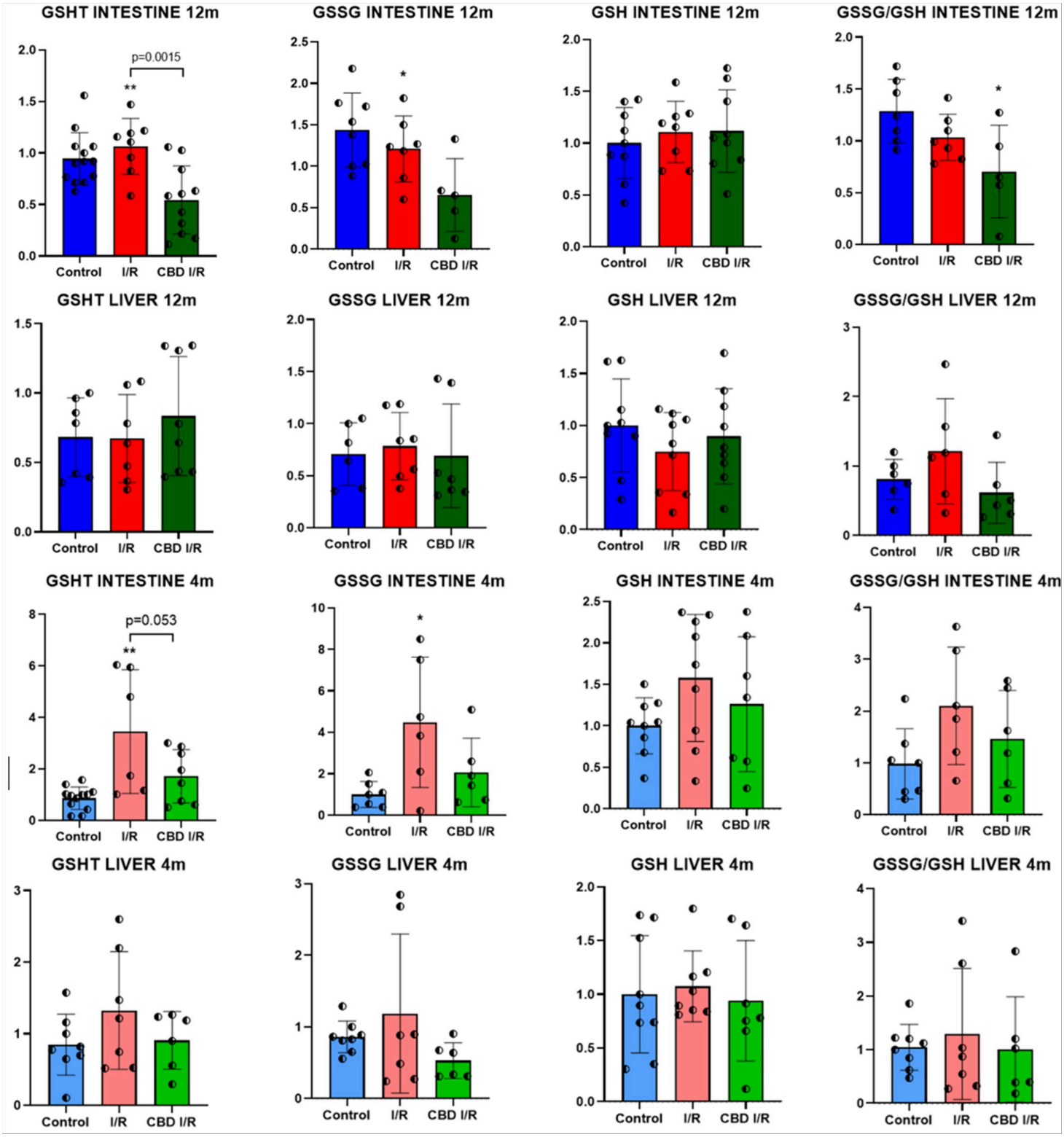

Figure 9

Intestinal and hepatic glutathione system metabolites. Quantification of total glutathione (GSHT), oxidized glutathione (GSSG), reduced glutathione (GSH), and GSSG/GSH ratio in the intestine and liver of 4-month (4M) and 12-month (12M) old rats. CBD treatment attenuated the massive oxidative burst and expansion of the oxidized glutathione pool in the intestine, while hepatic levels remained statistically stable at the 2-hour reperfusion window. Data are presented as mean ± SEM (n=6-8 per group).

Statistical differences were analyzed as described in the Methods section. *p<0.05; **p<0.01 vs. control group; ##p<0.01 vs. IRI group.

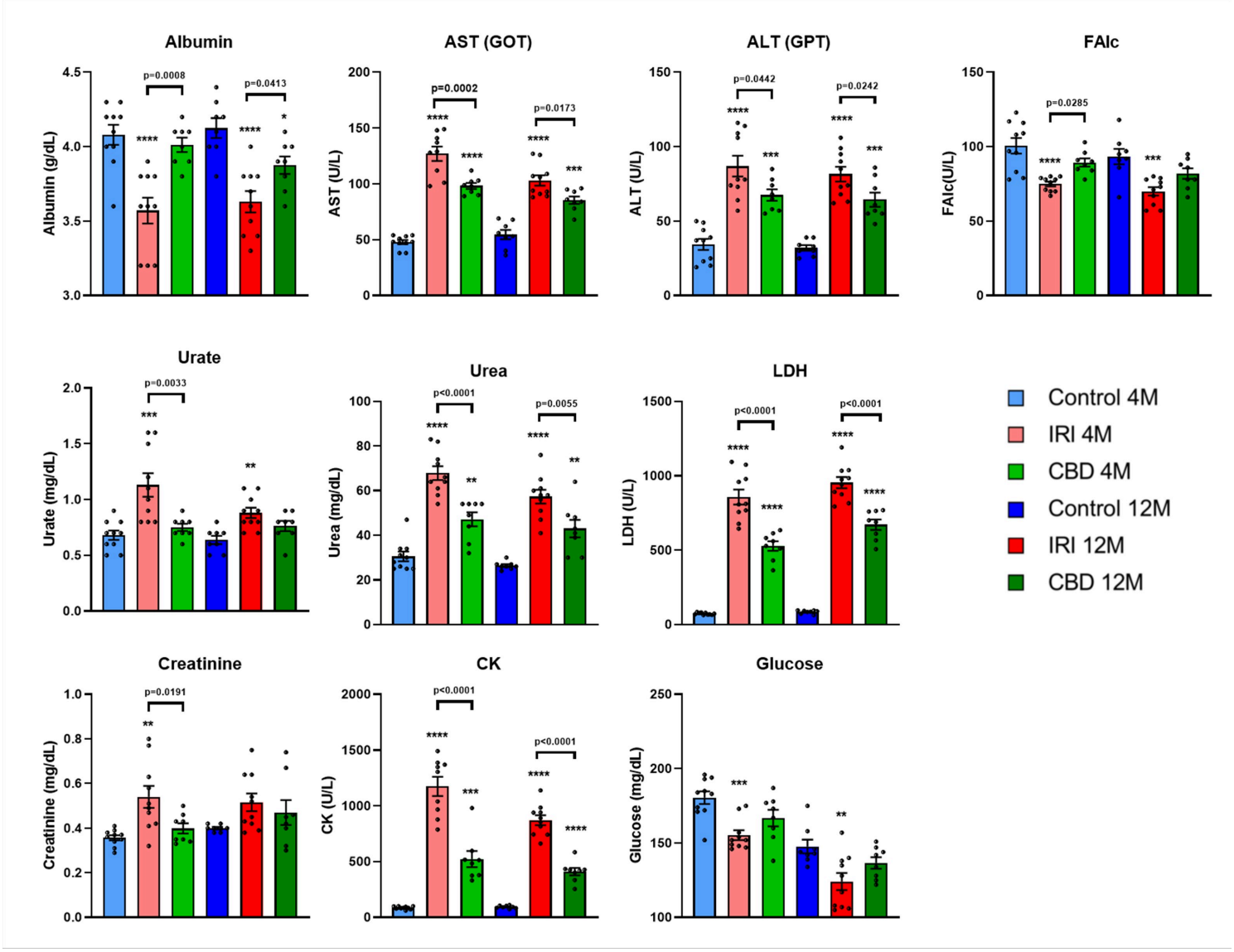

Figure 10

Serum biochemical profile in young and old rats. Circulating levels of hepatic, renal, and metabolic markers following intestinal ischemia-reperfusion (IRI), with or without CBD treatment. Prophylactic CBD significantly attenuated the i-IRI-induced release of intracellular enzymes (AST, ALT, LDH, CK) and improved synthetic/metabolic markers (Albumin, Glucose). Data are presented as mean ± SEM (n=6-8 per group). Statistical differences were analyzed as described in the Methods section. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 vs. control group of the same age.