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Original article

Honokiol remodeled the extracellular matrix and protected the intestinal tissue against ischemia-reperfusion injury in rats

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Abstract

Intestinal ischemia-reperfusion (IR) injury is a major clinical challenge due to its high morbidity and mortality rates. This study aims to demonstrate the effect of honokiol, a natural antioxidant compound, on intestinal IR injury in rats using histochemical and biochemical methods.

The protein-protein interaction (PPI) network construction and the honokiol-target network--reactome pathway analysis were performed using Cytoscape v3.10.1 software to validate inclusion of focused proteins in the study. 1 hour/2 hours of IR was applied on intestinal (jejunum) tissues. The tissues were further processed for biochemical measurement of total oxidant status (TOS) and antioxidant status (TAS). 5 mg/kg honokiol treatment was administered to rats after ischemia protocol. The tissues were fixed in formaldehyde and embedded in paraffin protocol. Sections were stained with vascular endothelial growth factor (VEGF), a disintegrin and metalloproteinase with thrombospondin motifs 15 (ADAMTS-15) and caspase-3 antibodies.

Analysis of the signaling network revealed that honokiol exerts a significant influence on the proposed mechanisms associated with IR through the VEGF, ADAMTS-15, and caspase-3 network. IR increased the TOS level and decreased the TAS level in ischemia and IR group, histopathologically damaged the intestinal tissues and led to epithelial degeneration, increased cell death, vascular dilatation and congestion. Honokiol treatment reduced the oxidant enzymes and supported the antioxidant system, and restored pathologies in the IR+honokiol group. Intestinal IR injury increased VEGF expression, ADAMTS-15 and caspase-3 expression in the ischemia and IR groups. Honokiol treatment after ischemia reduced the VEGF, ADAMTS15 and caspase-3 by restoring tissue integrity, preventing cell death and increasing cell matrix remodeling.

The administration of honokiol provided protection against intestinal IR injury by modulating apoptosis, angiogenesis, extracellular matrix remodeling processes through regulation of the VEGF, ADAMTS-15, and caspase-3 expression.

Keywords: antioxidant, honokiol, immunostaining, ischemia-reperfusion, jejunum

Introduction

Honokiol ($C_{18}H_{18}O_2$; 2-(4-hydroxy-3-prop-2-enylphenyl)-4-prop-2-enyl) is a naturally occurring bioactive neolignan polyphenolic compound derived from *Magnolia officinalis* (CHEN et al. 2021, Rauf et al. 2021). It has received significant attention in recent years for its diverse range of pharmacological properties. Studies have shown that honokiol possesses antioxidant, anti-inflammatory, anti-proliferative and anti--neoplastic effects (Ong et al. 2019, Rauf et al. 2021). It is involved in regulation of oxidative stress by scavenging free radicals and controlling active oxygen levels. It reduces the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a key enzyme in reactive oxygen species (ROS) production in neutrophils. It also weakens the acetylation of superoxide dismutase 2 (SOD2) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), leading to decreased ROS accumulation and enhanced mitochondrial biogenesis (CHEN et al. 2021). Additionally, honokiol exhibits anti-inflammatory effects by down-regulating mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways, inhibiting lipopolysaccharide (LPS)-induced iNOS and COX-2 expression, and blocking phosphatidylinositol 3-hydroxy kinase/protein kinase (PI3K/AKT) pathway activation. It further inhibits NF-κB signaling by preventing IκB kinase activation (Zhang et al. 2019).

Recent studies have highlighted the diverse therapeutic effects of honokiol, demonstrating its potential in alleviating gastrointestinal disorders (Li et al. 2023). Within the intestines, honokiol exerts a calcium-lowering effect by inhibiting the influx of external calcium through channels such as the transient receptor potential cation channel subfamily C member 4 (TRPC4) and voltage-gated calcium channels associated with the M receptor (Niu et al. 2022). Moreover, honokiol has been found to be protective against intestinal barrier dysfunction in severe acute pancreatitis by suppressing high-mobility group protein B1 (HMGB1) acetylation and modulating the JAK/STAT1 pathway (Li et al. 2023). Furthermore, it plays a crucial role in regulating apoptosis in various cell types, including those within the intestinal epithelium. It upregulates prostacyclin synthase protein expression and inhibits endothelial cell apoptosis (Zhang et al. 2007). It also regulates gastroenteric systems (Qiang et al. 2009), facilitates anti-inflammatory function, and supports the intestinal barrier (Deng et al. 2018). Notably, honokiol's influence extends to the gut microbiota composition, indicating its multifaceted impact on gastrointestinal health (Zhai et al. 2023).

Intestinal ischemia-reperfusion (IR) injury is a complex pathophysiological phenomenon that often arises during surgical procedures, trauma, and in cases of hemorrhagic shock. IR injury is known to carry high morbidity and mortality rates, posing a significant challenge in clinical settings. Therefore, the intestinal ischemia model is an extensively investigated model in experimental animals. In this model ischemia is artificially induced in the small intestine by complete vascular occlusion of the superior mesenteric artery (SMA) followed by reperfusion of the blood supply (Goldsmith et al. 2013 and Gonzalez et al. 2015). This condition affects the vascular structures in all intestinal tissue layers, in particular a decrease in the microcirculation gives rise to the destruction of apical villi (Gordeeva et al. 2017). Tissue damage is promoted primarily by IR then ischemic insult and free radicals. Neutrophils, platelets, endothelial factors, and inflammatory cytokines are all known to be involved in intestinal IR injury (Daniel et al. 2011). It is a known phenomenon that generation of free radicals impairs the equilibration between oxidants and antioxidants and this may initiate tissue damage. Reperfusion in the ischemic tissue triggers a cascade of different events which cause ischemia-induced tissue damage, and that is more moderate than the damage occurring after reperfusion (Ates et al. 2004). Therefore, previous experimental ischemia studies indicate that measuring the biochemical total antioxidant and oxidant values are informative methods used to determine tissue injury (Yazici et al. 2014).

Vascular endothelial growth factor (VEGF) is a pivotal signaling protein known for its potent angiogenic activity. It plays a central role in stimulating the formation of new blood vessels, a process essential for tissue repair and growth (Ferrara 2004). VEGF was shown to have a lower synthesis level in healthy tissues of adult humans and animals, but higher expression in the embryo and during physiological or pathological neovascularization. VEGF has the ability to induce vascular permeability (Ferrara et al. 2003 and Nagy et al. 2007). A disintegrin and metalloproteinase (ADAM) proteins, on the other hand, have multifaceted roles in cell biology, influencing processes such as cell adhesion, migration, proteolysis, and signaling (Edwards et al. 2008, Duffy et al. 2009). Previous studies showed that free radicals affect the action of proteases in ischemia and inflammation. Reactive oxygen and nitrogen species are produced during the neuroinflammatory phase of the ischemic injury. The major proteases involved in cell destruction include neutral proteases, such as caspases, and the adam lysins (ADAMs) and free radicals (Liu and Rosenberg 2005). Caspase-3 is a key regulatory protein in the caspase family, primarily responsible for orchestrating apoptosis, a critical process in programmed cell death (McIlwain et al.

2013). It is also an important component for B-cell homeostasis (Kuida et al. 1996, Woo et al. 1998, 2003). These proteins are integral to various physiological and pathological processes and play significant roles in cellular homeostasis and tissue response to injury. Understanding their interactions and involvement in intestinal IR injury is vital for advancing our knowledge of related pathologies and potential therapeutic interventions.

Despite honokiol's varied effects in specific cell types, its impact on intestinal tissue cells during IR injury has not been extensively investigated. In the light of previous studies, we aimed to investigate the protective effect of antioxidant compound honokiol in a rat model of intestinal IR injury by using VEGF, ADAMs and caspase markers immunohistochemically in addition to histopathological and biochemical methodologies.

Materials and Methods

Construction of merged PPI network

A honokiol target signaling pathway was constructed using a Search Tool for Interactions of Chemicals STITCH) database in Cytoscape v3.10.1 (https://cytoscape.org/). A protein-protein interaction (PPI) network including vascular endothelial growth factor (VEGFA), a disintegrin and metalloproteinase with thrombospondin motifs 15 (ADAMTS15) and caspase-3 was constructed with a Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database in Cytoscape software. For both analyses, maximum additional interactors and a minimum interaction score were set at 40 and 0.4 (medium confidence), respectively. Then these two networks were merged in order to elucidate the potential action mechanism of honokiol in relation to the focused proteins. Since the VEGFA network can be obtained through its receptors in the STRING database, FLT1 (Vascular Endothelial Growth Factor Receptor 1) and KDR (Vascular Endothelial Growth Factor Receptor 2) proteins were marked on the network.

Honokiol-targeted network-reactome pathway analysis

To demonstrate that honokiol targets the focused proteins (VEGFA, ADAMTS-15 and caspase-3) and cellular events, a network analysis was conducted using Cytoscape software v3.10.1 (https://cytoscape.org/). Honokiol target signaling pathway (maximum additional interactors: 40, confidence cutoff: 0.40) was constructed by using a STITCH database in cytoscape. PPI network including VEGFA, ADAMTS-15 and caspase-3 was constructed with a Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (maximum additional interactors: 40, confidence cutoff: 0.40) to predict protein interactions that contain direct and indirect associations of focused proteins in Cytoscape. The honokiol and the predicted target PPI networks were merged, and functional enrichment was applied. After the resulting pathway was filtered as a reactome pathway, the VEGFA, ADAMTS-15 and caspase-3 associated reactome analysis results were added as a node. Finally, the honokiol signaling pathway, focused proteins PPI and associated cellular events were visualized in Arena3Dweb (https://arena3d.org/) as multilayered graphs in 3D space. FLT1 and KDR marked in layers were VEGFA receptors.

Experimental design

Ethical approval was obtained from the Animal Experimentations Local Ethical Committee, Dicle University (2021/32). In this study, 40 three-month-old and healthy male Wistar Albino rats weighing 250-290 g were used. Rats were categorized into 4 groups, 10 rats per group. Honokiol extract was commercially purchased (catalog no: H4914, Sigma-Aldrich Inc Merck KGaA, Darmstadt, Germany). All procedures were performed under anesthesia with an intramuscular injection of ketamine (50 mg/kg; Ketalar; Parke Davis, Turkey) and xylazine (10 mg/kg; Rompun; Bayer AG, Germany). Honokiol extract was dissolved in dimethyl sulfoxide (DMSO) for treatment. All rats were fasted for 12 hours before the experiment. The abdominal region was shaved and a 3 cm abdominal midline incision was opened. In the intestinal IR injury model, SMA was occluded by a nontraumatic bulldog clamp.

Sham group: Rats were fixed on the operation table, and the abdomen with fascia was opened. SMA was observed and without any further occlusion, the abdomen was sutured. The rats were sacrificed by exsanguination under anesthesia. Jejunum tissues were kept for biochemical and histological staining.

Ischemia group: Rats were fixed on the operation table, and abdomen with fascia was opened. SMA was occluded for 1-hour to create ischemia with bulldog clamp. After 1 hour, clamp was removed and animals were sacrificed by exsanguinating under anesthesia. Jejunum tissues were retained for biochemical and histological staining.

Ischemia reperfusion (IR) group: Rats were fixed on the operation table, and the abdomen with fascia was opened. SMA was occluded for 1 hour to create ischemia with a bulldog clamp. After 1 hour, the clamp was removed and the intestine was reperfused for 2 hours.

After IR, the rats were sacrificed by exsanguination under anesthesia. Jejunum tissues were retained for biochemical and histological staining.

IR+honokiol group: Rats were fixed on the operation table, and the abdomen with fascia was opened. SMA was occluded for 1 hour to create ischemia with a bulldog clamp. After 1 hour, the clamp was removed and the intestine was reperfused for 2 hours. After IR, 5 mg/kg honokiol was administered to the rats via the intraperitoneal route. The administration and route of the honokiol was determined according to Yu et al. (2016). The rats were sacrificed by exsanguination under anesthesia. The jejunum tissues were retained for biochemical and histological staining.

Biochemical analysis

The jejunal tissue samples were thawed at room temperature. Working solution was added in a tube at 9 times the sample quantity (1/9). The samples were homogenized and centrifuged at 3000 rpm for 6 minutes. The supernatant was removed and calorimetrically analyzed using the "Rel Assay E" auto analyzer according to the method of Erel et al. (2005). Total antioxidant status (TAS) and total oxidant status (TOS) were measured using an automatic instrument. The TOS unit was defined as μmol H_2O_2Equiv./L and the TAS unit was recorded as mmol Trolox equivalent/L.

Histopathological analysis

The jejunal samples were fixed in zinc formalin solution, dehydrated in grading ascending ethanol series and embedded into paraffin wax 4 μm sections were stained with Haematoxylen-Eosin and imaged under a light microscope (Carl Zeiss Imager A2, Germany).

A semiquantitative histological evaluation scoring system was used to determine histopathological changes. The scoring system was developed according to the Schweizer et al. method (Schweizer et al. 1992). The criteria were mucosal damage (decomposition in the surface epithelium), vascular dilatation/congestion, hemorrhage, and inflammation. Each specimen was scored using a scale ranging from 0 to 3 (0: none, normal histological structure; 1: mild; 2: moderate; 3: severe) for each criterion. Two expert pathologists analyzed the specimens in a double-blinded manner.

Immunohistochemical methods

Sections were deparaffinized in xylene, rehydrated in descending ethanol series, and washed in distilled water. Endogenous peroxidase activity was blocked in 3% hydrogen peroxide solution (catalog no: TA-015-HP, ThermoFischer, Fremont, CA, US) for 30 minutes. Nonspecific binding was blocked by applying blocking solution (catalog no: TA-015-UB, ThermoFischer, Fremont, CA, US) for 8 min prior to incubation of primary antibodies VEGF and caspase-3 (#sc-7269, #sc-7272, Santa Cruz Biotech., US; respectively) and ADAMTS-15 (#PA5-48070, Invitrogen, US) for overnight. Secondary antibody (catalog no: TA-015-BN, ThermoFischer, Fremont, CA, US) was applied for 15 min. The sections were then exposed to streptavidin-peroxidase solution (catalog no: TA-015-HR, ThermoFischer, Fremont, CA, US) for 20 min. Diaminobenzidine (catalog no: TA-015-HCX, ThermoFischer, Fremont, CA, US) was used as a chromogen. The sections were counter-stained with Gill III Hematoxylin (Catalog no:107961, Sigma-Aldrich, St. Louis, MO, US), washed in tap water for 3 min and in distilled water for 2x3 minutes, mounted with mounting medium and imaged under a light microscope (Carl Zeiss Imager A2, Germany).

Statistical Analysis

Statistical analysis was performed with SPSS (Version 25.0, SPSS Inc., Chicago, IL, US). Data distribution was analyzed using the Shapiro-Wilk test. Descriptive statistics were presented as median (min-max). The groups were statistically compared using the Kruskal-Wallis test and the post-hoc Dunn's test. A value of $p<0.05$ was considered statistically significant.

Results

Merged PPI network analysis

In the network obtained by merging the PPI of honokiol and the proteins included in the study, it is evident that caspase-3 plays a central role as a key point of connection between honokiol and the proposed ischemia reperfusion (IR) mechanism. The network analysis confirmed that VEGFA, ADAMTS-15 and caspase-3, which were considered to play a significant role in the IR mechanism, are indeed involved in honokiol's action mechanism (Fig. 1).

Honokiol-Targeted Network-Reactome Pathway Analysis

The network map visualized in Arena3Dweb tools revealed that honokiol is involved in "Apoptotic cleavage of cellular proteins", "programmed cell death", "caspase activation via extrinsic apoptotic signaling pathway", "signaling by VEGF and extracellular matrix

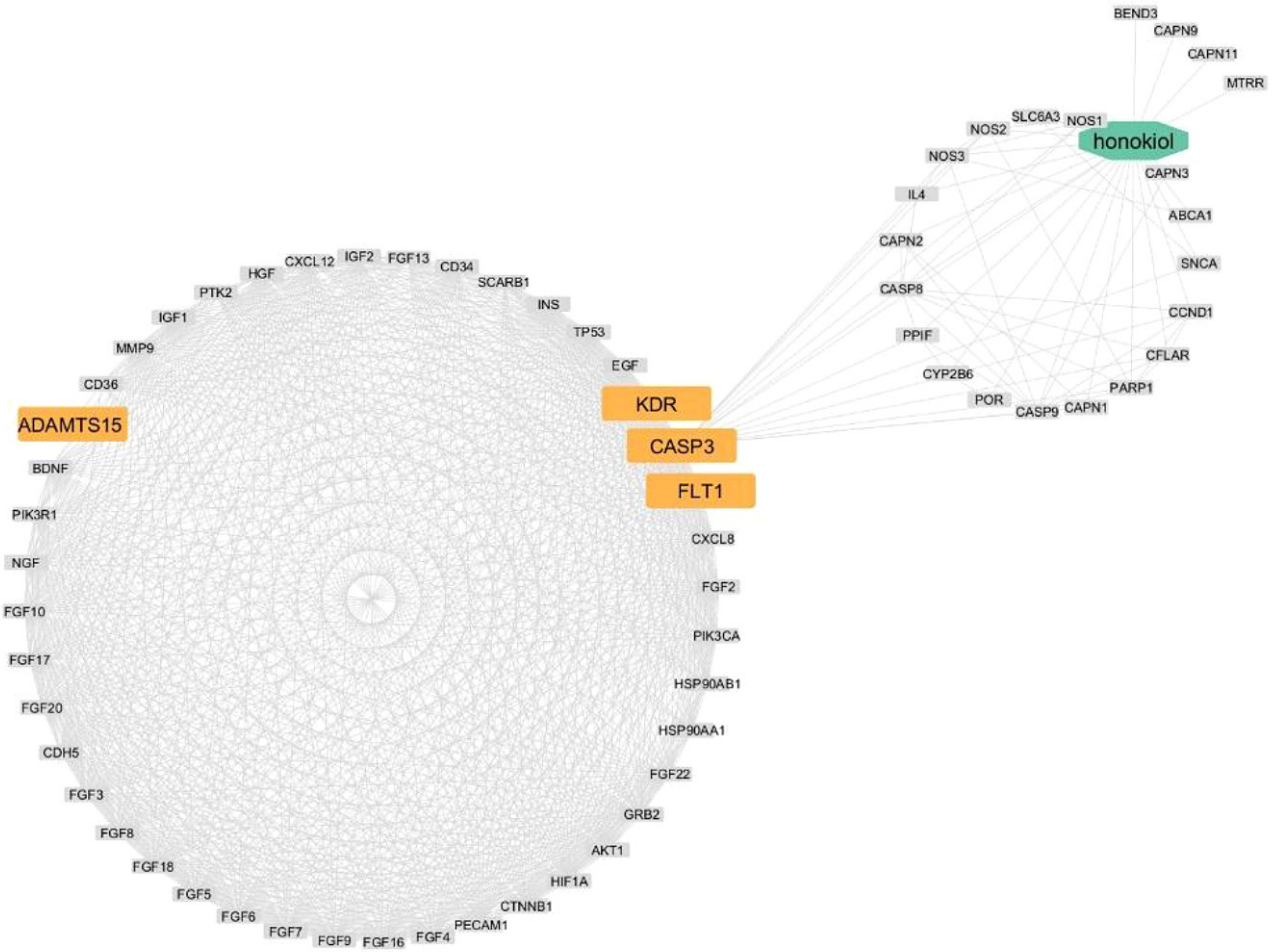

Fig. 1. Merged protein-protein interaction (PPI) network of the focused proteins: a disintegrin and metalloproteinase with thrombospondin motifs 15 (ADAMTS-15), Caspase-3, Vascular Endothelial Growth Factor (VEGFA) and VEGFA receptors: Vascular Endothelial Growth Factor Receptor 1 (FLT1), Vascular Endothelial Growth Factor Receptor 2 (KDR) PPI and honokiol pathway in rats.

organization" through VEGFA, ADAMTS-15 and caspase-3 PPI network. This analysis demonstrated that the honokiol, IR, and the focused mechanisms are in congruent alignment. As a result, it has been deduced that honokiol is a compound that affects the suggested mechanisms for IR through the focused protein network (Fig. 2).

Biochemical findings

TAS and TOS values are shown in Table 1. TOS values were the highest in the ischemia and IR groups, and the TAS value was lowest in these groups compared to the sham group. Honokiol treatment significantly improved the scores and favored the antioxidant system of the cell by its antioxidant activity ($p<0.05$).

Histopathological findings

Intestinal sections were stained with Hematoxylin Eosin and the images are shown in Fig. 3. Histopathology of the sham group showed that the jejunal villi are regular, protruding into the lumen. The mucosal layer was normal with a single layered columnar epithelium and regular intestinal glands. There was no pathology in the blood vessels and the connective tissue cells (Fig. 3A). Intestinal villi were degenerated with shedding of epithelial cells in the ischemia group. Collagen fibers and cells of connective tissue cells were degenerated. Vascular dilatation and congestion with inflammation and disrupted intestinal villi were observed (Fig. 3B). In the IR group, structural integrity of the mucosal layer was disrupted. The villi were degenerated with apoptotic epithelial cells. Vascular pathologies and inflammation were also seen in this group (Fig. 3C). In the IR+honokiol treated group, the intestinal villi and glands were restored. Epithelial cells were increased in number with a normal histological appearance. There was also prominent improvement in vessels and inflammation. Collagen fibers were also increased (Fig. 3D).

Histological scoring showed a similar finding with histological images. Epithelial degeneration, vascular dilation/congestion and inflammation were signifi-

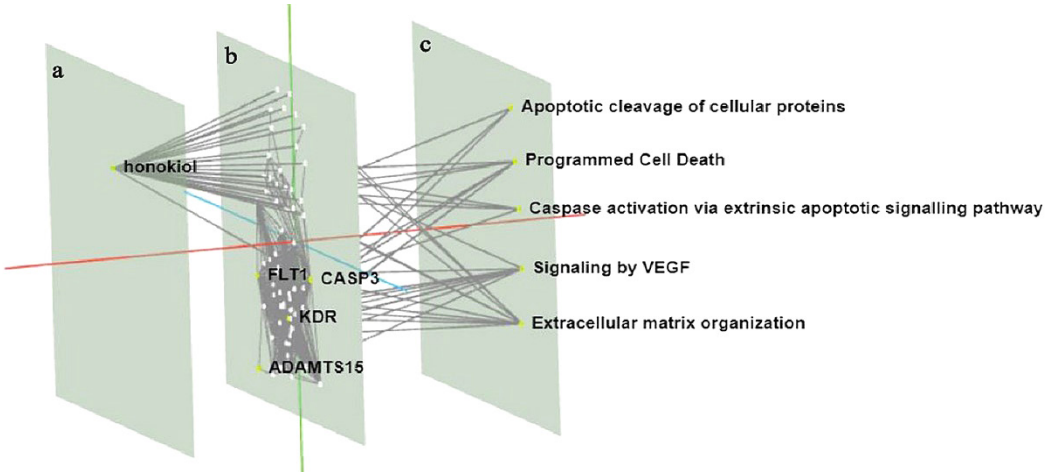

Fig. 2. Honokiol-targeted network-reactome pathway map in rats. Layers a, b, c represent honokiol protein-protein interaction (PPI) of targeted proteins: a disintegrin and metalloproteinase with thrombospondin motifs 15 (ADAMTS-15), Caspase-3, Vascular Endothelial Growth Factor A (VEGFA) and VEGFA receptors: Vascular Endothelial Growth Factor Receptor 1 (FLT1), Vascular Endothelial Growth Factor Receptor 2 (KDR) and biological functions of targeted proteins associated with ischemia-reperfusion (IR).

Table 1. Biochemical parameters antioxidant status (TAS) and total oxidant status (TOS) in rat sham, ischemia, IR and IR+honokiol groups.

Group	TAS	TOS
Sham	1.51±0.02	12.30±0.06
Ischemia	1.09±0.01*	22.45±0.03*
IR	0.75±0.03*	43.53±0.10*
IR+honokiol	1.44±0.04**	18.23±0.05**

Values are shown as mean±standard deviation. *: sham vs injury groups, ** injury groups vs honokiol

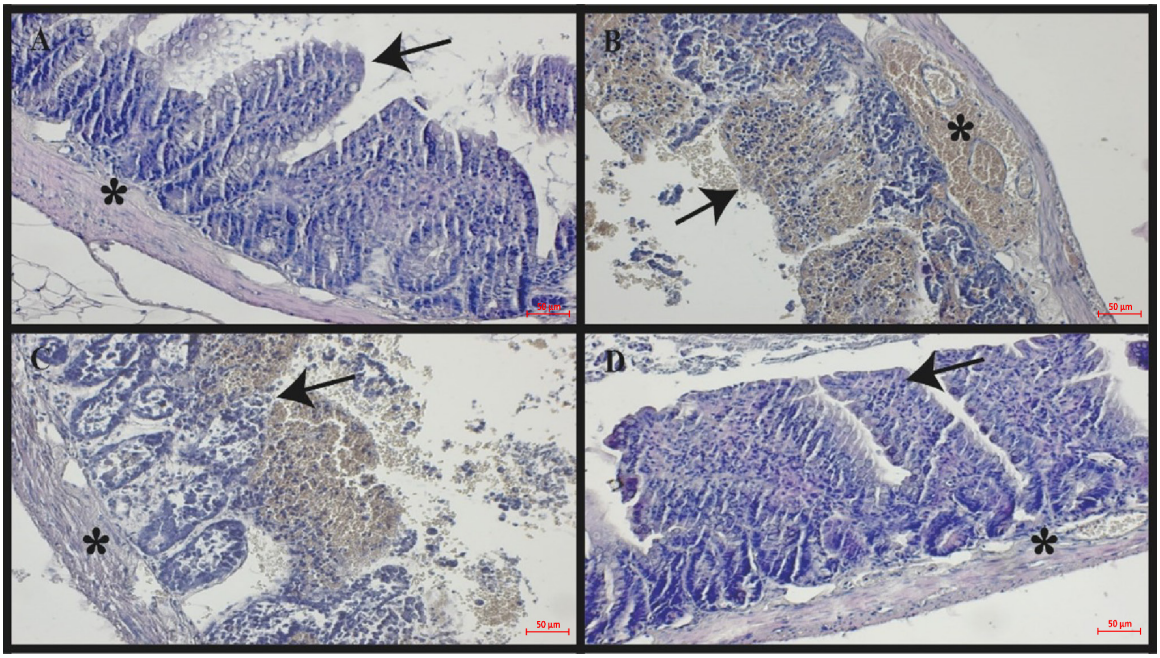

Fig. 3. Hematoxylin eosin staining of rat intestinal sections. A. Sham group: Normal appearance of intestine with epithelium cells (arrow) and intestinal layers (asterisk); B. Ischemia group: A significant loss in intestinal villi with degenerated and apoptotic changes in epitheial cells (arrow), dilatation and congestion in blood vessels with increased inflammatory cells (arrow, asterisk); C. IR group: Degenerated intestinal villi (arrow), inflammation (asterisk); D. IR+Honokiol group: Restored intestinal villi and intestinal glands (arrow), decreased inflammation (asterisk), Scale Bar = 50 µm.

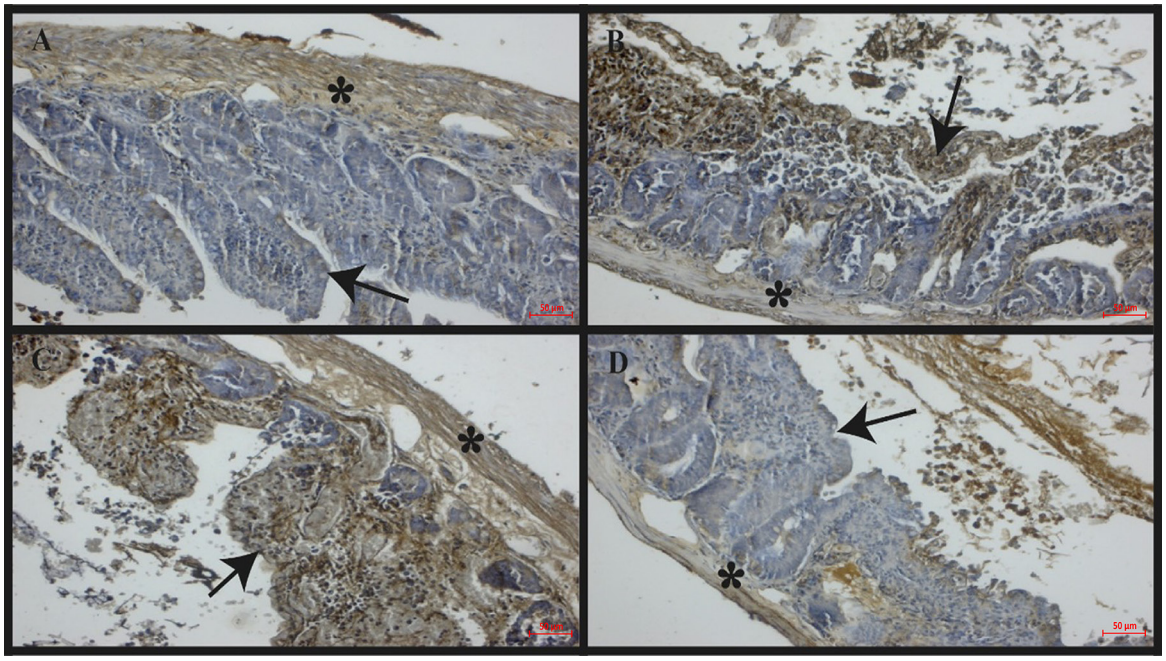

Fig. 4. Vascular Endothelial Growth Factor (VEGF) immunostaining of rat intestinal sections. A. Sham group: Mild VEGF expression in intestinal villi (arrow) and intestinal layers (asterisk); B. Ischemia group: Increased VEGF expression in degenerated villi and epithelial cells (arrow), and muscular layers (asterisk); C. IR group: VEGF expression was upregulated in degenerated villi and epithelial cells (arrow), and muscular layers (asterisk); D. IR+Honokiol group: Decreased VEGF expression in intestinal villi and epithelial cells (arrow), and muscular layers (asterisk); Scale Bar = 50 µm.

Table 2. Histopathologic scores for degenerated epithelium, congestion/dilatation and inflammation in rat sham, ischemia, IR and IR+honokiol groups.

Group	Degenerated epithelium	Congestion/Dilatation	Inflammation
Sham	0.36±0.50	0.47±0.50	0.80±.06
Ischemia	2.12±0.46*	2.54±0.13*	2.21±0.33*
IR	2.79±0.38*	2.83±0.25*	2.73±0.51*
IR+honokiol	1.45±0.52**	1.27±0.34**	1.01±0.48**

Values are shown as mean±standard deviation. *: sham vs injury groups, ** injury groups vs honokiol

cantly higher in the ischemia and IR groups compared to the sham group ($p<0.05$, respectively). After honokiol administration, intestinal pathologies were significantly decreased in the IR+honokiol group compared to the ischemia and IR groups. Honokiol treatment improved histological scores. Scores per group are shown in Table 2. Histological results support the hypothesis that honokiol, with its many biological activities, showed antioxidant and anti-inflammatory effects, protecting the tissue integrity of the intestine after IR injury.

Immunohistochemical findings

VEGF immunostaining

Immunostaining of VEGF antibody is shown in Fig. 4. In the sham group, VEGF expression was positive in the vascular endothelial cells (Fig. 4A). In the ischemia and IR groups, VEGF expression was highly increased in dilated endothelial cells and inflammatory cells (Fig. 4B and 4C, respectively). The IR+honokiol group showed decreased VEGF-positive expression in endothelial cells after IR injury (Fig. 4D).

ADAMTS-15 immunostaining

Immunostaining of ADAMTS-15 antibody is shown in Fig. 5. ADAMTS-15 expression was observed in the extracellular matrix of mucosa and submucosa layers in the sham group (Fig. 5A). In the ischemia and IR groups, ADAMTS-15 expression was significantly augmented in the extracellular matrix, which showing IR injury disrupted the matrix and induced matrix breakdown (Fig. 5B and 5C, respectively). The honokiol treated group showed a dramatically decreased expression of ADAMTS in the extracellular matrix of intestinal layers (Fig. 5D). Honokiol induced intestinal tissue renewal by suppressing the expression of ADAMTS-15.

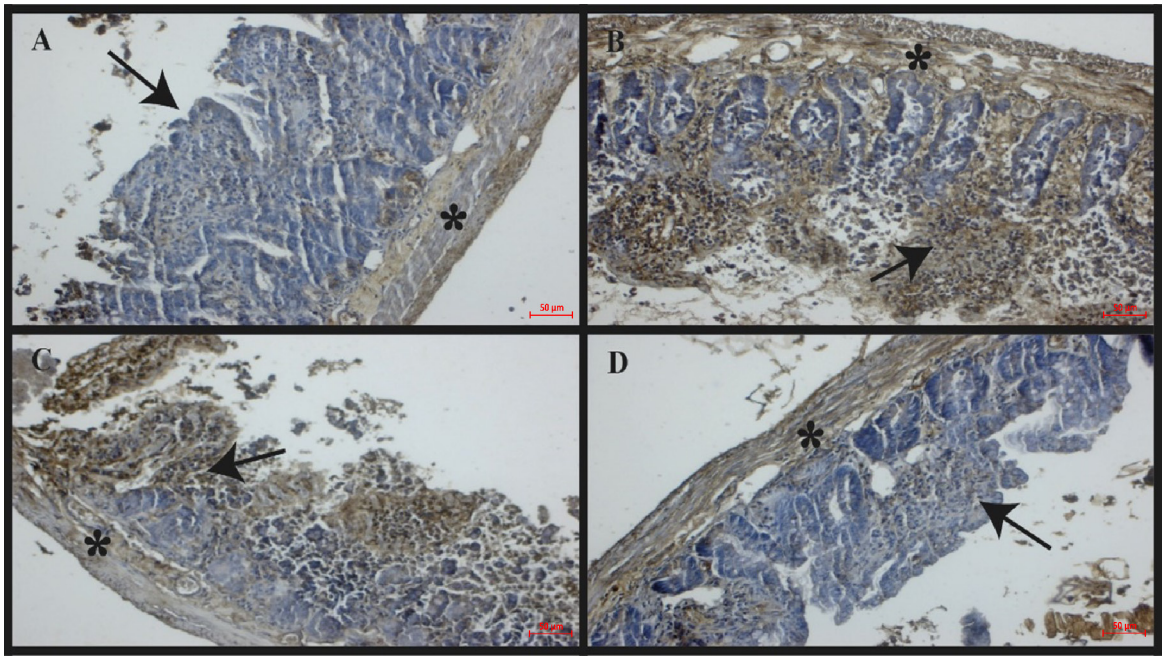

Fig. 5. ADAMTS-15 immunostaining of rat intestinal sections. A. Sham group: Mild ADAMTS-15 expression in intestinal villi (arrow) and intestinal layers (asterisk); B. Ischemia group: ADAMTS-15 expression was upregulated in degenerated villi and epithelial cells (arrow), and muscular layers (asterisk); C. IR group: High ADAMTS15 expression in degenerated villi and epithelial cells (arrow), and muscular layers (asterisk); D. IR+Honokiol group: ADAMTS-15 expression was downregulated in intestinal villi and epithelial cells (arrow), and muscular layers (asterisk); Scale Bar = 50 µm.

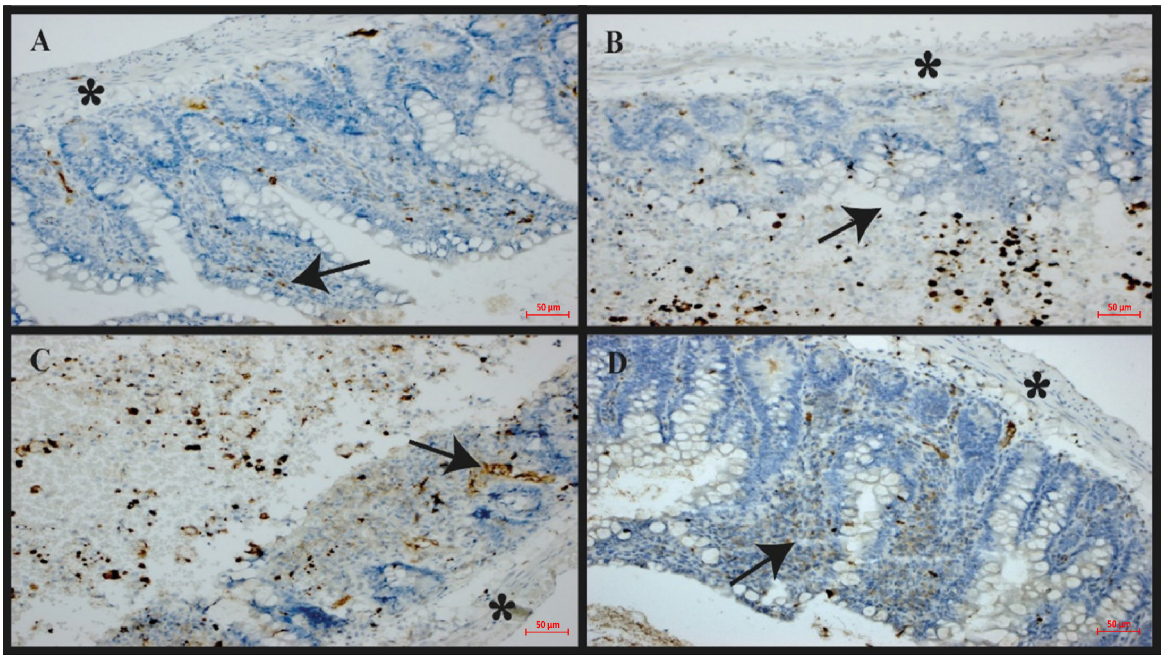

Fig. 6. Caspase3 immunostaining of rat intestinal sections. A. Sham group: Mild Caspase3 expression in epithelial cells (arrow) and negative expression in intestinal layers (asterisk); B. Ischemia group: High caspase3 expression in degenerated villi and epithelial cells (arrow), and muscular layers (asterisk); C. IR group: Overexpression of caspase3 in degenerated villi and epithelial cells (arrow), and muscular layers (asterisk); D. IR+Honokiol group: Decreased caspase3 expression in intestinal mucosal layer (arrow), and muscular layers (asterisk); Scale Bar = 50 µm.

Caspase-3 immunostaining

Immunostaining of caspase-3 antibody is shown in Fig. 6. The sham group showed mainly negative caspase3 expression (Fig. 6A). Due to the disruptive effects of IR injury, caspase-3 expression was very high in the ischemia and IR groups compared to the sham group, showing that many epithelial and stromal cells were apoptotic (Fig. 6B and 6C, respectively). In the IR+honokiol group, the level of caspase-3 expression

was significantly reduced in intestinal cells, showing that honokiol has anti-apoptotic effects against IR injury, promoting cell survival (Fig. 6D).

Discussion

Honokiol has anti-inflammatory activity through inhibition of protein kinase C, mitogen-activated protein kinase, and MAPK, NF-κB and PI3K/AKT signaling pathways (Cho et al. 2008, Chao et al. 2010, Zhang et al. 2019). Among the molecular players implicated in IR injury, the involvement of genes such as ADAMTS-15, VEGFA and caspase-3 is of particular interest. The PPI analysis and honokiol-targeted network-reactome pathway results definitively establishes honokiol as a potent compound that exerts a profound impact on the suggested mechanisms underlying IR through the VEGFA, ADAMTS-15 and caspase-3 network. Caspase-3 occupies a crucial position by linking the honokiol pathway to the focused protein pathway network. In addition to its essential function in apoptosis, this further underscores another significant reason for its inclusion in the study. It appears that honokiol may have indirect effects on the expressions of ADAMTS-15 and VEGFA, both of which are pivotal in tissue repair processes. However, additional research is necessary to establish a direct relationship between honokiol and these gene expressions.

TOS and TAS measurements serve not only as indicators of oxidative and antioxidative status during diagnosis but also play a crucial role in monitoring treatment progress (Demirpençe et al. 2014). TOS measurement provides a sensitive lipid peroxidation and oxidative stress index (Aycicek and Ipek 2008). Honokiol treatment led to a significant increase in TAS levels, accompanied by a decrease in TOS in the current study. Intestinal IR injury may often give rise to severe intestinal damage and increased intestinal permeability. A study of He et al. (2012) showed that intestinal IR causes intestinal morphological changes, such as intestinal mucosal injury, erosion, necrosis, interstitial congestion in the lamina propria of villi top, edema, inflammation, and mucosal and submucosal hemorrhage (He et al. 2012). Intestinal IR injuries are characterized by altered microvascular and epithelial permeability and villus damage (Spanos et al. 2007).

Honokiol is a biologically active compound that is also involved in many cellular pathways with tissue protective properties. Hu et al. showed that honokiol treatment prevented arterial thrombosis with nitric oxide synthase (NO) stimulation (HU et al. 2005). Another study also indicated that honokiol has a protective effect against drug induced gastric injury, by maintaining mitochondrial integrity, inhibiting apoptosis and inflammation in gastric tissues (Debsharma et al. 2023). This study revealed that intestinal IR injury caused microscopic intestinal damage such as mucosal destruction, villus degeneration and loss of epithelial cell, vascular dilatation and congestion and increased inflammation consistent with previous studies, honokiol treatment showed tissue protective effects on the intestine and promoted the tissue regeneration in the IR+honokiol group. Histological scoring also supported the microscopic findings in this study.

Gene expression, bacterial inoculation, hypoxia and cold stress together significantly regulate intestinal VEGF expression (Makino et al. 2001, Boutin et al. 2008). Hypoxia induces VEGF expression during IR injury. Canillioglu et al. studied ovarian and uterus tissue to record the expression of VEGF in an experimental IR model. They found that VEGF expression was increased in parallel to inflammation and tissue damage in ovary and uterine tissue (Canillioglu and Senturk 2020). Similarly, Zhou et al. (2020) studied cardiac IR injury and investigated the angiogenesis via expression of VEGF. They found that IR caused heart tissue damage and upregulated the expression of VEGF in cardiac tissues. Given the potent antioxidant properties of honokiol, the alterations in VEGF expressions, driven by the restoration of vascular integrity and inflammation reduction, strongly indicate the favorable influence of honokiol on angiogenesis.

The morphology of intestinal mucosa, especially the structure of villus and crypt, is one of the most important indicators of the digestive and absorptive capacity of the small intestine (Buchman et al. 1995). The goblet cell depletion and the decreased ratio of villus height to crypt depth induced by honokiol may reduce the secretion capacity to downregulate the inflammatory cytokines and delay the apoptosis process (Chen et al. 2009, Tang et al. 2011). ADAMTS are reported to secrete multi-site matrix-related zinc metalloendopeptidases with different roles in the division and modification of procollagen-N-propeptidases and ECM proteoglycans in inflammation and vascular biology in tissue morphogenesis and pathophysiological remodeling (Kelwick et al. 2015). ADAMTS-15 has been reported to have a potential role in angiogenesis (Kumar et al. 2012). In this study, a positive reaction was observed in the expression of ADAMTS-15 in the ischemia and ischemia reperfusion group with an increase in inflammation due to increased collagen fiber and edema in the extracellular matrix. Following the administration of honokiol and the subsequent development of extracellular matrix structure and decreased inflammation, ADAMTS-15 expression was found

to be an inducing effect on the protection of the mucosa and angiogenesis.

Zhang et al. observed that activation of caspase-3, an indicator of apoptosis, is usually marked in intestinal IR (Zhang et al. 2007). A study by Takeshita et al. found an increase in the levels of expression of the proapoptotic gene caspase-3 in the intestinal IR rats (Takeshita et al. 2010). Ischemic preconditioning, one of the most effective strategies for protection from intestinal IR, reduces apoptosis by upregulating the anti-apoptotic gene bcl-2 and inhibiting activation of caspase-3, one of the most remarkable apoptotic regulators (Taha et al. 2013). In this research, we observed a positive caspase-3 expression in the highly degenerative and apoptotic cells within the villi, as well as in the cells of the lamina propria and submucosa layers in the ischemia and ischemia-reperfusion groups. Our findings suggest that honokiol inhibits apoptosis by downregulating the caspase-3 expression in the ischemia-reperfusion injury in the rat intestine.

Conclusion

Ultimately, this research indicates that honokiol not only decisively hinders apoptotic progression by modulating cellular degeneration, but also exerts a profoundly positive influence on angiogenesis by stimulating the development of the extracellular matrix following IR. Taken together, treatment with honokiol confers protective effects on intestinal IR injury by modulating the VEGF, ADAMTS-15, and caspase-3 signaling axis. Further studies are needed to better understand the precise mechanisms and therapeutic potential of these interventions in the context of IR injury, and to develop effective strategies for clinical application.

Limitations

This study has some limitations. The study is conducted on rats, and animal models may not fully replicate the complexity of human physiology and the effectiveness of honokiol in humans needs to be investigated separately. For the optimal dosage and administration, a dose-response relationship and potential side effects associated with different concentrations should be further investigated. The long-term effects, including chronic exposure or repeated administration of honokiol should also be analyzed. Additional molecular techniques or imaging modalities to provide a more comprehensive understanding of tissue changes should be studied.

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