Anti-inflammatory and anti-apoptotic effect of nesfatin-1 on liver ischemia-reperfusion injury

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INTRODUCTION: *Severe local and systemic tissue injury develop during reperfusion, which is a period during which arterial blood flow and tissue oxygenation are re-established. In this study, we aimed to investigate the anti-inflammatory, antioxidant and protective effects of nesfatin in IR damage developing in liver.*
MATERIAL AND METHODS: *Twenty-four male Wistar-Albino rats were divided to three groups which contained eight rats in all groups. The rats were subjected to 30 minutes of hepatic pedicule occlusion followed by 2h of reperfusion to induce I/R damage. Nesfatin1 (10 μg/ kg) was administered, 30 min prior to ischemia and immediately before the reperfusion period.*
RESULTS: *The findings showed that while the blood levels of AST, ALT and LDH were markedly elevated in the I/R group, they returned to normal levels upon treatment in the Nesfatin group. While IL-1 α, IL-1β, IL-6, TNF-α and IFN- γ levels in blood and tissue were lower after therapy in the Nesfatin group compared to the I/R group, statistically significant decreases were only noted in IL-1β, IL-6, TNF-α and IFN- γ levels. TAS levels increased in the treatment group, while upon nesfatin treatment statistically significant decreases were noted in TOS and OSI levels. Histopathological investigations also showed statistically significant decreases in Bax and Caspase-3 staining intensity and the number of stained cells in the Nesfatin group.*
CONCLUSION: *The nesfatin has antioxidant activity and anti-inflammatory effect on improvement of liver functions and histopathological findings in liver ischemia and reperfusion injury.*

KEY WORDS: Anti-inflammatory, Anti apoptotic Liver ischemia-reperfusion injury, Nesfatin-1

Introduction

Ischemia-reperfusion injury was first defined in 1960 by Haimovici, and it indicates the severe local and systemic tissue injury which occurs during the period when tissue oxygenation is re-established [1]. Free oxygen radicals, such as hydroxyl (OH), superoxide anion (O_2), singlet oxygen ($1O_2$), hydrogen peroxide (H_2O_2) and nitric oxide (NO), which are released from the polymorphonuclear leukocytes, are held responsible from the ischemia and reperfusion injury [2,3]. While ischemia and reperfusion (I/R) injury may affect all organs, liver I/R injury is particularly common after major liver surgeries and transplantation.

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Nesfatin-1, first discovered in 2006 by Oh-I et al., is an 82 amino-acid-long protein that is derived from the NUCB2 protein released from the hypothalamic nucleus responsible for appetite control [4]. In the study of Ayada et al., Nesfatin-1 was demonstrated to have anti-inflammatory and anti-apoptotic characteristics in subarachnoid hemorrhage models induced in rats [5]. The protective efficacy of Nesfatin-1 in kidney ischemia-reperfusion injury was established in a study by Guanjun et al. [6]. To our knowledge, there is not any research that has investigated the protective efficacy of exogenous Nesfatin-1 in liver I/R injury. To contribute to the relevant literature, the present study aims to investigate the potential anti-inflammatory efficacy of exogenous Nesfatin-1 in experimental liver I/R model.

Material and Methods

Current study protocols and experimental methods were approved by local Institutional Ethics Committee of Experimental Animals in University of Afyon Kocatepe.

Experimental Design

Group 1 (N: 8): The abdomen was shaved following analgesia and anesthesia. The abdomen was incised over the midline after field sterilization. The hepatic pedicle was rotated with exploration while clamping or drug administration was not performed.
Group 2 (N: 8-I/R): 1 mL of serum physiological was administered intraperitoneally 30 minutes before the surgical procedure. The abdomen was incised over the midline after field sterilization. Hepatic pedicle was rotated with exploration. Atraumatic clamping was performed on the hepatic pedicle for 30 minutes. The clamp was removed after 30 minutes and 2 hours of reperfusion was ensured.
Group 3 (N:8, I/R+Nesfatin-1): 10 μg/kg Nesfatin-1 in 1 mL SF was intraperitoneally administered 30 minutes before initiation of the surgical procedure. The abdomen was incised over the midline after field sterilization. Hepatic pedicle was rotated with exploration. Atraumatic clamping was performed on the hepatic pedicle for 30 minutes. The clamp was removed after 30 minutes and 2 hours of reperfusion was ensured.
Before rats were killed, 6 mL of blood samples were collected for biochemical examinations and centrifuged to obtain serum. Serum samples were stored at -70°C for biochemical examinations. In addition, liver was removed for investigation.

Laboratory Analysis

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels were indicators of liver ischemia, were analyzed in an autoanalyzer (Cobas 6000, Roche, Switzerland). TNF-α, IL-1β, IL1-α, IL-6, IFN-γ (E-Bioscience, Vienna, Austria) and IL-18 (Booster, Fremont, USA) levels were analyzed by ELISA technique.

Measurement of Tissue Total Oxidant Status (Tos), Tissue Total Antioxidant Status (Tas) Tissue Total Antioxidant Status (Tas) and Tissue Oxidative Stress Index (Osi)

The TOS and TAS of the cell lysate were measured using an automated colorimetric measurement method for TOS and TAS. The ratio of TOS to TAS represents the OSI, an indicator of the degree of oxidative stress (OS). The OSI value is calculated according to the formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/g prot)/TAS (mmol Trolox Eq/g prot) ×100.

Histopathological Analysis

Tissue samples were fixed in 10 % neutral formalin solution, histologically processed and embedded in paraffin blocks. Sections were taken from these blocks at 5-micron thickness and were mounted on both normal and poly-l-lyzine coated slides. While the normal slides were stained with hematoxylin-eosin dye for general morphological evaluation. Immunohistochemical staining was performed for Bax, Bcl-2 and caspase-3. All cross-sections were investigated under a light microscope (Eclipse E-600 Nikon, Japan). While the liver tissues were investigated under 20x magnification objective, the increase in sinusoidal infiltration and Kupfer cells, sinusoidal congestion and the changes in hepatocyte morphology (cell shape and nucleus structure, cytoplasmic shrinkage and cytoplasmic viscosity) were scored between 0 to 4. Afterwards, all scores were summed up to obtain a single histological injury score. Bax, Bcl-2 and Caspase-3 immuno-positive cells in 6 randomly selected fields were counted and summed up under 20x objective using an Image Analysis Software (NIS Elements Nikon, Japan) analysis program. Afterwards, statistical analyses were performed both for H-E scorings and immuno-positive cell counts.

Statistical Analyses

The data were analyzed by using SPSS for Windows 15.0 software package (SPSS Inc., Chicago, Illinois, USA). Results for descriptive statistics were expressed as mean ± standard deviation (SD) or median [range (minimum-maximum)]. Statistical comparisons of continuous variables among the groups were performed using one-way analysis of variance (ANOVA) or Kruskal–Wallis test based on their distribution. Fisher's test was per-

formed for post hoc analysis after performing analysis of variance test. In cases where Kruskal-Wallis test yielded statistical significance, Bonferroni-corrected Mann–Whitney U-test was used to identify the groups which showed differences. A P value <0.05 was considered statistically significant.

Results

Evaluation of Biochemical Data Supporting Liver Injury

While the blood levels of AST, compared to the sham group, ALT and LDH were markedly elevated in the I/R group; in the Nesfatin group, they returned to normal levels upon treatment. Statistical analyses of the data indicated that the changes in AST, ALT and LDH levels were significant between the groups (Table I).

Evaluation of Blood IFN- γ, IL-1β, IL-6, TNF-α, and IL-1α Levels

A statistically significant decrease of blood levels of IFN-γ, IL-1beta, IL-6, TNF-α and IL-1α gamma were noted in the treatment group compared to the I/R group. Table II summarizes the blood IFN- γ, IL-1β, IL-6, TNF-α, and IL-1α levels and statistical analysis results of all groups.

Evaluation of Blood Total Antioxidant Status (Tas), Total Oxidant Status (Tos), and Oxidative Stress Index (Osi) Levels

Blood TOS levels were markedly elevated in the I/R group whereas they decreased in the Nesfatin group. The increase in the I/R group was significant compared to the control group, and the decrease in the Nesfatin group was significant compared to the I/R group (p= 0.010). Blood OSI levels in the control, I/R and Nesfatin groups were found to be 146.25 ± 27.46, 243.88 ± 41.81 and 180.14 ± 53.84, respectively. Statistically significant differences were noted between the I/R and control groups and between the Nesfatin and I/R groups (p= 0.001). Table III summarizes the blood TAS, TOS and OSI levels and statistical analysis results of all groups.

Evaluation Of Tissue IFN- γ, IL-1β, IL-6, TNF-α, and IL-1α Levels

A statistically significant decrease of tissue levels of IFN-γ, IL-6, TNF-α and IL-1α gamma were noted in the

Table I - *Enzyme results and statistical analyses showing liver injury between the groups.*

	Sham	IR	IR+NES	P
AST	514,28±232,88a	2063,33±863,57a,b	750,25±288,458b	0,002‡
ALT	459,41±167,76a	1471,31±990,24a,b	492,66±263,8b	0,009‡
LDH	2606,38±1249,26a	424,330±990,83a	3626,86±582,535	0,021†

† One-way analysis of variance (ANOVA) ve post hoc analysis ile LSD test.
‡ Kruskal-Wallis test ve post hoc analysis Mann–Whitney U-test.
Veriler aksi belirtilmedikçe ortalama ± standart sapma. Koyu P değerleri istatiksel olarak anlamli (P<0.05).

Table II - *Blood IL-1β, IL-6, IL-18, TNF-α, and IFN-γ results and their statistical analyses.*

	Sham	IR	IR+NES	P
IFNγ	18±0,57a,b	40,95±13,9a	34,24±4,47b	<0,001†
IL-1β	93,04±56,7a	211,5±113,69a,b	126,96±67,22b	0,043†
IL-6	16,74±4,84a	29,59±12,9a,b	16,82±5,54b	0,016†
TNF-α	26,43±1,71a	28,75±1,70a,b	26,22±1,70b	0,032†
IL-1α	42,4±22,73a	151,139±58,93a,b	52,54±22,79b	0,001‡

† One-way analysis of variance (ANOVA) ve post hoc analysis ile LSD test.
‡ Kruskal-Wallis test ve post hoc analysis Mann–Whitney U-test.
Veriler aksi belirtilmedikçe ortalama ± standart sapma. Koyu P değerleri istatiksel olarak anlamli (P<0,05).

Table III - *TOC, TAC, and OSI data and their statistical analysis results.*

	Sham	IR	IR+NES	P
TOS	1,37±0,135a	2,03±0,38a,b	1,52±0,48b	0,010†
TAS	0,96±0,15	0,83±0,14	0,85±0,09	0,171†
OSI	146,25±27,46a	243,88±41,81a,b	180,14±53,84b	0,001†

† One-way analysis of variance (ANOVA) ve post hoc analysis ile LSD test.
‡ Kruskal-Wallis test ve post hoc analysis Mann–Whitney U-test.
Veriler aksi belirtilmedikçe ortalama ± standart sapma. Koyu P değerleri istatiksel olarak anlamli (P<0,05).

Table IV - *Tissue IL-1β, IL-6, IL-18, TNF-α, and IFN-γ results and their statistical analyses.*

	Sham	IR	IR+NES	P
IFNγ	849,49±180,320a	1212,44±196,24a,b	998,91±81,37b	0,002†
IL-1β	1708,4±533,56	1992,11±726,87	1892,93±547,19	0,720†
IL-6	1418,06±279,42a	1790,2±298,26a	1571,22±193,24	0,041†
TNF-α	1447,55±367,76a	3521,52±1256,65a,b	2094,85±472,57b	<0,001†
IL-1α	922,85±222,044a	1521,081±461,55a,b	1145,64±146,41b	0,007†

† One-way analysis of variance (ANOVA) ve post hoc analysis ile LSD test.
‡ Kruskal-Wallis test ve post hoc analysis Mann–Whitney U-test.
Veriler aksi belirtilmedikçe ortalama ± standart sapma. Koyu P değerleri istatiksel olarak anlamli (P<0,05).

Table V - *Tissue TOC, TAC, and OSI data and their statistical analysis results.*

	Sham	IR	IR+NES	P
TOS	6,01±0,95a	8,61±2,49a,b	6,37±1,23b	0,021‡
TAS	6,84±1,59a	3,84±1,78a,b	6,53±2b	0,011†
OSİ	91,74±25,59a	302,09±241,62a,b	108,12±45,58b	0,009‡

† One-way analysis of variance (ANOVA) ve post hoc analysis ile LSD test.
‡ Kruskal-Wallis test ve post hoc analysis Mann–Whitney U-test.
Veriler aksi belirtilmedikçe ortalama ± standart sapma. Koyu P değerleri istatiksel olarak anlamli (P<0,05).

Table VI - *Histopatological evaluation and scoring of the groups.*

	Sham	IR	IR+NES	P
INJURY SCOR	1±0,92a,b	8,13±1,45a,c	3,75±0,88b,c	<0,001†
BAX	8,88±5,22a,b	234,75±76,16a,c	147±41,98b,c	<0,001†
BCL-2	140,38±41,24a	42,75±13,99a,b	142,13±29,84b	<0,001†
KASPAZ-3	19,63±11,38a,b	165,13±40,1a,c	98,63±24,89b,c	<0,001†

† One-way analysis of variance(ANOVA) ve post hoc analysis ile LSD test.
‡ Kruskal-Wallis test ve post hoc analysis Mann–Whitney U-test.
Veriler aksi belirtilmedikçe ortalama ± standart sapma. Koyu P değerleri istatiksel olarak anlamli(P<0,05).

treatment group compared to the I/R group. However, there was no statistically significant difference of tissue levels of IL-1β between the groups (p= 0.720). Table IV summarizes the tissue IFN- γ, IL-1β, IL-6, TNF-α, and IL-1α levels and statistical analysis results of all groups.

Evaluation of Tissue Total Antioxidant Status (Tas), Total Oxidant Status (Tos), and Oxidative Stress Index (Osi) Levels

Statistically significant differences of tissue TOS levels were noted between the I/R and control groups and between the Nesfatin and I/R groups (p= 0.021). Tissue TAS levels in the control, I/R and Nesfatin groups were found to be 6.84 ± 1.59mmol Trolox Equivalent/g-protein, 3.84 ± 1.78mmol Trolox Equivalent/g-protein and 6.53 ± 2mmol Trolox Equivalent/g-protein, respectively. Statistically significant differences were noted between the I/R and control groups and between the Nesfatin and I/R groups (p= 0.011). Tissue OSI levels in the control, I/R and Nesfatin groups were found to be 91.74 ± 25.59, 302.09 ± 241.62 and 108.12 ± 45.58, respectively. Statistically significant differences were noted between the I/R and control groups and between the Nesfatin and I/R groups (p= 0.009). Table V summarizes the tissue TAS, TOS and OSI levels and statistical analysis results of all groups.

Histopathology Evaluation

Hematoxylin-Eosin Evaluation

When the experimental groups were investigated in terms of hepatocyte morphology, liver appearance was observed to be normal in the control group while the hepatocyte morphology in samples obtained from the ischemia group indicated cytoplasmic condensation and alterations in cell shape, sinusoidal congestion and increases in both kupfer cell count and sinusoidal infiltration. Histological injury was significantly different between the control and ischemia groups (p<0.05). These parameters improved in the Nesfatin-treated group and were significantly different compared to the ischemia group (p<0.05). Despite such improvements, there still was a significant difference between the Nesfatin and control groups (p<0.05) (Fig. 1).

Immunohistochemical Evaluation (Bax, Bcl-2 And Caspase-3)

The effects of Nesfatin on apoptosis induced by ischemia-reperfusion injury in liver were immunohistochemically evaluated using Bax, Bcl-2 and caspase-3 primary antibodies. Cytoplasmic staining was considered positive if there were Bax, Bcl-2 and caspase-3 immunostaining. Staining was particularly apparent around the vessels (v. centralis). Bax immunostaining results indicated positive staining in very few cells in the control group, while the expression of Bax as a pro-apoptotic protein was significantly increased in the ischemia group (p<0.05). Staining intensity was also elevated in this group. Bax staining intensity and the number of cells with positive staining were significantly decreased in the Nesfatin-treated group (p<0.05). Bcl-2 immunostaining results indi-

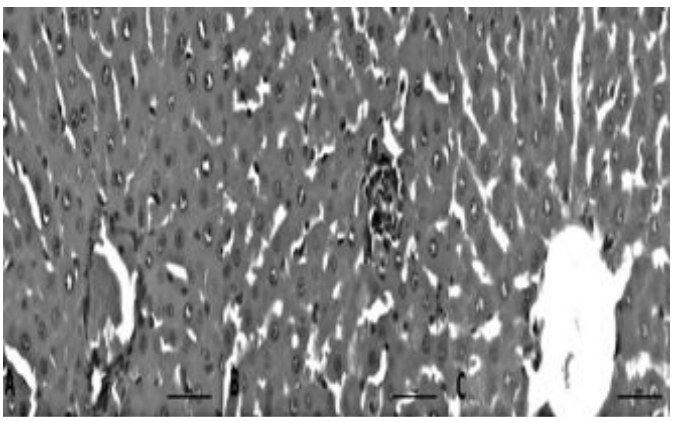

Fig. 1: While the morphological appearance was normal in control group (A), increases were noted in the kupffer cell count and sinusoidal infiltration in ischemia group (B). Sinusoidal congestion was also visible. Decreases were noted in the kupffer cell count and infiltration in the nesfatin group (C). (HEx400, scale bar 50 μm).

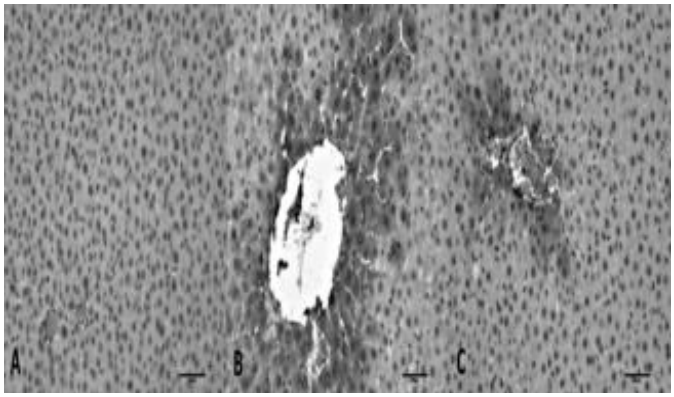

Fig. 2: Staining was not noted for Bax expression in control group (A). Multiple Bax-positive cells can be seen in ischemia group (B). Abundant Bax-positive cells and staining intensity observed in nesfatin group (C). (x200, scale bar 50 μm).

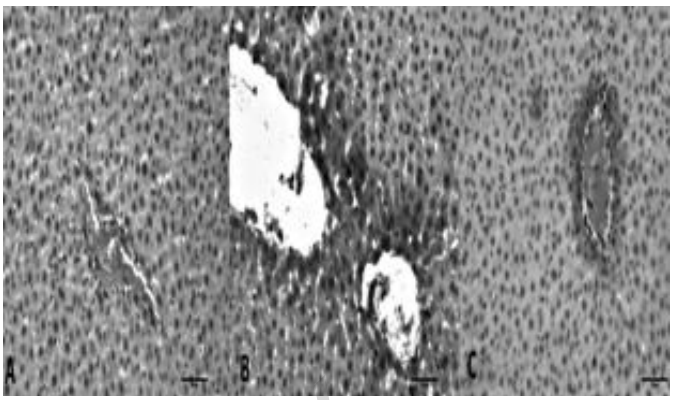

Fig. 4: Limited staining was noted for Caspase-3 expression in control group (A). Multiple Caspase-3-positive cells can be seen in ischemia group (B). Abundant Caspase-3-positive cells and staining intensity observed in nesfatin group (C). (x200, scale bar 3 μm).

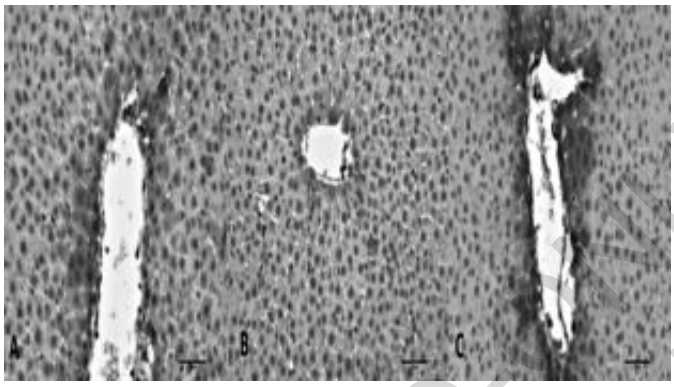

Fig. 3: Staining was not noted for multiple Bcl-2 expression in control group (A). Significant decrease in Bcl-2 positive cell count in ischemia group (B). Abundant Bcl-2-positive cells and staining intensity observed in nesfatin group (C). (x200, scale bar 2 μm).

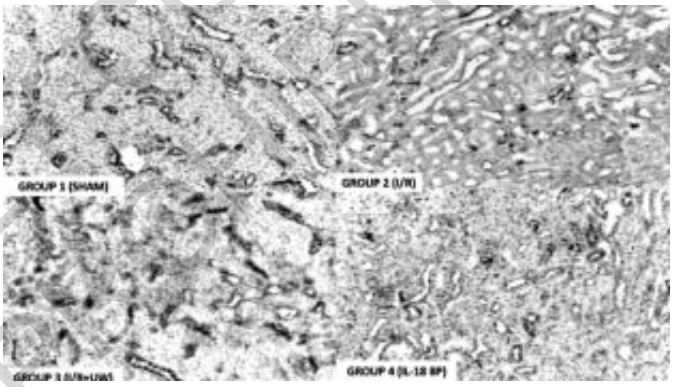

Fig. 5.

cated that the number of Bcl-2-expressing cells was significantly lower in the ischemia group and statistically significant differences were noted between the groups. Bcl-2 expression was not significantly different between the control and Nesfatin groups ($p>0.05$). Caspase-3 immunostaining results indicated that there were only a few cells with positive staining in the control group, while this figure was markedly higher in the ischemia group (Figs. 2, 4). The difference between two groups was statistically significant ($p<0.05$). The number of cells positively stained for caspase-3 was significantly lower in the Nesfatin group compared to the ischemia group ($p<0.05$). A significant difference was also noted between the control and nesfatin groups ($p<0.05$) (Table VI).

Discussion

Nesfatin-1 is released from the hypothalamic nucleus, a part of brain responsible from appetite control [7]. In addition to being released in the central nervous system, immunohistochemical studies indicated that NUCB2, the precursor of nesfatin, is also released in the peripheral nervous system and adipose tissue, stomach mucosa, pancreatic beta cells and testicle tissue [8]. The anti-apoptotic, anti-inflammatory and antioxidant efficacy of nesfatin was clearly demonstrated in the experimental study of Ozsavci et al. [9]. In the study of Ayada et al., TNF-α, interleukin-1β (IL-1β) and interleukin-6 (IL-6) levels increased while the levels of antioxidant enzymes decreased in the brain tissue of experimental subjects with induced subarachnoid hemorrhage. On the other hand, apparent improvement was noted in oxidative brain damage and neurological injuries in the treatment group [5]. In another study, Guanjun Jiang et al. investigated the protective efficacy of Nesfatin-1 in experimental kidney ischemia-reperfusion injury models and reported favorable results [6].

The levels of liver enzymes increase markedly in the presence of liver ischemia and reperfusion injury. Among all

liver enzyme tests, ALT is the most sensitive marker of hepatotoxicity [10]. In the present study, AST and ALT levels as markers of liver toxicity were significantly elevated in the I/R group compared to the control group. On the other hand, significant decreases were noted in the AST and ALT levels of the treatment group. The decrease in AST and ALT levels were accompanied by a statistically insignificant decrease in LDH levels. We believe that this is due to the presence of LDH isoenzymes in different tissues. Pro-inflammatory cytokines, such as IL-1β, IL-6, IL-1α, TNF-α and IFN-γ, play significant roles in the development of damage in liver I/R injury [11]. TNF-α and IL-1β levels increase during early phase of the injury, which induces generation of the other cytokines and chemokines. TNF- α is the cytokine that plays the central role in development of injury. It is also responsible for distant organ injury. The mechanisms through which TNF-α causes hepatocellular injury are still unclear. Proposed mechanisms include direct mitochondrial toxicity and induction of necrosis. It also induces chemotaxis by the release of epithelial neutrophil-activating protein–78 and production of superoxide radicals by kupffer cells [12]. IL–1 is released in response to TNF-α. It induces Kupffer cells to produce more TNF-α and increases the production of free oxygen radicals in neutrophils. It was previously proven that IL–1 antagonists reduce I/R damage in liver by decreasing TNF-α levels. IL–6, on the other hand, is released from kupffer and sinusoid endothelial cells. It induces the release of acute phase reactants (C reactive protein, α-1 antityripsin, fibrinogen) from hepatocytes [13,14]. In experimental liver I/R models based on one-hour periods of ischemia and reperfusion, Lee et al. reported a marked increase in the levels of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, compared to the levels in the control group [15]. In another study performed by Samiye Yabanoğlu Çiftçi et al., similar results were obtained despite the differences in the duration of ischemia and reperfusion [16]. Consistent with the literature, in the present study, IL-1β, IL-6, IL-1α, TNF-α and IFN-γ blood levels were significantly higher in the I/R than the control group. While in the treatment group, IL-1β, IL-6, IL-1α and TNF-α levels significantly decreased by nesfatin treatment, blood IFN-γ levels, on the other hand, showed a statistically insignificant decrease but still suggested improvement with nesfatin treatment. Analyses of tissue pro-inflammatory cytokine levels showed that IL-1α, TNF-α and IFN-γ levels were markedly elevated in I/R group and significantly decreased with nesfatin treatment. The decrease in tissue IL-1β and IL-6 levels in treatment group was not statistically significant, but still suggested the beneficial effects of nesfatin.

Total oxidant and antioxidant status provide detailed information on oxidative stress during I/R injury. Oxidative stress in liver I/R injury develops as a result of the oxidant-antioxidant imbalance, which occurs in relation to an increase in the levels of oxidants or a decrease in the levels of anti-oxidants. Kaplan et al. investigated the effects of ellagic acid on TAS, TOS and OSI in liver I/R injury [17]. In that study, TAS and TOS levels were significantly altered in the I/R group, but the change in OSI levels was not statistically significant. Tüfek et al. found that dexmedetomidine treatment decreased TOS and OSI levels and increased TAS levels in liver I/R damage [18]. In the present study, blood levels of TOS and OSI were significantly increased in the I/R group compared to the control group. On the other hand, a statistically significant decrease was noted in the nesfatin group. TAS blood levels were not significantly different between the I/R and control groups or between the nesfatin and I/R groups. However, when compared to the I/R group, the increase noted in TAS levels in the treatment group was in favor of the antioxidant and anti-inflammatory properties of nesfatin. Tissue TOS and OSI levels were also significantly increased in the I/R group compared to the control group. While these levels were significantly decreased in the nesfatin group compared to the I/R group. Similarly, TAS levels were significantly lower in the IR group compared to the levels in the control group, while the comparisons between the nesfatin and I/R groups indicated a significant increase in the nesfatin group.

J.F.K. Kerr, an Australian pathologist, was the first to define the main context of apoptosis in 1972 [19]. A cell's predisposition to apoptosis depends on the Bcl-2 family of genes. Bcl-2 family consists of 2 groups of genes, which display opposite pro-apoptotic and anti-apoptotic characteristics. A cell with a higher amount of pro-apoptotic proteins is more prone to apoptosis, whereas a cell with a higher amount of anti-apoptotic proteins is less prone to entering apoptosis. Pro-apoptotic members of this family include Bad, Bax, Bid, BclXs, Bak, Bim, Puma and Noxa, all of which are cytoplasmic proteins. They induce apoptosis by increasing the release of cytochrome-c and apoptosis inducing factor (AIF). Anti-apoptotic members include Bcl-2, Bcl-xL and Mcl-1. These proteins are found in the external membrane of the mitochondria, in the endoplasmic reticulum and nuclear membrane. They aid in pore formation and regulate ion transport. They are particularly responsible for the control of intracellular calcium levels. They also inhibit apoptosis by blocking the release of caspase precursors, AIF and cytochrome-c. Caspases are cysteine proteases which are inactive in a cell but activate each other through proteolytic mechanisms. Caspases are classified into three groups as initiator caspases (Caspase 2,8,9,10), effector caspases (Caspase 3,6,7) and inflammatory caspases (Caspase 1,4,5,11,12,13,14) [20-22]. In an experimental kidney ischemia reperfusion injury model developed by Guanjun Jiang et al., a significant amount of injury was noted in the I/R group when the histological injury was compared between the I/R and control groups, while significant improvements were noted

in the nesfatin group compared to the I/R group. Bax and caspase-3 analyses also indicated a significant increase in the I/R group compared to the control group and a significant decrease in the nesfatin group compared to the I/R group. Consistently, the Bcl-2 analysis showed that there was a significant decrease in I/R compared to control group, and a significant increase in the treatment group compared to the I/R group [6]. In line with previous findings in the literature, the analyses of histological injury in this study indicated that there was a severe injury in the I/R group compared to the control group, and significant improvements occurred in the nesfatin group compared to I/R group. Bax and caspase-3 analyses, on the other hand, demonstrated that there were significant increases in the I/R group compared to the control group and significant decreases in the nesfatin group compared to the I/R group. Bcl-2 analyses also indicated that there were significant decreases in the I/R group compared to the control group and significant increases in the nesfatin group compared to the I/R group.
In conclusion, the histopathological and biochemical analyses performed in the present study indicated that Nesfatin-1 has anti-inflammatory, antioxidant and protective properties against ischemia-reperfusion injury in experimental liver-ischemia reperfusion models.

Riassunto

Durante la riperfusione, con il ripristino del flusso ematico arterioso e l'ossigenazione dei tessuti, si sviluppa una grave lesione tissutale locale e sistemica. In questo studio, abbiamo indagato sperimentalmente gli effetti antiinfiammatori, antiossidanti e protettivi di nesfatin in relazione al danno ischemia/riperfusione (I/R) che si sviluppa nel fegato.
Per questo studio sono stati impiegati 24 ratti Wistar-Albino maschi, divisi in tre gruppi di otto individui ciascuno. I ratti sono stati sottoposti a 30 minuti di occlusione del peduncolo epatico seguiti da 2 ore di riperfusione per indurre il danno I / R.
Nesfatin1 è stata somministrata alla dose di 10 μg / kg, 30 minuti prima dell'induzione dell'ischemia e immediatamente prima del periodo di riperfusione nel 3° gruppo, mentre il primo gruppo è stato utilizzato come controllo senza clampaggio del peduncolo, ed il secondo è stato sottoposto a 30' di ischemia per clampaggio senza somministrazione di Nesfatin. .
I risultati hanno mostrato che mentre i livelli ematici di AST, ALT e LDH erano marcatamente elevati nel gruppo I / R, ritornavano a livelli normali dopo trattamento nel gruppo Nesfatin. Mentre i livelli di IL-1 α, IL-1β, IL-6, TNF-α e IFN-γ nel sangue e nei tessuti erano inferiori dopo la terapia nel gruppo Nesfatin rispetto al gruppo I / R, le diminuzioni statisticamente significative sono state osservate solo in IL Livelli -1β, IL-6, TNF-α e IFN-γ. I livelli di TAS sono aumentati nel gruppo di trattamento, mentre sul trattamento con nesfatin sono state notate diminuzioni statisticamente significative nei livelli TOS e OSI. Le indagini istopatologiche hanno anche mostrato diminuzioni statisticamente significative dell'intensità di colorazione di Bax e Caspase-3 e il numero di cellule colorate nel gruppo Nesfatin.
L'attività antiossidante e l'effetto anti-infiammatorio del nesfatin determina dunque un miglioramento delle funzioni epatiche e dei risultati istopatologici nell'ischemia epatica e nel danno da riperfusione.

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