

# Melatonin Ameliorates Burn-Induced Liver Injury by Modulation of Nrf2 and Nf- $\kappa$ B Signaling Pathways

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## Abstract

Severe thermal injury is a common traumatic injury that results in local tissue damage and systemic response and represents a serious clinical problem. A skin burn leads to dysfunction of organs distant from the original burn skin injury, including the liver. The pathophysiology of burn-induced liver injury is very complicated and remains unclear. Increasing evidence implicate activation of the inflammatory response along with the oxidative stress as the main mechanisms of hepatic injury in burns. Melatonin is a multifunctional molecule that might counteract all pathophysiologic steps and displays beneficial effects against cellular damage, induced by thermal injury. This review summarises protective effect of melatonin on hepatic injury related with suppression of oxidative stress, inflammatory response and apoptosis induced by thermal skin injury. Special emphasis is focused on modulating effect of melatonin on Nuclear Erythroid 2-related factor 2 (Nrf2) and Nuclear Factor-kappa B (NF- $\kappa$ B) signaling pathways, related with activation of cellular defense against oxidative stress and reduction of proinflammatory mediator production. The overall findings suggest that melatonin should be exploited as a therapeutic tool to prevent or reverse the harmful effects of thermal trauma.

**Keywords:** NF- $\kappa$ B; Nrf2; Oxidative stress; Inflammation; Melatonin; Liver injury; Burns

## Introduction

A severe burn is a devastating injury affecting every organ system and leads to complications with poor outcome. Liver plays an important role in metabolism, inflammatory response, homeostasis and host defense mechanisms. It is also a major organ responsible for initiating Multi-Organ Dysfunction Syndrome (MODS) following burn injury [1,2]. The pathophysiology of burn-induced liver injury is complex and not well understood. In this regard, several pathogenetic mechanisms have been proposed in particular ischemia/reperfusion, generation of lipid metabolites, cytokine overproduction, depletion of glutathione and mitochondrial dysfunction. These complex mechanisms cause liver dysfunction, damage of hepatic parenchyma and, eventually, cell death [3-5].

Taking into consideration the role of the lipid peroxidation in burn-induced hepatic injury, the interference with hepatic

oxidative status and the inflammatory response, it is logical to hypothesize that therapy with substances possessing antioxidant, anti-inflammatory and anti-apoptotic effects may be beneficial in reducing these complications.

Melatonin (N-acetyl-5-methoxytryptamine) is secreted in the pineal gland and extra pineal tissues. Melatonin possesses a wide variety of biological effects such as chronobiological, sedative and anxiolytic. Melatonin may act as an antioxidant and a potent scavenger of both oxygen and nitrogen reactive molecules. The free radical scavenging activity of melatonin is receptor independent process whereas the indirect effect likely involves specific receptors [6]. Melatonin stimulates the activity of enzymes involved in antioxidant defense and reduces the production of proinflammatory mediators [7,8]. Melatonin is a small, highly lipophilic and hydrophilic molecule. It passes freely through membranes and distributes in all subcellular compartments [5]. These evidences suggest that these pleiotropic functions of melatonin may be used clinically under conditions where its circulating levels are reduced [9,10]. Because of its antioxidant, anti-inflammatory and anti-apoptotic effects, exogenous melatonin attenuates ischemia/reperfusion and hepatotoxins induced liver injury [11-13].

## Antioxidative and anti-inflammatory effect of melatonin

Melatonin participates in the regulation of important physiological processes. This hormone is known to regulate the circadian rhythms induced by a short light dark cycle and the circadian rhythms [14,15]. A number of studies demonstrate that melatonin is an interesting pharmacological agent in the treatment of inflammatory conditions [8-16]. The beneficial effect of melatonin in these studies is associated with: 1) antioxidant effect of this indole and 2) modulation of the inflammatory response.

A large body of evidence indicates that melatonin is a major Scavenger of Radical Oxygen Species (ROS) and Reactive nitrogen (RNS) species and activator of antioxidant enzymes [17-19]. Under normal conditions ROS and RNS are produced in mitochondria and they are neutralized by non enzymatic

scavengers and antioxidant enzymes. Melatonin, vitamins C and E, reduced glutathione and uric acid are non enzymatic scavengers [20,21]. Antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), or Glutathione Reductase (GR) are important in the defense against the damaging effect of ROS and RNS [17,22]. Oxidative stress occurs as a result of an imbalance between the excessive production of ROS and RNS and impaired ability of tissue to detoxify free radical in burns. Accumulated ROS and RNS in tissues have direct toxic effect on cells: damage nuclear DNA, membrane lipids and cytosolic proteins [23-25]. The harmful effects of ROS and RNS have been shown in experimental thermal trauma [5,9]. These alterations often results in the production of more ROS and lipid peroxides. This leads to cellular dysfunction and/or cell death. Cells possess defense antioxidant system that protect against oxidative stress and suppress oxidative injury. Such antioxidants as glutathione can scavenge ROS and restrict cellular alteration. Activation of antioxidant enzymes is an adaptive defense mechanism against oxidative stress.

Nuclear Factor erythroid 2-related factor (Nrf2) is a transcription factor activated by oxidative stress that has an important role in regulation of the expression of detoxifying and antioxidant genes. Activated Nrf2 translocates to nucleus, binds to Antioxidant Response Element (ARE), and enhances the up-regulation of a network of cytoprotective enzyme such as Heme Oxygenase-1 (HO-1), Glutamate Cysteine Ligase (GCL), thioredoxin reductase, glutathione-S-transferase and NAD(P) H:quinone oxidoreductase that mediate cell survival [26]. Other Antioxidant Enzymes (SOD and CAT) and non-enzymatic scavengers such as glutathione are also involved in scavenging ROS. Nrf2/ARE signaling plays an important role in the cell protection from ROS. Nrf2/ serve as a novel target for designing therapy to prevent and treat diseases in which oxidative stress is involved. In addition, Nrf2 signaling pathway can attenuate proinflammatory stimuli leading to restrict inflammatory injury [26,27].

ROS also activate the transcription factor NF- $\kappa$ B and inflammatory signaling pathways leading to increased production of inflammatory mediators [28]. NF- $\kappa$ B is sequestered in the cytoplasm of cells, bound to its inhibitor I $\kappa$ B. NF- $\kappa$ B is activated by degradation of this inhibitor by ROS, proinflammatory cytokines and bacterial products. After liberation, NF- $\kappa$ B translocates to the nucleus binding to DNA and enhances the expression genes of pro inflammatory cytokines (tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukins (IL-1 $\beta$ , IL-6, IL-8), adhesion molecules (VCAM-1, ICAM-1), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) [29,30]. Therefore, therapeutic agents that inhibit the activation of NF- $\kappa$ B-mediated inflammatory response may be an attractive strategy for limiting the damaging effect of the proinflammatory mediators under pathological conditions.

Recently, melatonin has been shown to activate signal transduction pathways leading to the activation of antioxidant enzymes and to the reduction of inflammatory mediators [16]. Melatonin upregulates the expression of Nrf2, involved in the induction of antioxidant enzymes such as SOD, CAT, GPx, GST and

GR [7]. Since, melatonin is known to activate these antioxidant enzymes. It is possible that antioxidant effect of melatonin is mediated by Nrf2 transcriptional factor [13,16].

Melatonin modulates the NF- $\kappa$ B inflammatory signaling pathway. Melatonin plays a key role in the induction of anti-inflammatory cytokine and suppression and pro-inflammatory signaling [16]. It reduces gene expression and synthesis of proinflammatory cytokine such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 and prostaglandins [31,32]. In addition, melatonin is also reported to modulate the processes of apoptosis and necrosis, to stimulate the production of Vascular Endothelial Growth Factor (VEGF), and to activate the process of angiogenesis [33].

Melatonin upregulates Nrf2 expression and increases antioxidant activities, reduces NF- $\kappa$ B activation and inhibits iNOS and pro-inflammatory cytokine expression in rats with dimethylnitrosamine (DMN)-induced liver injury [14]. Melatonin could modulate inflammation by decreasing NF- $\kappa$ B activation and oxidative stress by increasing Nrf2 expression that might be related, at least in part, with its hepatoprotective effect.

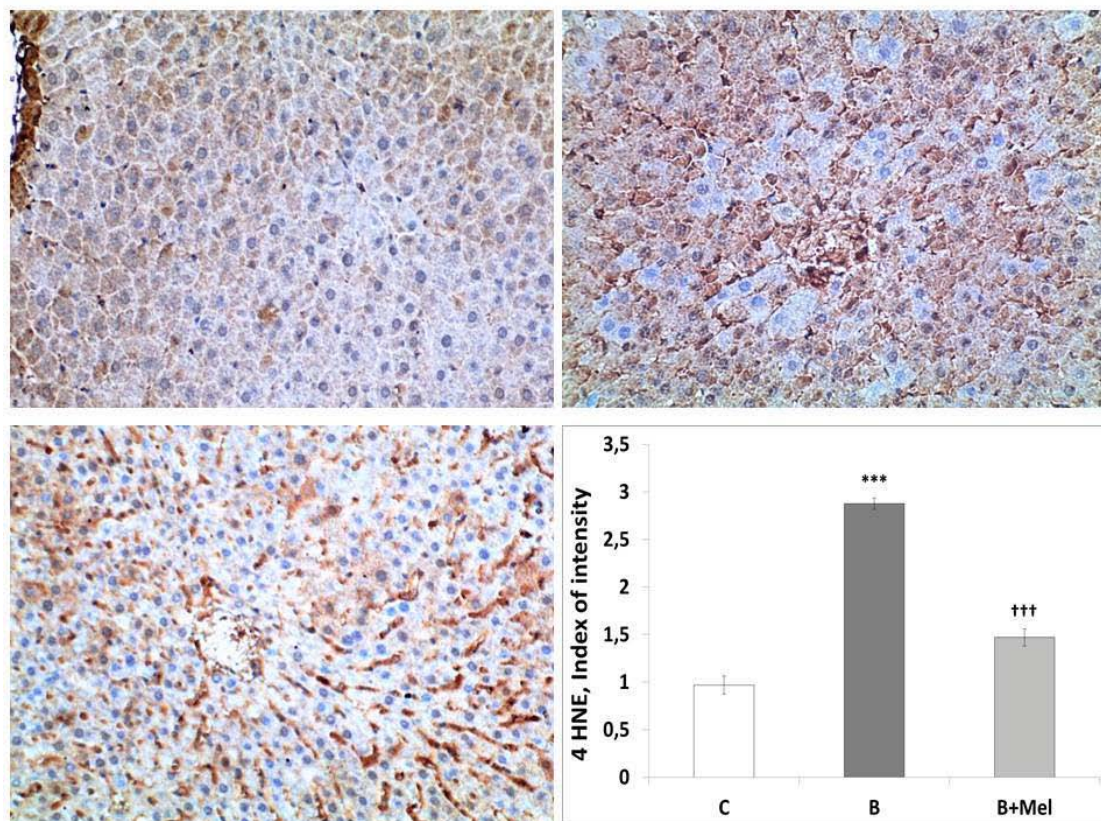
### **Hepatoprotective effect of melatonin in thermal skin injury**

Burns initiate Systemic Inflammatory Response Syndrome (SIRS) where the generation of pro-inflammatory cytokines and free radicals causes a progressive distant organs dysfunction [1,3,34,35]. Experimental studies have shown that application of melatonin protects liver tissue against the damage caused by thermal trauma [6,7]. Melatonin based on its small molecular size and high lipophilic capacity reduces liver injury in burn rat model [33,36] and other models of oxidative stress [15,28,37,38].

Overproduction of free radicals along with unbalanced hepatic antioxidant defense capacity initiates lipid peroxidation and directly damages plasma and intracellular membranes which alters membrane-bound proteins. [33,36,39]. Both ROS and RNS enhance the production of reactive aldehydes such as 4-HNE with potent pro-inflammatory and pro-apoptotic properties [40]. Increased levels lipid peroxidation and oxidative injury is demonstrated by increased expression of 4-HNE in liver in burn rat model (Figure 1). The activation of peroxidative mechanisms results in cellular dysfunction, apoptosis or necrosis in hepatocytes with the release of the hepatic enzymes into circulation. Serum Aminotransferase (ALT and AST) activities increases markedly. Early postburn and these elevations attenuate with melatonin (10 mg/kg), thus indicating melatonin's role in burn-induced hepatoprotection.

Several studies associated with an increased antioxidant activity shows to counteract the oxidative stress-induced by thermal injury [41,42]. CuZnSOD expression as front-line antioxidant increases as compensatory reaction early post-burn when oxidative stress increases [43,44]. HO-1 is the inducible isoform of heme oxygenase, and it is reported to protect against liver injuries, too [45]. Our results shows that HO-1 protein is expressed in hepatocytes surrounding the central vein in liver (Figure 2).





**Figure 1:** Effect of melatonin on the expression of 4-hydroxy-nonenal in liver after thermal trauma

C-controls; B-burned non treated; B + Mel- burned treated with melatonin

\*\*\*  $p < 0.001$  - B vs C ; †††  $p < 0.001$  - B + MelvsB

Immunohistochemistry detection for 4-hydroxynonenal (4-HNE) in liver

**A:** It is localized in both Sinusoidal Endothelial Cells (SECs) and hepatocytes in the control group. The staining intensity of 4-HNE positive cells was weak.

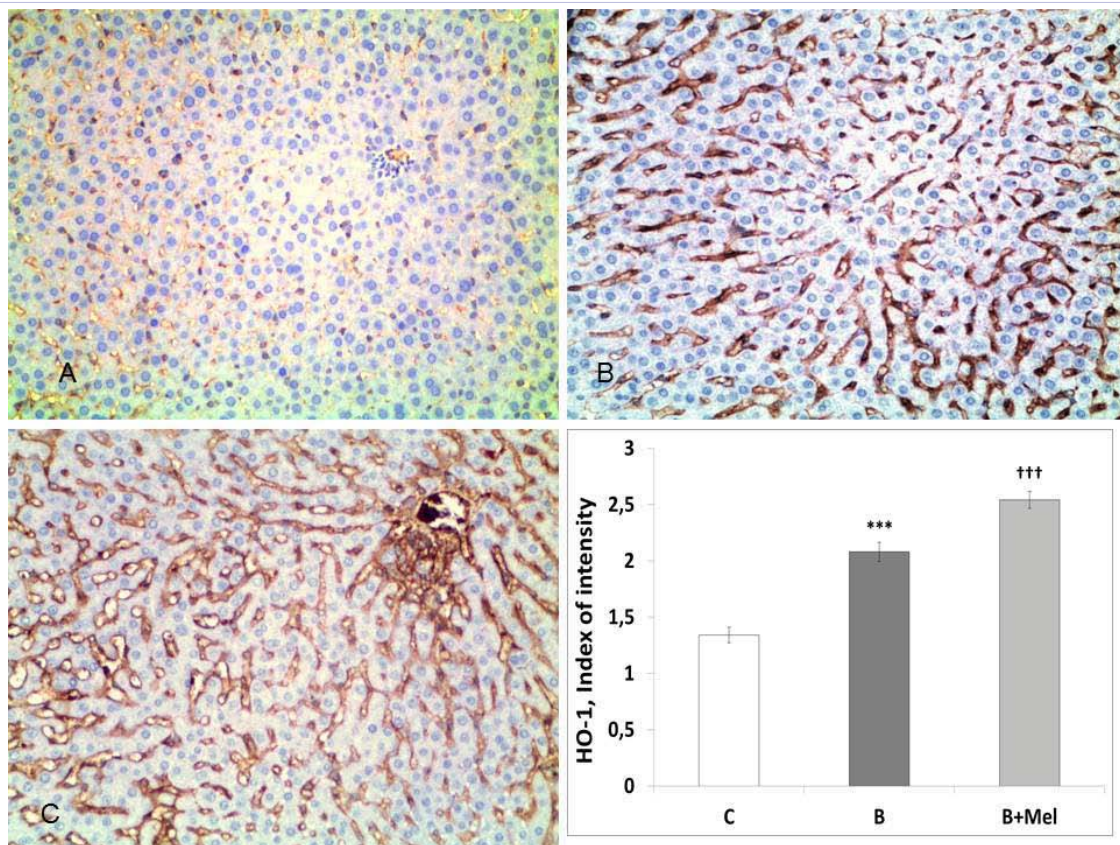
**B:** In the burned group, induction of 4-HNE positive cells was principally in the sinusoidal cells and mainly in hepatocytes around the central vein. It was moderate to strong in the individually cells.

**C:** In the burned group reacted with melatonin, induction of 4-HNE positive cells was principally in SEC and some hepatocytes around the central vein. The number of 4-HNE positive cells was lower than in the burned non treated. (Immunohistochemistry, x 400).

Indeed, HO-1 expression and levels of products of lipid peroxidation (MDA and 4-HNE) in liver were elevated within 24 h after burns [46]. Because HO-1 is induced by oxidative stress, it could be speculated that the increased expression of HO-1 in liver is likely related to overproduction of ROS/RNS after burns. Melatonin reduces the levels of lipid peroxidation products, improves liver histopathological changes and augmented the increase of HO-1 expression in burned rats [47]. These findings suggest that the protective mechanism of melatonin against burn-induced liver injury could be closely related with HO-1 overexpression and increased cellular antioxidant capacity. Both endogenous HO-1 and melatonin-induced HO-1 may enhance the overall cellular antioxidant capacity and prevent oxidative stress induced cytotoxicity in liver after burns. Several studies have reported that HO-1 exerts protective effect against a variety of pathological conditions, such as ischemia/ reperfusion, endotoxin, hepatotoxins [37,38]. The cytoprotective effect of HO-1 under pathological conditions and oxidative stress depends on biliverdin, a free radical scavenger or CO anti-inflammatory

anti apoptotic gas, both of which are degradation products of heme catabolized by HO-1 [48,49].

Stress-activated Nrf2 transcription factor plays predominant role and mediates potent HO-1 and SOD activation. Melatonin increases Nrf2 expression and it is even more intensive in the cytoplasm of hepatocytes, but it is also present in the nuclei of endothelial cells after burns (Figure 3). Nrf2 is localized in the cytoplasm bound to Keap1, a cytoskeleton-associated protein. A possible mechanism by which melatonin increases Nrf2 expression is related to the dissociation of Nrf2/Keap1 (repressor) and translocation of Nrf2 to the nucleus, where it binds to ARE in the promoter of the genes involved in the antioxidant protection [50]. Melatonin may ameliorate burn-induced liver injury through inhibition of free-radical activating lipid peroxidation and elevation of expression of the transcription factor Nrf2 and some antioxidant enzymes such as HO-1 and SOD. The activation of HO-1 and Nrf2 transcriptional factor is demonstrated in other models of hepatic and oxidative stress [26,51,52,53].



**Figure 2:** Effect of melatonin on the expression of Heme-oxygenase-1 in liver after thermal trauma

C-controls; B-burned non treated; B + Mel- burned treated with melatonin

\*\*\*  $p < 0.001$  - B vs C ; †††  $p < 0.001$  - B + Mel vs B

Immunohistochemistry detection for heme-oxygenase-1 (HO-1) in liver

**A:** It is localized in SECs of the liver in the control group. The staining intensity of HO-1 positive cells was weak.

**B:** In the burned group, induction of HO-1 positive cells was in the sinusoidal cells. It was moderate to strong in the individually cells.

**C:** In the burned group treated with melatonin, induction of HO-1 positive cells was principally in the sinusoidal cells. The number of HO-1 positive cells was higher than in the burned non treated. (Immunohistochemistry, x 400).

Melatonin activates Nrf2/HO-1 signaling pathway and acts as a natural inducer of an antioxidant protection (Figure 4). The stimulation of protective mechanisms of the cells by activating Nrf2 transcription antioxidant factor is a new mechanism for protection against burn-induced liver damage. These results demonstrate that melatonin could induce the activation of HO-1/Nrf-2 pathway as a part of the hepatoprotective effects of melatonin in burns. Further research of the molecular mechanisms of the Nrf2 activation offers opportunities to develop a new therapeutic approach to therapy victims with thermal trauma.

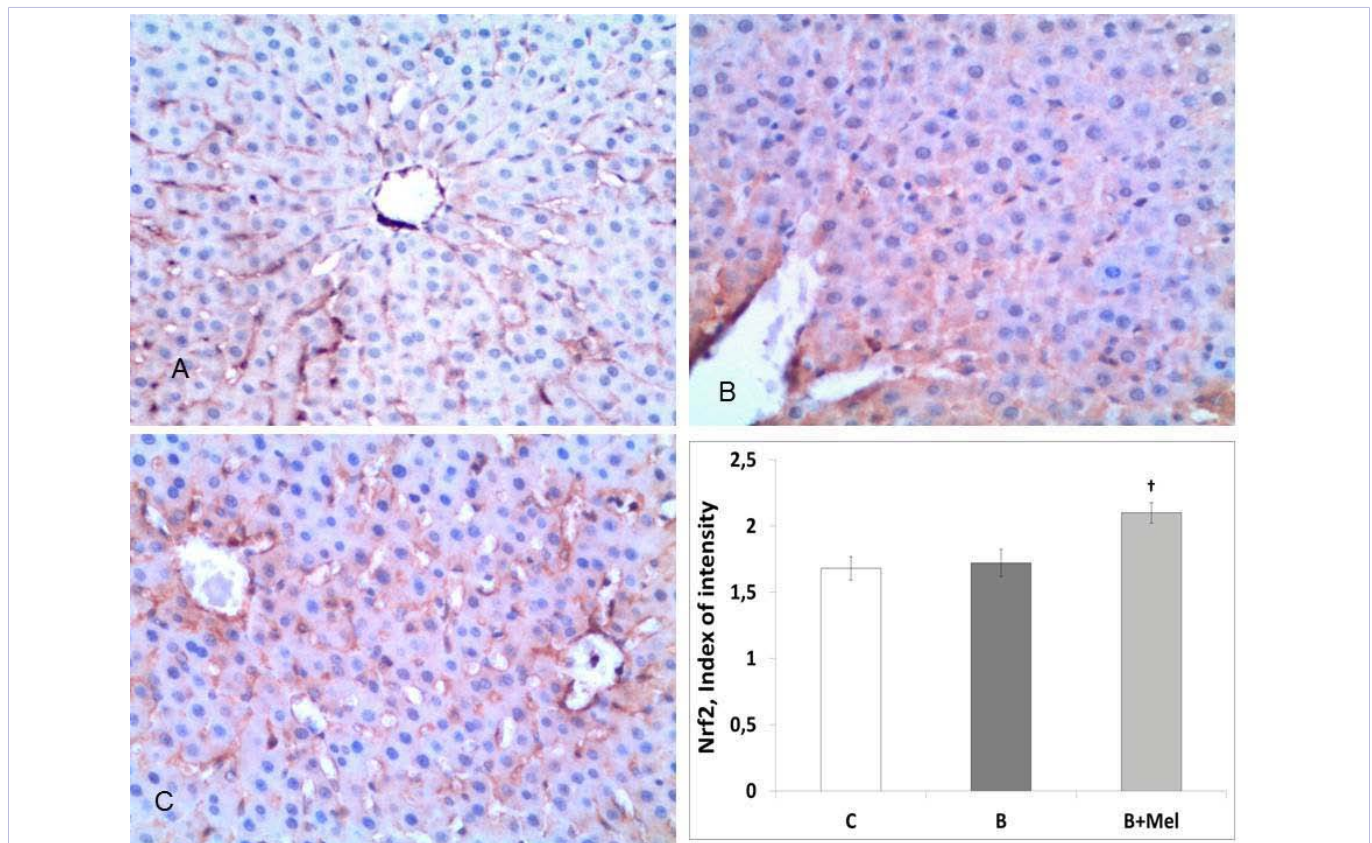
Following a severe burn injury, the systemic inflammatory response encompasses the release of large quantities of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6 or TNF- $\alpha$  [1,54,55]. The level of anti-inflammatory cytokines, such as IL-10, released in an attempt to counter-regulate the effects of the pro-inflammatory cytokines, decreases or remains unchanged [56,57]. The unbalance in pro-/anti-inflammatory cytokine production leads to alteration in function and structure of liver [3].

High TNF- $\alpha$  level may activate cell-death pathways and promotes the production of reactive oxygen species and lipid peroxidation and thus hepatic injury [29]. Furthermore, hepatocytes are much more sensitive to TNF- $\alpha$  and free radicals damaging effect when their antioxidant capacity is low. It appears that oxidative stress plays an important role in the primary cell and tissue destruction, and thus the secondary inflammatory reaction.

ROS/RNS may activate NF- $\kappa$ B transcription factor and increase the expression of pro-inflammatory cytokines. TNF $\alpha$  has a cytotoxic activity and may cause hepatic injury. On the other hand, increased production of TNF- $\alpha$  and free radicals may increase the expression of NF- $\kappa$ B transcription factor [58]. Probably, vicious cycle is formed between oxidative stress and inflammation by NF- $\kappa$ B activation. Such a positive relationship between markers of lipid peroxidation and inflammation in liver is found in other experimental models of thermal trauma [6].

Melatonin has the capability of scavenging both oxygen and nitrogen-based reactants including ONOO- and blocking NF- $\kappa$ B





**Figure 3:** Effect of melatonin on the expression of Nrf2 transcriptional factor in liver after thermal trauma.

<sup>†</sup> $p < 0.001$ - B + Mel vs B

Immunohistochemistry detection for of nuclear factor erythroid 2-related factor 2 (Nrf2) in liver

A. It is localized in the cytoplasm and in the nucleus of the SECs of the liver in the control group. The staining intensity is moderate.

B. B: In the burned group, induction of Nrf2 positive cells was in cytoplasm of the SECs in individual hepatocytes. It was moderate to strong in the individually cells. In the burned group treated with melatonin, induction of Nrf2 positive cells was in the cytoplasm and in the nucleus in both hepatocytes and SECs. The number of HO-1 positive cells was higher than in the burned non treated. (Immunohistochemistry, x 400).

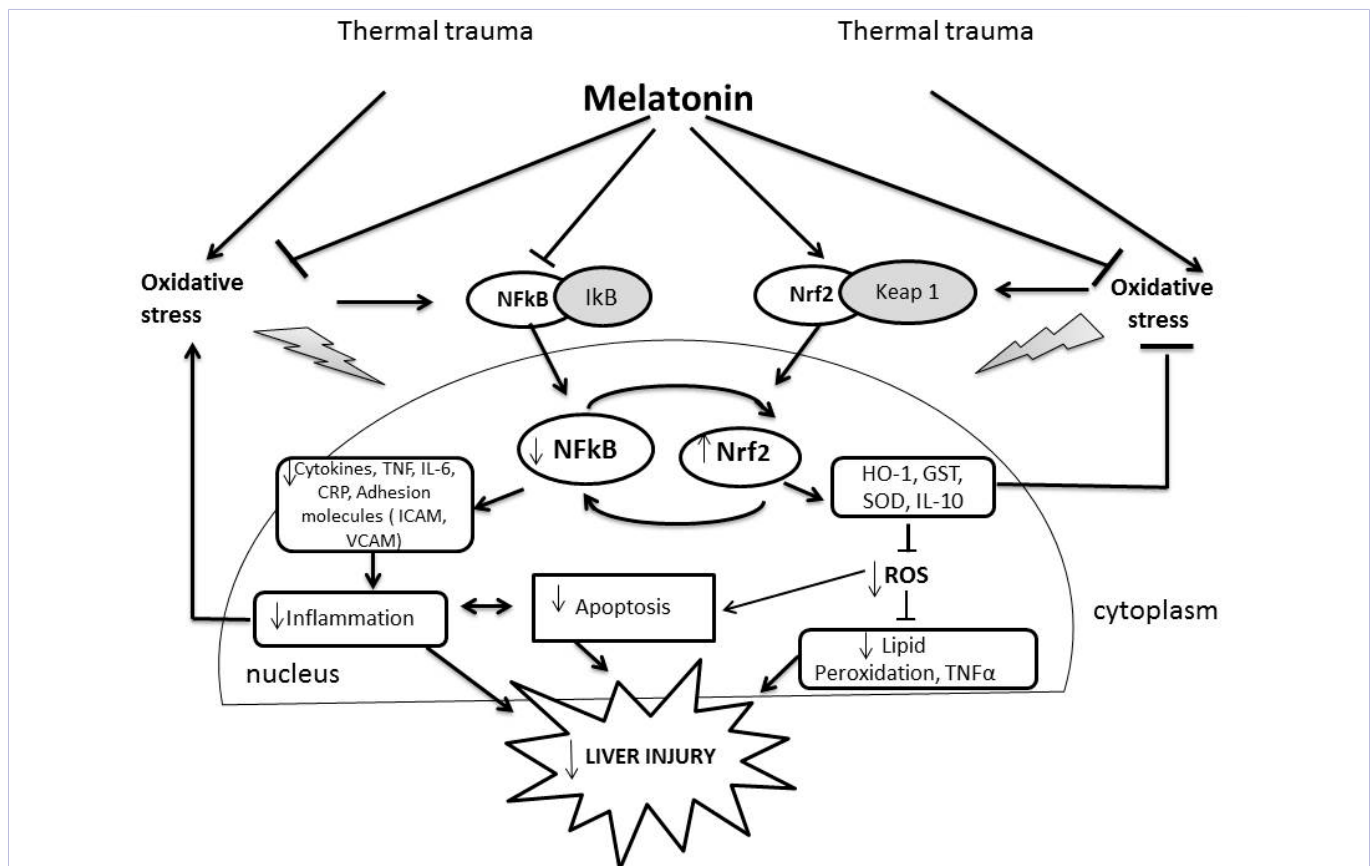
transcriptional factor which induces pro-inflammatory cytokine expression [16]. Both TNF- $\alpha$  level and NF- $\kappa$ B expression in liver are reduced after melatonin administration [58]. These findings suggest that NF- $\kappa$ B activation promotes hepatic TNF- $\alpha$  production so TNF- $\alpha$  elevation further stimulates liver NF- $\kappa$ B transcriptional factor activation after burns. Both form a positive feedback loop and stimulate liver injury. After melatonin inhibition of the increased TNF- $\alpha$  level and NF- $\kappa$ B expression there is only restriction of liver injury evidenced by increased plasma transaminase (AST and ALT) activities and histological changes. Melatonin suppresses burn-induced oxidative injury, avoids thiol level depletion, increases antioxidant defense and restricts liver morphology disorders [32,39]. The morphological changes of inflammation such as edema, leukocyte infiltration and cell vacuolization are reduced in animals treated with melatonin [36].

Melatonin presents with other means to reduce inflammation-induced liver damage. Thus, it prevents translation of TNF- $\alpha$ -binding of NF- $\kappa$ B and thereby, suppresses the production and release of proinflammatory mediators such as TNF- $\alpha$ , other cytokines and adhesion molecules [59]. These data demonstrate

that melatonin protection of burn-induced liver may be attributed by its antioxidant and anti-inflammatory properties.

The decreased TNF $\alpha$  level is accompanied by a marked rise of anti-inflammatory interleukin 10 (IL-10) in the animals, pretreated with melatonin [55,56] and effect may be due to its anti-inflammatory properties [16]. Thus, melatonin affects the pro- /anti-inflammatory balance mitigating hepatic damage from uncontrollable inflammation. This may be one of the main mechanisms for the hepatoprotective effect of melatonin after burns.

Melatonin treatment may attenuate oxidative stress, inflammation and delays deterioration of liver structure and function in rats during early response following burn trauma. Further studies would be directed to the possible useful applications of melatonin in specific, pharmacologically tested dosages to patients after severe acute thermal injury. If proven effective, melatonin may be an attractive therapy, since it is a natural, inexpensive, widely available, orally applicable, and relatively safe product.



**Figure 4:** Schematic representation of the modulating effect of melatonin on Nf- $\kappa$ B and Nrf2 signaling pathways and its role in restricting the burn-induced hepatic injury.

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