Burn-Induced Multiple Organ Injury and Protective Effect of Lutein in Rats

Huda O. AbuBakr,1,4 Samira H. Aljuaydi,1 Shimaa M. Abou-Zeid,2 and Amanallah El-Bahrawy3

Abstract—Thermal injury may lead to multiple organ dysfunction through release of proinflammatory mediators and reactive oxygen radicals. This study investigated the effects of thermal injury on remote organs of rats and the possible protective effect of lutein. Thermal trauma was induced in the back of rats by exposing them to 90 °C bath for 10 s. Rats were sacrificed 48 h after burn, and blood samples were collected to monitor liver and kidney function. Tissue samples from liver, kidneys, and lungs were taken for studying oxidative stress parameters, gene expressions of TNF-α and Casp-3, besides histopathological examination. Skin scald injury caused significant elevations of liver and kidney function biomarkers in the serum. In tissue samples, increments of MDA, GPx, and 8-OHdG were recorded while GSH level and the activities of CAT and SOD were suppressed. The expressions of TNF-α and caspase-3 mRNA were increased, and histopathological results revealed remote organ injury. Oral administration of lutein (250 mg/kg) resulted in amelioration of the biochemical and molecular changes induced by burn as well as the histopathological alterations. According to the findings of the present study, lutein possesses antioxidant, anti-inflammatory, and anti-apoptotic effects that protect against burn-induced damage in remote organs.

KEY WORDS: scald; lutein; protection; oxidative stress; TNF-α; Casp-3.

INTRODUCTION

In forensic and clinical medicine, it is important to study the mechanisms of organ dysfunction resulting from burns. Severe thermal injury is caused by full-thickness burn greater than 10% of the total body surface area (TBSA) or when burn area is greater than one third of the TBSA [1]. Burn is a post-traumatic inflammatory disease characterized by both local effects and deleterious systemic effects in all the organ systems distant from the original wound [2, 3].

The pathophysiological mechanism of such remote organ injury in severe burn remains unclear. However, animal studies documented that reactive oxygen species (ROS) and reactive nitrogen species (RNS) mediated by elevated proinflammatory mediators released from immunocytes and necrotic cells play important roles in the development of distant organ damage [4]. It has been demonstrated that burn injury is associated with lipid peroxidation induced by oxygen radicals, which is an autocatalytic mechanism leading to oxidative damage of cellular membranes, and their destruction can lead to the production of toxic reactive metabolites and cell death [5].

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It has been suggested that the activation of a pro-inflammatory cascade after major burn is responsible for the development of immune dysfunction, susceptibility to sepsis, and multiple organ dysfunction [6]. Hyperactivation of neutrophils induced by the inflammatory response also contributes to oxidative damage of distant organs [7]. Moreover, the enhanced production of pro-inflammatory mediators by macrophages plays an important role in damaging remote organs [8].

An inflammatory cascade is triggered by severe thermal injury causing production of potentially dangerous pro-inflammatory mediators in excess including interleukin (IL)-1, IL-6, reactive nitrogen intermediates (RNI), tumor necrosis factor (TNF)-α, prostaglandin E2 (PGE2), and transforming growth factor (TGF)-β [8].

As the remote organ damage occurring after thermal injury is mediated by both reactive oxygen radicals and activated immune cells, the anti-oxidants given in the post-burn period are expected to exert protective effects against thermal injury-induced oxidative tissue damage and multiple organ dysfunctions [9].

Lutein is a carotenoid synthesized by plants, bacteria, and algae. It is not synthesized in the body; therefore, it must be supplied through the diet like egg yolks or dark green leafy vegetables, such as spinach [10]. Lutein protected the erythrocyte membrane from the harmful effects of benzo(a)pyrene by scavenging the released free radicals and activating cellular anti-oxidant enzymes [11]. Administration of lutein protected the liver of rats against the toxic insult of paracetamol, carbon tetrachloride, and ethanol as evidenced by reduction of serum AST, ALT, and LDH activities [12, 13].

To the best of our knowledge, lutein exerted anti-inflammatory effect against LPS-induced inflammation [14] and retinal ischemic/hypoxic injury [15]. Moreover, lutein reduced lipid peroxidation and attenuated pro-inflammatory cytokine production through NF-κB activity in the liver and eyes of guinea pigs after being fed a hypercholesterolemic diet [16].

Based on these findings, it is hypothesized that lutein will represent a potential therapeutic option for therapy of burn-induced remote organ dysfunction. Therefore, the present study was performed to investigate the role of oxidative damage, inflammatory response, and apoptosis in remote organ injury induced by scald in rats and the potential therapeutic effects of lutein on biomarkers of remote organ dysfunction. Liver and kidney functions were monitored in addition to measurement of malondialdehyde (MDA) and glutathione (GSH) levels and activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) in liver, kidneys, and lungs. Oxidative DNA damage was assessed by measuring 8-OHdG. Moreover, expressions of TNF-α and caspase-3 were studied in these organs together with recording the histopathological alterations.

**MATERIALS AND METHODS**

The research was conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals approved by the Committee on the Ethics of Animal Experiments, University of Sadat City, Egypt.

**Chemicals**

Lutein was obtained from Nature’s Bounty, Inc., USA, as 40 mg soft gels. Test kits for AST, ALT, MDA, GSH, CAT, and SOD were purchased from Biodiagnostic, Egypt. Kit of serum LDH was purchased from Salucea (Netherlands). 5,5′-dithiobis 2-nitrobenzoic acid (DTNB), GSH, and NaN₃ for GPx activity and 8-OHdG standard for determination of oxidative DNA damaged were purchased from Sigma Aldrich.

**Animals**

A total of 40 adult male rats (200–250 g) were used in this investigation. The animals were provided by the Animal Care Unit of Vacsera Pharmaceutical Company, Agouza, Egypt. The animals were fed *ad libitum* with standard diet and water throughout the experimental work. They were housed separately in plastic cages and maintained under standard conditions (a 12-h light/dark cycle; temperature maintained at 23 ± 2 °C).

**Experimental Design**

The animals were randomly allocated into four equal groups (10 rats each) as follows: control (sham) group, burn group, lutein group, and lutein-burn group. Under brief ether anesthesia, the dorsum of all animals was shaved closely 24 h before the experiment. The sham group received three daily oral doses of 1 ml normal saline and served as control. The burn group was subjected to a scald injury under brief ether anesthesia by dipping the dorsum of rats in water bath at 90 °C for 10 s, resulting in partial thickness second-degree burn involving
30% of the TBSA. The animals, then, received physiological saline solution 10 ml/kg S/C to be compensated from fluid loss [2]. The lutein-burn group received the same dose of lutein as that of the lutein group for 3 days and subjected to scald injury in the second day, as mentioned before. In order to rule out the effects of anesthesia, the same protocol was applied in the control and lutein groups, except that the dorsa were dipped in a 25 °C water bath for 10 s. All animals were sacrificed at 48 h after burn injury. Blood was used for biochemical analysis. Liver, kidneys, and lungs were divided into two parts: First parts were taken and stored at −80 °C for biochemical and gene expression studies. Other parts of tissue samples were fixed in 10% neutral formalin and taken for histopathology.

Methods

Liver and Kidney Function Tests

Liver injury was assessed by measurement of the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) [18, 19]. Kidney dysfunction was assessed by measuring the serum levels of BUN and creatinine [20, 21].

Oxidative Stress Parameters

Oxidative stress was assessed by measurement of malondialdehyde (MDA) [22] and reduced glutathione (GSH) [23] levels and activities of catalase (CAT) [24], superoxide dismutase (SOD) [25], and glutathione peroxidase (GPx) [26].

Oxidative DNA Damage

Isolation and hydrolysis of tissue DNA were performed as previously described by Lodovici et al. [27]. The hydrolyzed mixture was centrifuged, and the supernatant was injected into the HPLC. The separation of 8-OdDG was performed with anLC/Agilent 1200 series HPLC apparatus (USA) using UV detectors. For chromatographic separation, we used C18 reverse phase columns in series (Supelco, 5 pm, I.D. 0.46 × 25 cm); the eluting solution was H2O/CH3OH (85: 15 v/v) with 50 mM KH2PO4, pH 5.5 at a flow rate of 0.68 ml/min. The UV detector was set at 245 nm. The resulting chromatogram identified the concentration from the sample as compared to that of the standard.

Quantitative Real-Time PCR Evaluation for Tumor Necrotic Factor Alpha (TNF-α) and Caspase 3 (Casp 3) Expression

Total RNA isolation was performed in the liver, kidney, and lung tissues using QIAmp RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s manual. The concentration and purity of the total RNA samples were obtained by using a Nanodrop ND-1000 spectrophotometer. The isolated RNA was used for cDNA synthesis using reverse transcriptase (Fermentas, EU). Real-time PCR (qPCR) was carried out using the reaction mixture of 1 μl cDNA, 0.5 mM of each primer (TNF-α, Cas-3, and GAPDH as an internal control), iQ SYBR Green Premix (Bio-Rad 170–880, USA) in a total volume of 20 μl. PCR amplification and analysis were achieved using Bio-Rad iCycler thermal cycler and the MyiQ real-time PCR detection system. All templates were amplified using the following LightCycler protocol. The primers and their sequence published in GenBank are shown in Table 1.

Histopathological Examination

Tissue samples from the liver, kidneys, and lungs fixed in 10% neutral-buffered formalin were prepared for histopathological examination according to Bancroft et al. [29]. Sections were microscopically scored and derived semi-quantitatively as follows: –, none; +, slight < 20%; ++, moderate < 50%; ++++, severe > 50% of examined sections.

Statistical Analysis

Statistical analysis was performed by using SPSS 15.0. The obtained values were given as means ± S.E. of the mean. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by post hoc analysis (Duncan’s test) and
GraphPad software Instat (version 2). The level of significance was set at $P \leq 0.05$.

**RESULTS**

Liver and Kidney Function Findings

The activities of transaminases and LDH in serum were significantly elevated ($P < 0.05$) after the thermal injury compared to the sham group. Also, BUN and creatinine concentrations in serum of rats exposed to thermal injury were found to be significantly higher than those of the sham group ($P < 0.05$). Administration of lutein ameliorated the rise in liver and kidney function markers (Table 2).

Tissue Oxidant/Anti-oxidant Biomarkers

Lipid peroxidation expressed as malondialdehyde was significantly high ($P < 0.05$) in the liver, kidneys, and lungs of the sham group as a result of the scald injury. This effect was ameliorated by lutein administration (Fig. 1a). Glutathione level in the liver, kidneys, and lungs was reduced by burn injury ($P < 0.05$), while lutein administration replenished GSH level back toward control values (Fig. 1b). The tissue activities of CAT (Fig. 1c) and SOD (Fig. 1d) in rats with burn injury were significantly low in the sham group ($P < 0.05$). On the other hand, the activity of GPx (Fig. 1e) in the tissues was elevated after burn injury ($P < 0.05$). Lutein administration restored the changes in anti-oxidant enzyme activities in the direction of control levels.

Oxidative DNA Damage Findings

The level of oxidative DNA damage in the liver, kidneys, and lungs significantly increased ($P \leq 0.05$) in the burn group as evidenced by increased 8-OHdG concentration. However, lutein administration resulted in alleviation of elevated 8-OHdG concentration as shown in the lutein-burn group (Fig. 2).

TNF-α and Caspase-3 Gene Expression

The relative expression of TNF-α gene significantly increased ($P \leq 0.05$) in the liver, kidneys, and lungs of burn groups to 51.8-, 2.1-, and 8.4-fold, respectively, while lutein could decrease its expression. In addition, the relative expression of Casp-3 significantly increased in the liver, kidneys, and lungs of rats exposed to burn injury and reduced by lutein treatment to 22.3-, 5.7-, and 1.9-fold, respectively (Fig. 3).

Histopathological Findings

Table 3 presents the semi-quantitative scoring of histopathological lesions in the liver, kidneys, and lungs. Normal morphology of hepatic parenchyma from sham and lutein-treated rats are shown in Fig. 4a and b, respectively. Liver of burn exposed group showed severe congestion of the portal veins with fibrin thrombi and margination of inflammatory cells to the endothelium lining of

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Accession number</th>
</tr>
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<tbody>
<tr>
<td>TNF-α</td>
<td>ACACACGAGACGCTGAAGTA</td>
<td>GGAACAGTCGGAGCTCTT</td>
<td>NM_012675.3</td>
</tr>
<tr>
<td>Casp3</td>
<td>CATGCACATCTCCTCGTG</td>
<td>CCCACTCCCCAGTCTTTT</td>
<td>NM_012922.2</td>
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**Table 1. Primer Sequences of TNF-α and Casp3 Genes Designed by Primer3**

**Table 2. Liver and Kidney Function Markers in Serum of Different Groups**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Lutein</th>
<th>Burn</th>
<th>Lutein-Burn</th>
</tr>
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<tbody>
<tr>
<td>ALT (μ/l)</td>
<td>45.4 ± 3.326</td>
<td>44.4 ± 3.696</td>
<td>75 ± 4.289&lt;sup&gt;*&lt;/sup&gt;</td>
<td>60 ± 2.324&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST (μ/l)</td>
<td>79 ± 2.549</td>
<td>76 ± 3.146</td>
<td>138 ± 4.626&lt;sup&gt;*&lt;/sup&gt;</td>
<td>110.2 ± 5.083&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDH (μ/l)</td>
<td>904 ± 21.587</td>
<td>875 ± 20.616</td>
<td>1602 ± 23.537&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1200 ± 25.149&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>40 ± 2.449</td>
<td>30.4 ± 2.926</td>
<td>108 ± 5.745&lt;sup&gt;†&lt;/sup&gt;</td>
<td>77.2 ± 3.813&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.26 ± 0.043</td>
<td>1.25 ± 0.026</td>
<td>2.44 ± 0.051&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1.74 ± 0.085&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE, $n = 5$

<sup>*</sup> $P < 0.05$ significant vs. sham group

<sup>**</sup> $P < 0.05$ significant vs. both sham and burn groups

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blood vessels, congestion of hepatic sinusoids, subcapsular hemorrhage, and inflammatory cell infiltration in the portal area (Fig. 4c). In the lutein-burn group, slight congestion of portal veins was observed (Fig. 4d).

While sham (Fig. 5a) and lutein-treated (Fig. 5b) groups had a regular morphology of renal parenchyma with well designated glomeruli and tubules, burn-induced severe interstitial hemorrhage occurs between the renal tubules (Fig. 5c). Kidney of the lutein-burn group showed only slight interstitial hemorrhage in the renal parenchyma (Fig. 5d).

Normal lung bronchi and alveoli were observed in the sham (Fig. 6a) and the lutein-treated (Fig. 6b) groups. Lungs of burn-exposed group revealed congestion of blood vessels, severe interstitial hemorrhage, and inflammatory cell infiltration between the
lung alveoli (Fig. 6c). Lungs of the lutein-burn group showed only slight interstitial hemorrhage in the lung parenchyma (Fig. 6d) and congestion of blood vessels.

DISCUSSION

In the emergency medicine, burns are considered one of the most severe forms of traumatic clinical issues.
Thermal injury causes both local tissue destruction and systemic mediator-induced response, manifested by various cellular and molecular interactions. These interactions include neutrophil and macrophage excitation, oxygen radicals, overproduction of cytokines, remarkable decrease of total anti-oxidant status and scavenging capacity, and mitochondrial dysfunction [30, 31].

Macrophages and biochemical cytokines are the central elements involved in thermal injuries, in which macrophage cells are major producers of proinflammatory...
mediators including prostaglandin E2, IL-6, and TNF-α [8, 32]. Thermal injury also causes formation of reactive oxygen species (ROS), including hydroxyl radical, superoxide anion, and hydrogen peroxide, and reactive nitrogen species, such as peroxynitrite and nitric oxide (NO) [33].

The data presented in this study demonstrated that scald injury induced in rats produced remote organ damage as evidenced by liver and kidney dysfunction, oxidative damage, and apoptosis. The ability of lutein to protect against thermal trauma was evidenced by significant decreases in serum AST, ALT, and LDH activities, serum BUN and creatinine levels, tissue MDA and 8-OHdG levels with elevation of tissue GSH level, and activities of CAT and SOD. Moreover, tissue expressions of TNF-α and caspase-3 were reduced by lutein treatment demonstrating its anti-inflammatory and anti-apoptotic effects.

Our results revealed hepatic dysfunction in rats subjected to scald injury as evidenced by elevated activities of serum ALT (a specific marker for hepatic parenchymal injury), AST (a nonspecific marker for hepatic injury), and LDH (a marker of non-specific cellular injury) in addition to the histopathological alterations observed in the liver. Similar findings were previously recorded in rats with thermal injury [34–37].

The administration of lutein was found to have protective effect against the hepatic injury induced by scald injury. The hepatoprotective effect of lutein was previously reported in rats intoxicated with paracetamol, carbon tetrachloride, and ethanol as evidenced by reduction in serum ALT, AST, and LDH activities [12, 13]. In addition, Li et al. [38] reported that lutein reduced hepatic injury induced by arsenic in mice.

When compared with control rats, thermal injury induced significant elevations in the serum levels of BUN and creatinine and histopathological changes in the kidney indicating the development of renal injury. Similar findings were previously demonstrated in rats with thermal trauma [34, 35, 37].

Lutein treatment down-regulated serum BUN and creatinine levels and alleviated the pathological changes in the kidney. Our findings are in line with Liu et al. [39] who reported that lutein reduced the levels of serum BUN and creatinine in rats with ischemia/reperfusion injury. The protective effect of lutein on liver and kidney may be
attributable to its action as a powerful anti-oxidant and free radical scavenger [40].

The development of the systemic inflammatory response syndrome represents a major cause of death in patients with full thickness, third-degree burns that exceed 25% of the TBSA. One of the causative agents responsible for the development of burn shock and distant organ injury in animal models of burn trauma are oxygen radicals. The distant organs involved in this thermal injury during experimental skin burn are thought to be the lungs, heart, liver, kidneys, and gastrointestinal mucosa [1]. After thermal trauma, neutrophils diffusely invade these organs and constitute the main source of reactive oxygen species [41].

Our findings demonstrated that burn injury induced oxidative tissue damage in the liver, kidneys, and lungs evidenced by increased MDA concentration and decreased GSH levels in these organs together with reduced CAT and SOD activities, while GPx activity was elevated. Moreover, the burn injury increased the level of tissue8-OHdG.

Our results are in accordance with those previously reporting increase of MDA level and suppression of anti-oxidant enzyme activities due to thermal injury [1–3, 34, 36, 42–44].

It is well documented that reactive oxygen species, such as hydroxyl radical, superoxide radical, and hydrogen peroxide, are enhanced after thermal injury, and they induce tissue damage through lipid peroxidation, oxidation of protein sulfuryl groups, and damage of DNA strands [45]. Lipid peroxidation is a process mediated by free radicals leading to changes of membrane fluidity and permeability that enhance rates of protein and DNA degradation, which will eventually lead to cell lysis [46]. MDA is the major marker of lipid peroxidation, and there is a good correlation between its concentration and the degree of burn complications, including shock and remote organ damage [43, 47].

Glutathione is the main constituent of protective mechanisms in the cell against lipid peroxidation, while the reduced GSH is the major component of endogenous non-protein sulfhydryl pool that scavenges free radicals in the cytoplasm [48]. The reduction of the activity of anti-oxidant enzymes by burn injury may be attributed to their consumption in combating oxidative stress by...
targeting the oxygen free radicals. The elevation of GPx activity after burn may be the result of oxygen-responsive element (ORE) activation in GSH-Px gene due to high oxygen level [49]. The elevation of 8-OHdG in different tissues is indicative of oxidative DNA damage [27].

It has been demonstrated that anti-oxidants which maintain the concentration of reduced GSH block lipid peroxidation restore the cellular defense mechanisms and therefore protect against the oxidative cellular damage. In accordance with our findings, lutein has been reported to protect against the oxidative stress induced by lipopolysaccharide [40, 50], benzo(a)pyrene [11], paracetamol and CCl₄ [12], hypercholesterolemic diet [16], and ethanol [13]. In addition, Serpeloni et al. [51] demonstrated that lutein improved anti-oxidant defense in vivo and protects against DNA damage and chromosome instability induced by cisplatin in mice.

Lutein has higher anti-oxidant properties than other carotenoids. The anti-oxidant activities of lutein are nearly 10- and 15-folds of those of β-carotene and lycopene [52], respectively. The anti-oxidant properties of lutein are due to its free radical scavenging activity [11], replenishing non-enzymatic anti-oxidants like glutathione and vitamin C and increasing anti-oxidant enzyme activities, including SOD, CAT, GST, and GR [40, 50].

In severe burns, all body homeostatic mechanisms are subjected to stressful conditions associated with both local and remote effects, finally leading to intense inflammation, tissue damage, and infection. The non-specific host immunity is then activated by local production of proinflammatory cytokines. Thus, cytokines play an important role in the post-burn pathophysiological process [53]. After burn injury, many cytokines are induced rapidly, including IL-1, IL-6, and TNF-α [33, 47, 54].

In the present study, tissue relative TNF-α gene expression significantly elevated 48 h after thermal trauma, whereas its expression was ameliorated by lutein administration. Our findings agree with those previously recorded by Jin et al. [55], Sener et al. [43], Toklu et al. [2], Belyarova et al. [56], Liu et al. [36], Rocha et al. [37], Shen et al. [57], and Zhang et al. [35]. Contrary to our findings, Bortolin et al. [58] reported no change in TNF-α gene expression after burn injury although hepatic histopathological alterations were noticed.

Experimental and clinical studies have shown that the response to thermal injury is perceived by macrophages and monocytes which in turn secrete waves of cytokines; TNF-α is one of the first-wave cytokines resulting in multiple organ damage [59, 60]. These cytokines activate inflammatory cells (neutrophils, macrophages/monocytes, platelets, mastocytes) that release large amounts of toxic ROMs, which cause cellular injury via several mechanisms, including peroxidation of membrane lipids as well as oxidative damage of proteins and DNA [61]. The increased circulating level of TNF-α is a stronger predictor of local tissue damage as well as systemic effects involving organs distant from the burn area [8, 62].

The anti-inflammatory effect of lutein recorded in this study is in accordance with Hadad and Levy [14] who demonstrated the anti-inflammatory effect of lutein against LPS induced inflammation. Lutein stimulated a reduction of lipid peroxidation and attenuated pro-inflammatory cytokine production through NF-κB activity in the liver and eyes of guinea pigs after being fed a hypercholesterolemic diet [16]. Moreover, Li et al. [15] reported the anti-inflammatory role of lutein after retinal ischemic/hypoxic injury.

Lutein exerts anti-inflammatory effects and inhibits the production of pro-inflammatory mediators because of its enhanced bioavailability. It suppressed secretion of the pro-inflammatory mediators NO synthase (iNOS), cyclooxygenase-2 (COX-2), NADPH oxidase (NOX), and TNF-α from mouse macrophages exposed to LPS [14].

Apoptosis is important in the pathophysiological alterations occurring after major thermal injuries. Following burns, almost every tissue or organ is affected and manifests a systemic apoptotic response [63]. Generally, apoptosis is caused by ROS through activation of caspases, rise in mitochondrial permeability with the release of cytochrome C, and cleavage of poly(ADP-ribose) polymerase [64]. ROS may trigger release of lysosomal contents leading also to apoptosis. Hydrogen peroxide can freely diffuse across lysosomal membrane where it reacts with iron inside the lysosome forming the potent hydroxyl radical, finally disrupting the lysosomal membrane causing leakage of contents. Apoptosis is induced after moving of the protease cathepsin D from the lysosome to the cytosol [65].

In the current study, we examined the apoptosis by measuring caspase-3 gene expression and found that thermal trauma increased hepatic, pulmonary, and renal apoptosis. Similar findings were previously reported [1, 66–68]. On the other hand, Bortolin et al. [58] reported that burn injury induced hepatic histopathological alterations but did not change caspase-3 gene expression.

Administration of lutein to rats with thermal injury reduced caspase-3 expression in the liver, kidneys, and lungs. Our results corroborate with previous reports where
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lutein protected HT-29 cells against oxidative stress and apoptosis induced by the mycotoxin deoxynivalenol [69] and IEC-6 cells against methotrexate-induced apoptotic cell injury [70]. The anti-apoptosis effect of lutein may be attributed to inhibiting the activation of pro-apoptotic markers, such as caspases-3, -8, and -9, and enhancing anti-apoptotic marker (Bcl-2) expressions [71].

Our findings demonstrated that burn injury caused histopathological changes in the liver, kidneys, and lungs. Thermal injury induces a systemic inflammatory response starting at the liver resulting in hepatic dysfunction, damaging of hepatic parenchyma and eventually cell death, subsequently initiating multi-organ dysfunction syndrome [72, 73]. Kidney is one of the most frequently involved organs in that circumstance in which patients suffered from burns exceeded 20% of body surface tend to develop acute kidney injury (AKI), even acute kidney failure [74]. Furthermore, the lung is an important organ in the inflammatory response process in burned patients, due to their great capacity in producing inflammatory mediators and radical oxygen species and neutrophil recruitment [75].

CONCLUSION

In conclusion, the current investigation revealed that thermal injury in rats is associated with oxidative stress, inflammatory response, apoptosis, and subsequent remote organ damage. Lutein administration provided multi-organ protection through suppression of proinflammatory and oxidative pathways resulting in concomitant reduction in lipid peroxidation, oxidative DNA damage, apoptosis, and increase in tissue anti-oxidant defense.

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Authors’ Contributions Huda O. AbuBakr and Samira H. Aljuaydi have participated in performing biochemical analysis of oxidant/anti-oxidant biomarkers and gene expression. Shimaa M. Abou-Zeid has performed the experimental design and participated in biochemical analysis of liver and kidney functions and oxidative stress. Amanallah El-Bahrawy has performed histopathological examination. All authors have contributed in writing this article. All authors critically read and revised the manuscript and approved its submission for publication.

COMPLIANCE WITH ETHICAL STANDARDS

Competing Interests. The authors declare that they have no competing interests.

Consent for Publication. All authors have reviewed the manuscript and approved its submission for publication.

Ethics Approval and Consent to Participate. Ethics approval and consent to participate this study were approved by the Animal Use and Care Committee at Faculty of Veterinary Medicine, University of Sadat City, Egypt.

REFERENCES


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