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Anethum graveolens Prevents Liver and Kidney Injury, Oxidative Stress and Inflammation in Mice Exposed to Nicotine Perinatally

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Abstract: Perinatal exposure to nicotine imbalances the redox status in newborns. This study investigated the effect of *Anethum graveolens* (dill) extract on oxidative stress and tissue injury in the liver and kidney of mice newborns exposed to nicotine perinatally. Pregnant mice received nicotine (0.25 mg/kg) on gestational day 12 to day 5 after birth and/or *A. graveolens* extract on gestational day 1 to day 15 after birth. Newborn mice exposed to nicotine showed multiple histopathological alterations in the kidney and liver, including inflammatory cell infiltration and degenerative changes. Nicotine exposure increased hepatic and renal reactive oxygen species (ROS), lipid peroxidation, tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS) ($p < 0.001$), and decreased antioxidant defenses ($p < 0.001$). *A. graveolens* supplementation significantly prevented liver and kidney injury, suppressed ROS generation ($p < 0.001$), lipid peroxidation ($p < 0.001$) and inflammatory response ($p < 0.001$), and enhanced antioxidant defenses. In addition, *A. graveolens* upregulated hepatic and renal Nrf2 and HO-1 mRNA and increased HO-1 activity in normal and nicotine-exposed mice. In conclusion, *A. graveolens* protects against perinatal nicotine-induced oxidative stress, inflammation and tissue injury in the liver and kidney of newborn mice. *A. graveolens* upregulated hepatic and renal Nrf2/HO-1 signaling and enhanced antioxidant defenses in mice.

Keywords: smoking; dill; nicotine; Nrf2; oxidative stress; cytokines

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1. Introduction

Cigarette smoke contains more than 4000 compounds, including aldehydes, carbon monoxide, nicotine and other compounds that cause perinatal damage [1,2]. Smoking during pregnancy can cause serious consequences such as perinatal/neonatal death, miscarriage, premature birth, impaired fetal growth, congenital anomalies and neural tube defects [3,4]. Nicotine is a chiral alkaloid accumulates in the leaves of tobacco and represents ~0.6%–3.0% of the dry weight [5]. It is metabolized within the liver and can cross the blood brain barrier (BBB) where it induces malformations [6]. The use of new delivery systems as well as non-combustible products containing nicotine for recreational purposes and as replacement therapies for smoking cessation has recently increased [7]. Owing to the increased use of nicotine-delivery systems, concerns regarding the impact of nicotine on the health during all stages of development have risen [7].

Oxidative stress and declined antioxidant defenses play a role in mediating the toxic effects of nicotine and in tobacco smoking-linked alterations during pregnancy [8,9]. Perinatal nicotine exposure resulted in increased lipid peroxidation (LPO) and declined cellular defenses in different brain regions of mice [10,11]. In the same context, female rats that received chronic administration of nicotine exhibited a redox imbalance in blood and tissues [12], and oxidative DNA damage was provoked in peripheral blood lymphocytes treated with nicotine [13]. Therefore, counteracting oxidative stress might be effective in preventing the prooxidant and other deleterious effects of nicotine exposure during pregnancy.

Anethum graveolens L. is an annual herb of the Apiaceae family and commonly called dill. It grows mostly in the Mediterranean region, Europe and Asia [14]. It is rich in antioxidants such as flavonoids, alkaloids, anthocyanins, tannins and saponins [15–17]. This herb has been traditionally used for the relief of digestive disorders, hyperlipidemia and inflammation and stimulation of lactation [14]. Oral supplementation of dill extract prevented liver injury and oxidative stress in a rat model of carbon tetrachloride (CCl_4) hepatotoxicity [18]. In addition, treatment with *A. graveolens* extract attenuated gentamicin-induced kidney injury in rats [19]. Although the beneficial effect of dill on liver and kidney injury has been demonstrated before, nothing has yet been reported on its ability to prevent liver and kidney injury in mice exposed perinatally to nicotine. Therefore, this study investigated the protective effects of *A. graveolens* extract against perinatal nicotine-induced oxidative stress and tissue injury in the liver and kidney of mice newborns.

2. Materials and Methods

2.1. Chemical and Reagents

Nicotine was purchased form SOMATCO (Riyadh, Saudi Arabia). Thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA), pyrogallol, 5,5′-dithiobis (nitrobenzoic acid) (DTNB), 2,2-diphenyl-1-picrylhydrazyl (DPPH), reduced glutathione (GSH) and O-phosphoric acid were supplied by Sigma (St. Louis, MO, USA). Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine were supplied by Spinreact (Girona, Spain) and primers for polymerase chain reaction (PCR) were purchased from Vivantis Technologies (Selangor, Malaysia). TRIzol, cDNA synthesis kit and SYBR green master mix were supplied by ThermoFisher Scientific (Waltham, MA, USA). All other chemicals were supplied by Sigma (USA) or standard commercial suppliers.

2.2. Preparation of A. graveolens Extract and Determination of Total Phenolics, Flavonoids and Radical-Scavenging Activity

A. graveolens was ground to a fine powder. Five hundred g of the plant material was macerated in 1 L 70% (*v*/*v*) methanol for 48 h at 4 °C, filtered and dried using a rotary evaporator. The content of total phenolics was assayed using Folin Ciocalteu method [20]. *A. graveolens* extract (1 mL) was mixed with Folin-Ciocalteu reagent (1 mL) and 2% sodium carbonate (3 mL) was added. The mixture was incubated for 2 h at room temperature (RT) and the absorbance was measured at 760 nm. A standard curve, established using different concentrations of gallic acid, was used for the quantification of total phenolics. To assay the content of flavonoids, the aluminum trichloride method [21] was used. In this method, the extract was mixed with 2% aluminum chloride, and the mixture was incubated for 60 min at RT. The absorbance was measured against a blank at 420 nm and quercetin was used to make the standard curve.

To determine the radical-scavenging activity of the extract, an in vitro DPPH radical assay was conducted as previously described [22]. Equal volumes of DPPH (200 μM in ethanol) and the extract were mixed, incubated for 30 min at 37 °C and the absorbance was measured at 515 nm. Gallic acid was used as a standard.

2.3. Experimental Animals and Treatments

Twelve-week-old Swiss–Webster strain mice obtained from the animal house of King Saud University were included in this investigation. The study was conducted according to the guidelines of the National Institutes of Health (NIH publication No. 85-23, revised 2011) and approved by the Ethics Committee of King Saud University (Riyadh, Saudi Arabia). The mice were housed under standard conditions of temperature and humidity under 12 h light/dark cycle and supplied food ad libitum. Three females and one male were kept in each cage and the males were excluded after pregnancy.

The pregnant mice were then allocated into four groups as follows:

Group 1 (Control).

Group 2 (*A. graveolens*): received *A. graveolens* extract (500 mg/kg) [23] by oral gavage from pregnancy day 1 (PD1) until day 15 postnatal (PN).

Group 3 (Nicotine): received 0.25 mg/kg nicotine [10,24] dissolved in saline subcutaneously (s.c.) from PD12 to PN15.

Group 4 (*A. graveolens* + Nicotine): received 0.25 mg/kg nicotine [10,24] s.c. from PD12 to PN15 and 500 mg/kg *A. graveolens* by oral gavage from PD1 to PN15.

A. graveolens extract was dissolved in distilled water and mice in group I received the vehicle orally. Mice in group II received saline s.c. from PD12 to PN15. The newborns of each group were culled to 8 on PN0 and kept with their mothers until PN21. The newborns were sacrificed by decapitation at PN22. Blood was collected and serum was prepared by centrifugation at 3000 rpm for 15 min for the assay of aminotransferases, creatinine and urea. The mice were dissected, and liver and kidney were collected, and samples were fixed in 10% neutral buffered formalin for histopathological examination. Other samples were kept at −80 °C in RNAlater for RNA isolation, and others were homogenized (10% *w*/*v*) in cold phosphate buffered saline (PBS), centrifuged and the supernatant was collected and stored at −80 °C.

2.4. Biochemical Assays

ALT, AST, creatinine and urea were determined in serum using commercially available assay kits (Spinreact, Girona, Spain), following the manufacturer's instructions. Reactive oxygen species (ROS) were assessed immediately using H_2DCF-DA as previously reported [25]. Briefly, tissue homogenate (100 µL), PBS (1 mL) and 5 µL H_2DCF-DA (10 µM final concentration) were mixed, incubated for 30 min at 37 °C and the fluorescence intensity was determined at 490 and 540 nm. Malondialdehyde (MDA), a marker of LPO, was assayed in liver and kidney according to the method of Mihara and Uchiyama [26]. In this method, tissue homogenate (200 µL) was mixed with 0.6% TBA (400 µL) and 1% O-phosphoric acid (1.2 mL), incubated at 95 °C for 45 min and n-butanol (0.8 mL) was added. The mixture was vortexed for 1 min and the upper layer was separetd by centrifugation at 2000 rpm for 10 min, and the absorbance was measured at 535 nm. GSH was assayed based on its reaction with DTNB generating a yellow-colored product. The absorbance of the product was measured at 412 nm [27]. The assay method of superoxide dismutase (SOD) was based on the inhibition of pyrogallol autoxidation by SOD. This inhibition is directly proportional to SOD activity [28]. Determination of catalase (CAT) activity was based on the decomposition of hydrogen peroxide (H_2O_2). The decrease in absorbance was continuously monitored at 240 nm for 3 min [29]. The activity of heme oxygenase-1 (HO-1) was assayed according to the method described by Abraham et al. [30]. In this method, the tissue samples were mixed with glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate (NADPH), hemin and glucose-6-phosphate dehydrogenase, incubated for 1 h at 37 °C, and the absorbance was measured at 464 nm.

2.5. Determination of Inflammation Markers

Interleukin (IL)-6 and tumor necrosis factor (TNF)-α were assayed in the liver and kidney of mice using R&D Systems (Minneapolis, MN, USA) enzyme-linked immunosorbent assay (ELISA) kits. This assay employs sandwich ELISA using microplates coated

with monoclonal antibody specific for either TNF-α or IL-6. The samples were added, the plate was washed, and a specific enzyme-linked antibody was added. Following washing, a blue color was developed by the addition of the substrate solution. The color turned yellow after the addition of a stop solution and the absorbance was measured at 450 nm. The intensity of the color is proportional to the amount of the assayed cytokine.

2.6. Histopathological Study

Liver and kidney samples fixed in 10% neutral buffered formalin for 24 h were dehydrated, cleared in xylene and embedded in paraffin wax. Five-µm sections were cut using a microtome and processed for hematoxylin and eosin (H&E) staining [31] and then examined using a light microscope.

2.7. Gene Expression

The effect of *A. graveolens* on mRNA abundance of Nrf2, HO-1, TNF-α, IL-6 and inducible nitric oxide synthase (iNOS) was determined by quantitative real-time polymerase chain reaction (qRT-PCR). Briefly, total RNA was isolated from the liver and kidney samples of the mice using TRIzol. The isolated RNA was quantified at 260 nm on a nanodrop and samples with A260/A280 of ≥1.8 were selected for cDNA synthesis. SYBR green master mix and the list of primers in Table 1 were used for cDNA amplification by ABI 7500 real-time PCR System (Applied Biosystems, Waltham, MA, USA) in a total reaction volume of 20 µL. The obtained data were analyzed using the $2^{-\Delta\Delta Ct}$ method [32] and normalized to β-actin.

Table 1. Primers used for quantitative real-time polymerase chain reaction (qRT-PCR).

Gene	GenBank Accession Number	Primer Sequence (5′-3′)	Product Size (bp)
TNF-α	NM_001278601.1	F: CCCTCACACTCACAAACCAC R: ACAAGGTACAACCCATCGGC	133
IL-6	NM_031168.2	F: ACAAAGCGAGAGTCCTTCAGAG R: GAGCATTGGAAATTGGGGTAGG	108
iNOS	NM_010927.4	F: GCCACCTTGGTGAAGGGACT R: ACGTTCTCCGTTCTCTTGCAG	111
Nrf2	NM_010902.3	F: TCCTATGCGTGAATCCCAAT R: GCGGCTTGAATGTTTGTCTT	103
HO-1	NM_010442.2	F: GGGCTGTGAACTCTGTCCAAT R: GGTGAGGGAACTGTGTCAGG	162
β-actin	NM_007393.5	F: GTGCTATGTTGCTCTAGACTTCG R: ATGCCACAGGATTCCATACC	174

2.8. Statistical Analysis

The results were presented as mean ± standard error of the mean (SEM). Multiple comparisons were tested using one-way analysis of variance (ANOVA) followed by Tukey's test post hoc analysis on Graphpad Prism 7 (San Diego, CA, USA) and a p value below 0.05 was considered significant.

3. Results

3.1. Total Phenolics, Flavonoids and Radical Scavenging Activity of A. graveolens

Determination of total phenolics and flavonoids (Figure 1A) revealed that the extract contains 86.7 ± 1.56 mg gallic acid equivalents/g and 53.00 ± 1.02 mg quercetin equivalents/g, respectively. The scavenging activity against DPPH• revealed a concentration-dependent efficacy of *A. graveolens* (Figure 1B).

3.2. A. graveolens Prevents Liver and Kidney Injury in Mice Newborns Exposed to Nicotine

Perinatal exposure to nicotine increased serum activities of ALT (Figure 2A), AST (Figure 2B), creatinine (Figure 2C) and urea (Figure 2D) significantly ($p < 0.001$) in mice

newborns. In contrast, oral supplementation of *A. graveolens* extract ameliorated serum ALT, AST, creatinine and urea in nicotine-induced newborn mice.

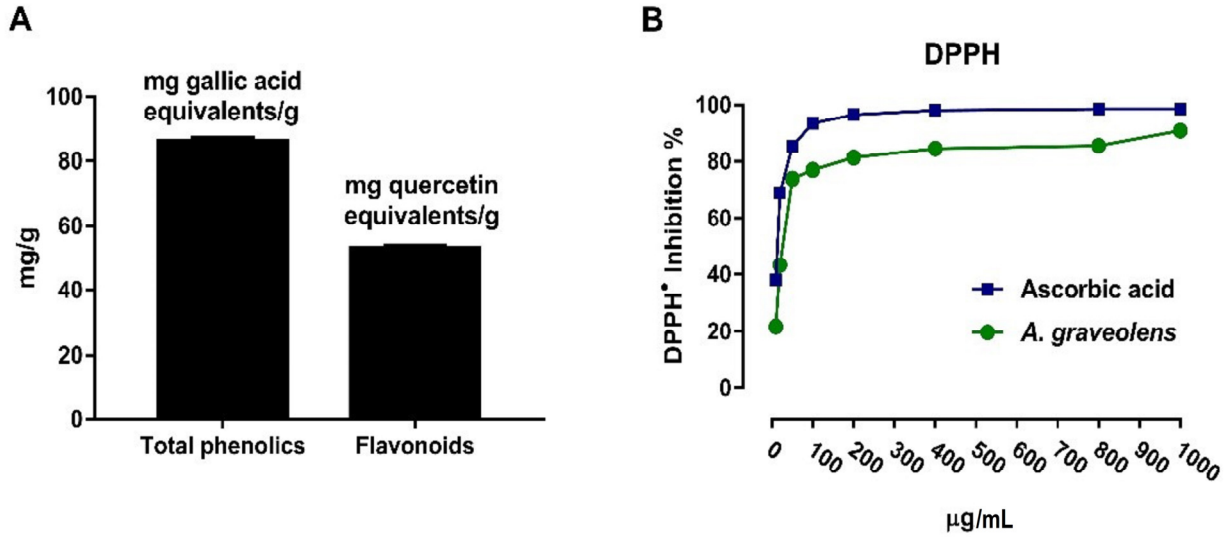

Figure 1. Total phenolics and flavonoids content (**A**) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•)-scavenging activity *A. graveolens* (**B**). Data are the mean values of triplicate and expressed as mean ± standard deviation (SD).

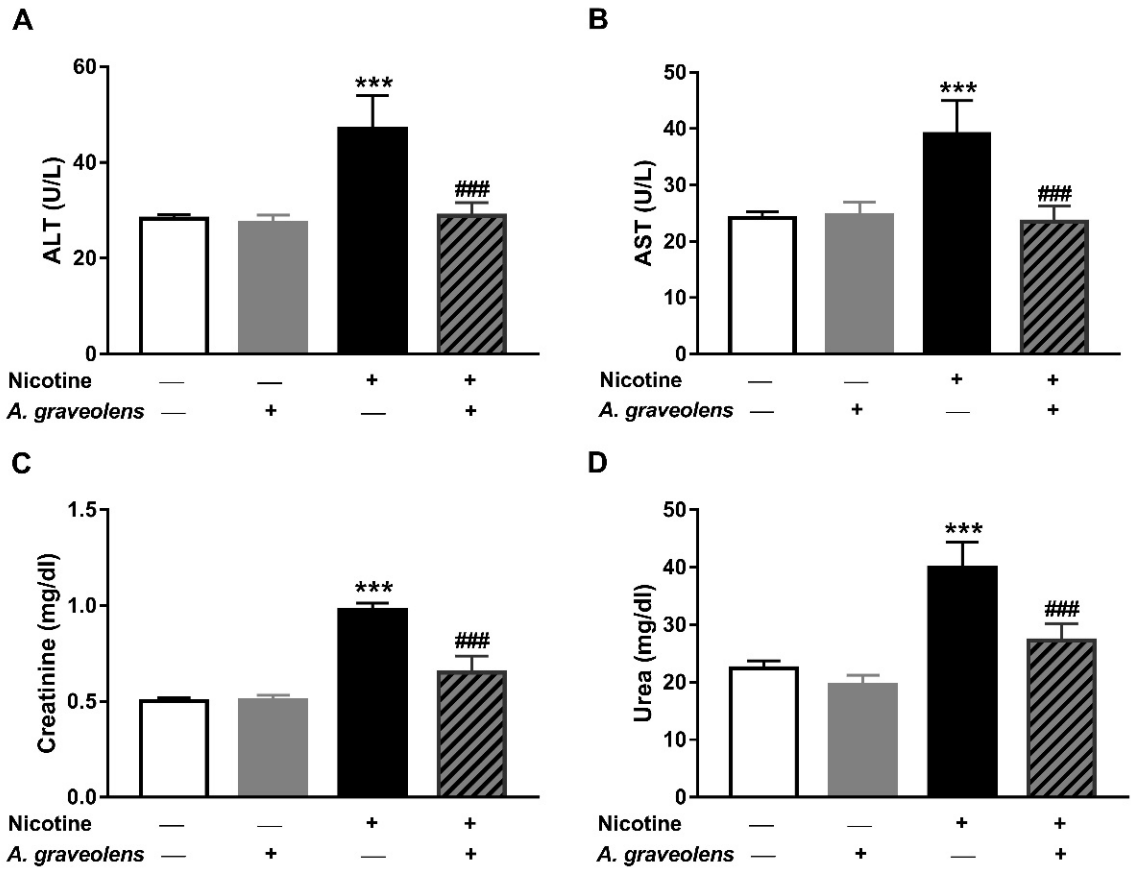

Figure 2. *A. graveolens* ameliorated serum aminotransferase (ALT) (**A**), aspartate aminotransferase (AST) (**B**), creatinine (**C**) and urea (**D**) in nicotine-exposed mice newborns. Data are mean ± standard error of the mean (SEM), $n = 8$. *** $p < 0.001$ versus Control and ### $p < 0.001$ versus nicotine.

The histological examination added support to the hepato- and renoprotective effects of *A. graveolens* extract in newborn mice. While the control (Figure 3A,B) and *A. graveolens* extract-supplemented mice (Figure 3C,D) exhibited normal structure of the hepatic lobules, hepatocytes and sinusoids, nicotine-exposed newborns showed inflammatory cells infiltration, central vein congestion and degenerative changes (Figure 3E,F). Treatment with *A. graveolens* extract markedly prevented nicotine-induced liver histological alterations in the newborn mice (Figure 3G,H).

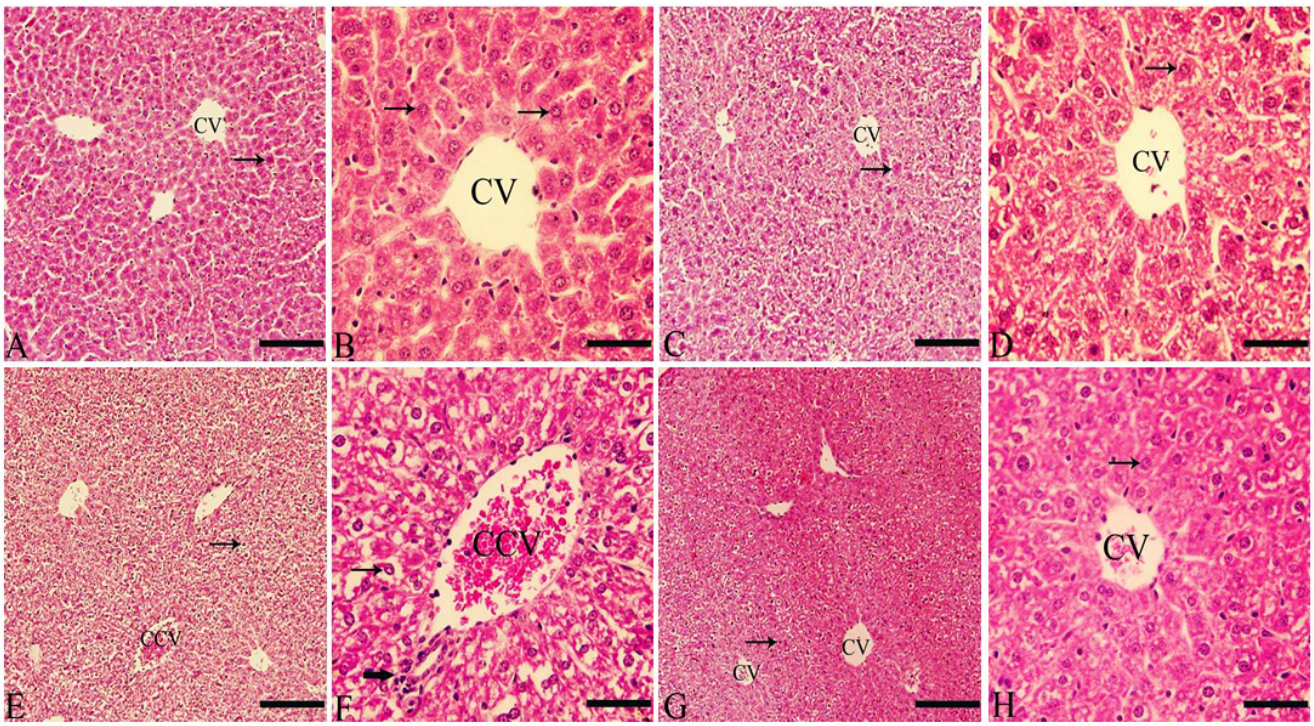

Figure 3. Photomicrographs of hematoxylin and eosin (H&E)-stained sections in the liver of (**A**,**B**) Control and (**C**,**D**) *A. graveolens*-treated mice showing normal hepatic cells (thin arrow), central vein (CV), inflammatory cells (thick arrow), (**E**,**F**) nicotine-exposed newborns showing congested central vein observed (CCV), degeneration of some hepatic cells and (**G**,**H**) nicotine-exposed newborns treated with *A. graveolens* showing ameliorated liver structure. [Scale bar = 50 μm (**A**,**C**,**E**,**G**) and 200 μm (**B**,**D**,**F**,**H**)].

Nicotine exposure resulted in multiple alterations in the kidney of newborns, including inflammatory cells infiltration, hemorrhage and desquamation (Figure 4E,F). Treatment with *A. graveolens* extract markedly prevented nicotine-induced kidney injury in the newborn mice (Figure 4G,H). Sections in the kidney of both the control (Figure 4A,B) and *A. graveolens* extract-supplemented mice (Figure 4C,D) exhibited normal structure of the renal tubules and corpuscles.

3.3. A. graveolens Attenuates Oxidative Stress in Liver and Kidney of Mice Newborns Exposed to Nicotine

ROS levels exhibited a significant elevation in the liver and kidney of nicotine-exposed mice ($p < 0.001$) as illustrated in Figure 5A,B, respectively. Oral supplementation of *A. graveolens* extract decreased hepatic and renal ROS in nicotine-treated newborn mice. Hepatic and renal MDA, a LPO marker (Figure 5C,D), was significantly increased in nicotine-exposed mice. These alterations were reversed in the group that received *A. graveolens* extract.

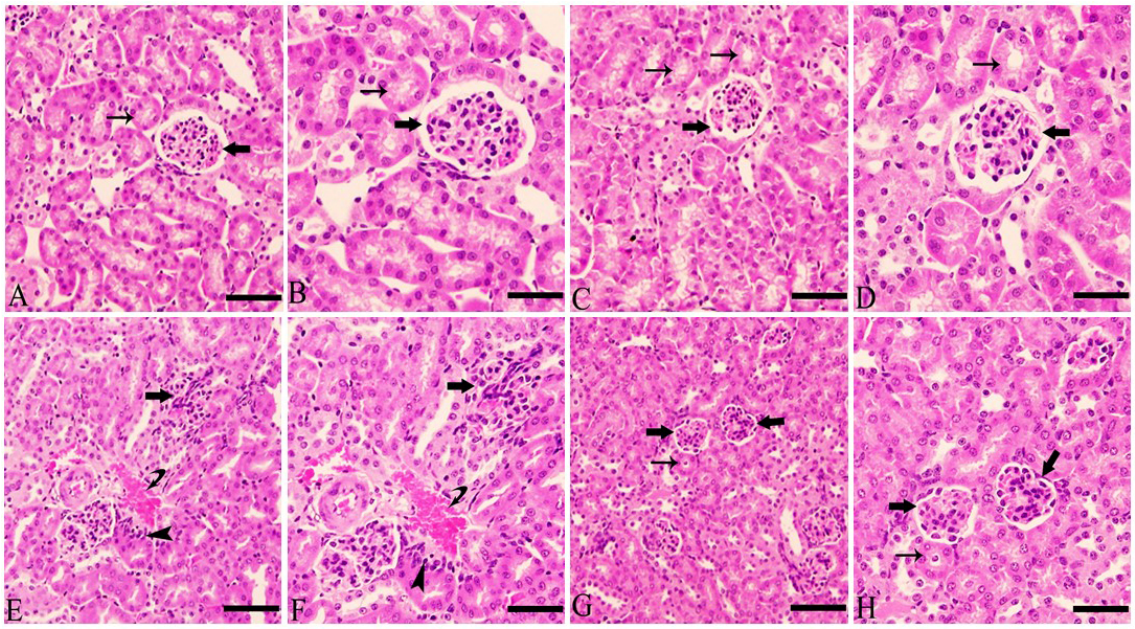

Figure 4. Photomicrographs of H&E-stained sections in the kidney of (**A**,**B**) Control and (**C**,**D**) *A. graveolens*-treated mice showing normal glomeruli (thick arrow) and renal tubules (thin arrow), (**E**,**F**) nicotine-exposed newborns showing inflammatory cells (arrowhead) and hemorrhage (curved arrow) and (**G**,**H**) nicotine-exposed newborns treated with *A. graveolens* showing ameliorated kidney structure. [Scale bar = 50 µm (**A**,**C**,**E**,**G**) and 200 µm (**B**,**D**,**F**,**H**)].

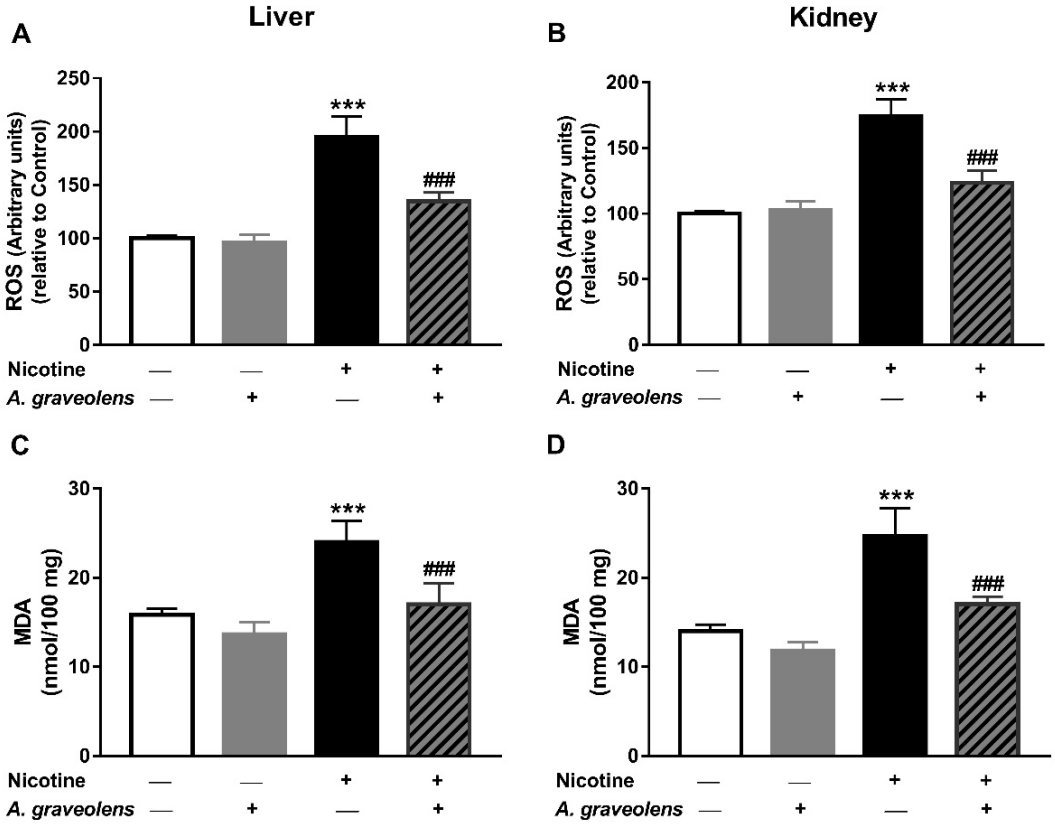

Figure 5. *A. graveolens* decreased reactive oxygen species (ROS) and malondialdehyde (MDA) in liver (**A**,**C**) and kidney (**B**,**D**) of nicotine-exposed mice newborns. Data are mean ± SEM, $n = 8$. *** $p < 0.001$ versus Control. ### $p < 0.001$ versus Nicotine.

In contrast, mice exposed to nicotine exhibited a decrease in hepatic (Figure 6A) and renal (Figure 6B) GSH content and the activity of SOD (Figure 6C,D, respectively) and CAT (Figure 6E,F, respectively) significantly when compared with the control group ($p < 0.001$). Perinatal supplementation of *A. graveolens* extract increased GSH and enzymatic antioxidants in the liver and kidney of nicotine-exposed mice. Of note, *A. graveolens* extract had no effect on ROS, MDA and antioxidant defenses in the liver and kidney of normal mice.

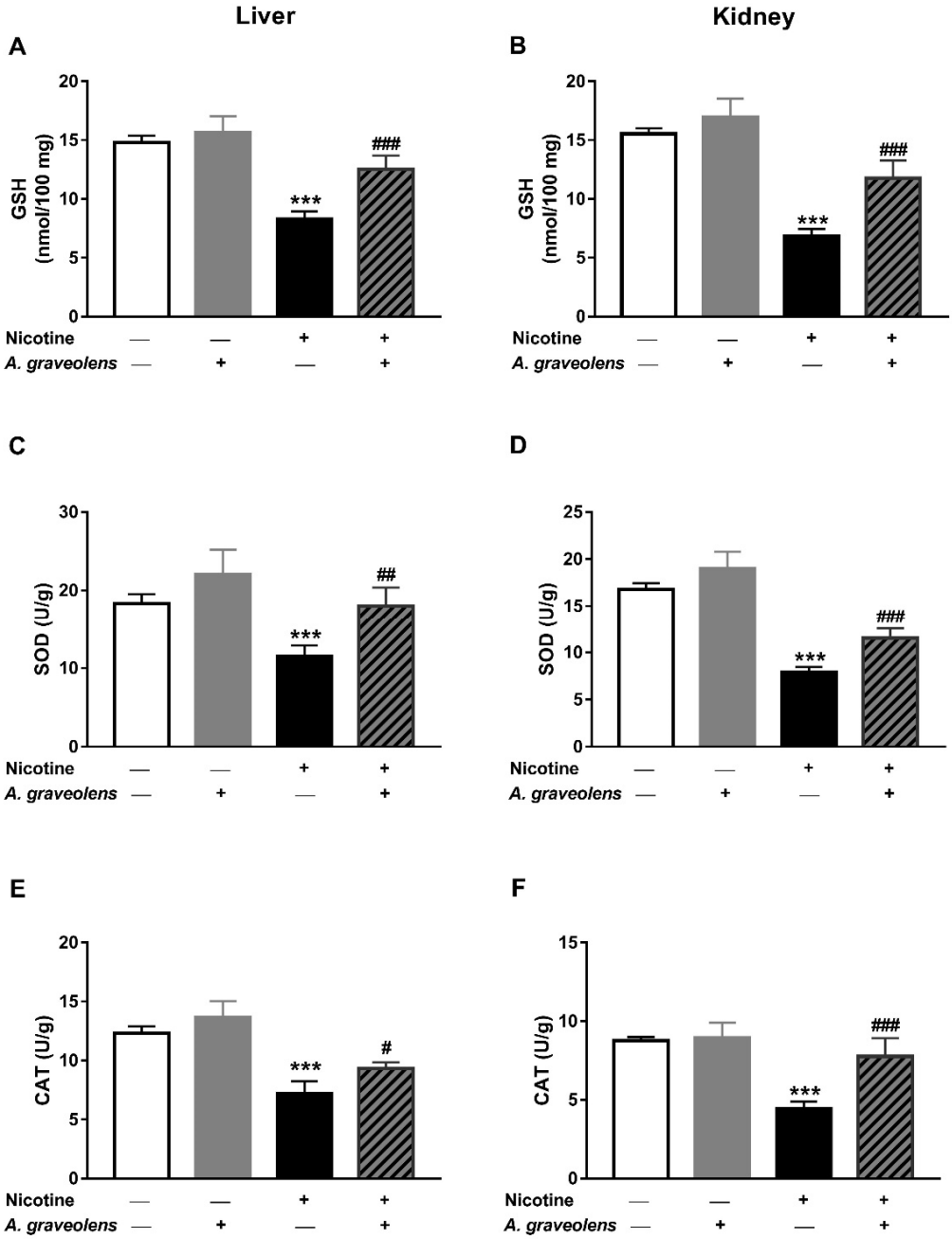

Figure 6. *A. graveolens* and increased glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) in liver and kidney of nicotine-exposed mice newborns. Data are mean ± SEM, $n = 8$. *** $p < 0.001$ versus Control and ### $p < 0.001$ versus nicotine: (**A**) Liver GSH; (**B**) Kidney GSH; (**C**) Liver SOD; (**D**) Kidney SOD; (**E**) Liver CAT; (**F**) Kidney CAT.

3.4. A. graveolens Mitigates Inflammation in Liver and Kidney of Mice Newborns Exposed to Nicotine

Changes in the expression of TNF-α, IL-6 and iNOS were determined to explore the effect of *A. graveolens* extract on nicotine-induced inflammatory response in the liver and

kidney of newborn mice. The results revealed a significant upregulation of TNF-α, IL-6 and iNOS mRNA in the liver (Figure 6A,C,E, respectively) and kidney (Figure 7B,D,F, respectively) of nicotine-exposed newborns ($p < 0.001$). TNF-α and IL-6 levels were determined in the liver and kidney using ELISA (Figure 8). Both cytokines showed a significant elevation in the liver (Figure 8A,C, respectively) and kidney (Figure 8B,D, respectively) of nicotine-exposed newborns. *A. graveolens* extract significantly downregulated TNF-α, IL-6 and iNOS in the liver and kidney of nicotine-exposed mice.

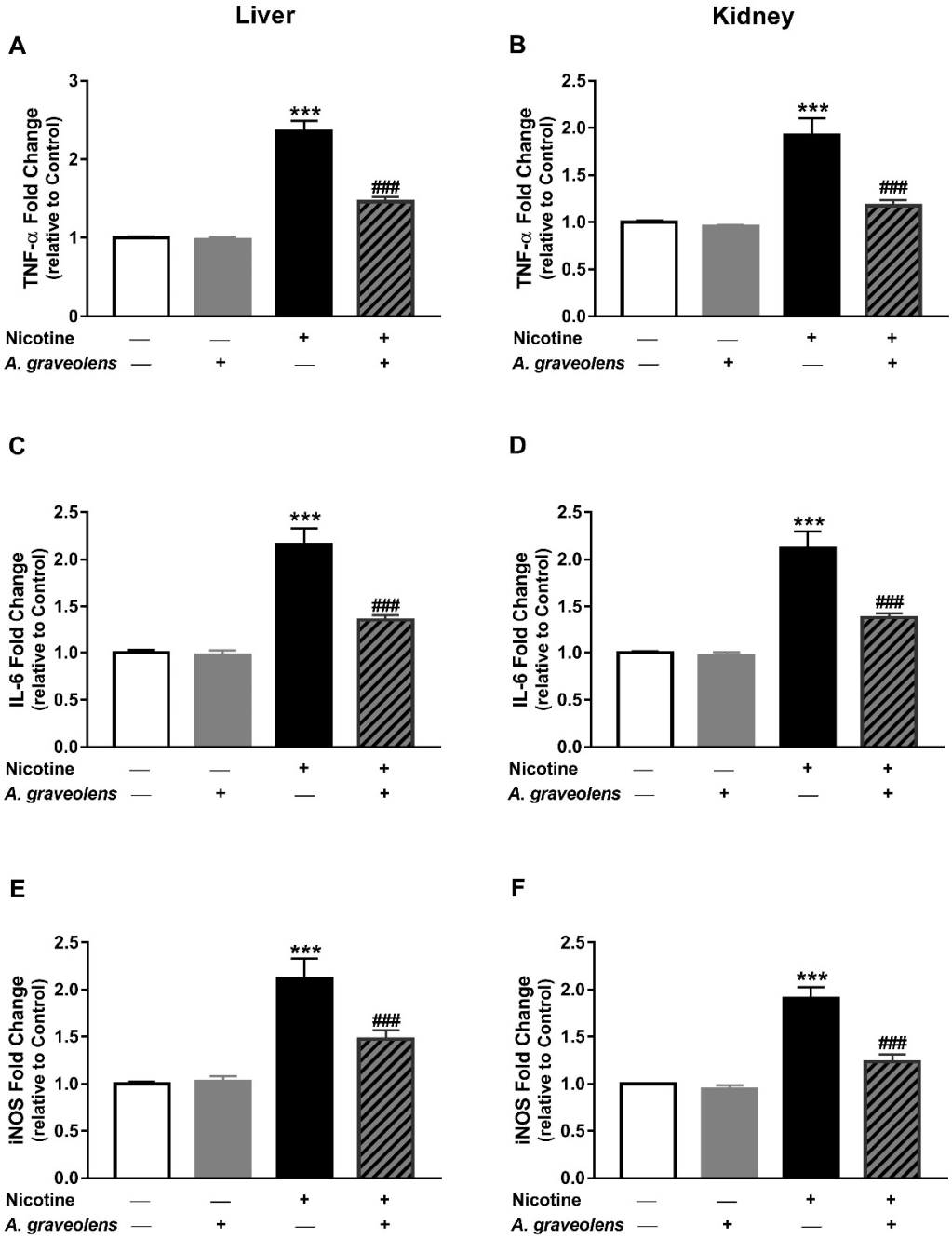

Figure 7. *A. graveolens* decreased tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS) mRNA abundance in liver and kidney of nicotine-exposed mice newborns. Data are mean ± SEM, $n = 8$. *** $p < 0.001$ versus Control and ### $p < 0.001$ versus nicotine: (**A**) Liver TNF-α; (**B**) Kidney TNF-α; (**C**) Liver IL-6; (**D**) Kidney IL-6; (**E**) Liver iNOS; (**F**) Kidney iNOS.

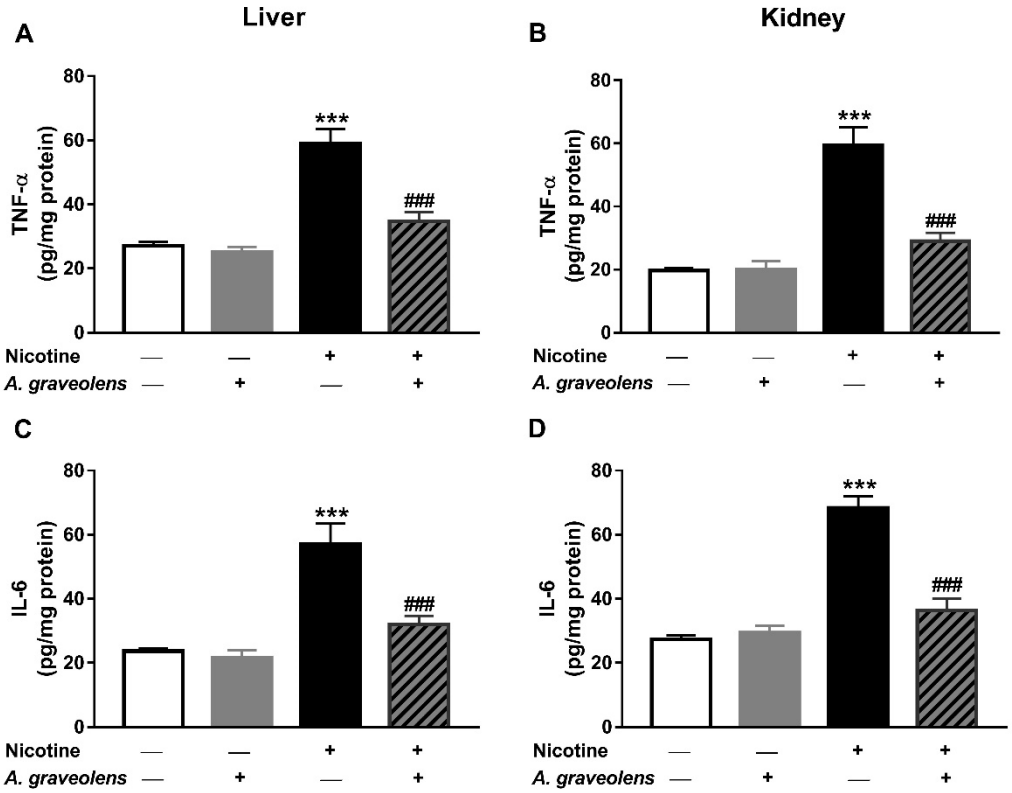

Figure 8. *A. graveolens* decreased TNF-α and IL-6 protein levels in liver (**A**,**C**) kidney (**B**,**D**) of nicotine-exposed mice newborns. Data are mean ± SEM, $n = 8$. *** $p < 0.001$ versus Control and ### $p < 0.001$ versus nicotine.

3.5. A. graveolens Upregulates Nrf2/HO-1 Signaling in Liver and Kidney of Mice Newborns Exposed to Nicotine

Nrf2 mRNA abundance exhibited a significant decrease in the liver (Figure 9A) and kidney (Figure 9B) of nicotine-exposed mice when compared to the control group ($p < 0.001$). HO-1 mRNA and activity were also decreased in the liver (Figure 9C,E, respectively) and kidney (Figure 9D,F, respectively) of the newborns that were exposed to nicotine perinatally. Treatment with *A. graveolens* extract remarkably ameliorated hepatic and renal Nrf2 and HO-1 expression and activity in nicotine-exposed mice. Notably, *A. graveolens* extract significantly upregulated Nrf2 and HO-1 in both the liver and kidney of normal newborn mice.

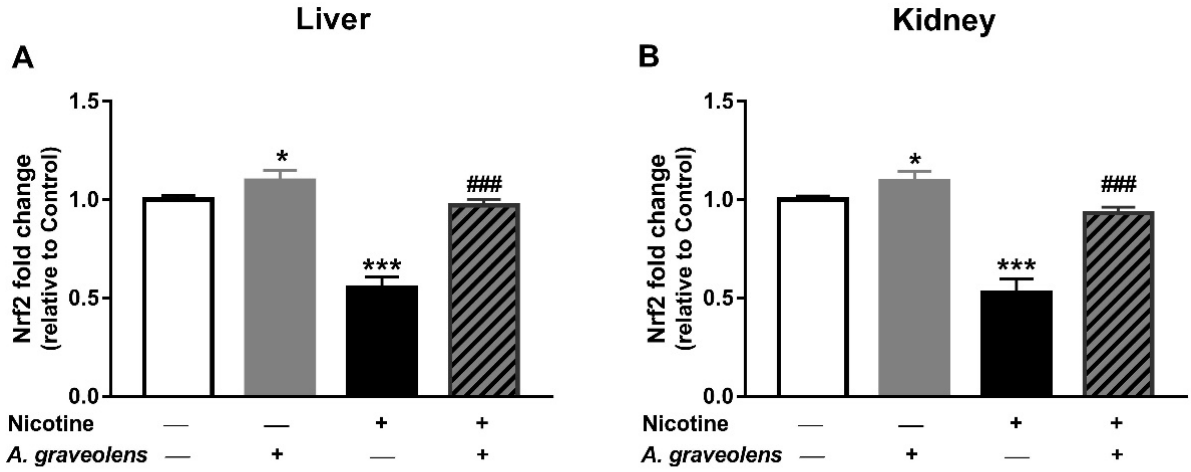

Figure 9. *Cont.*

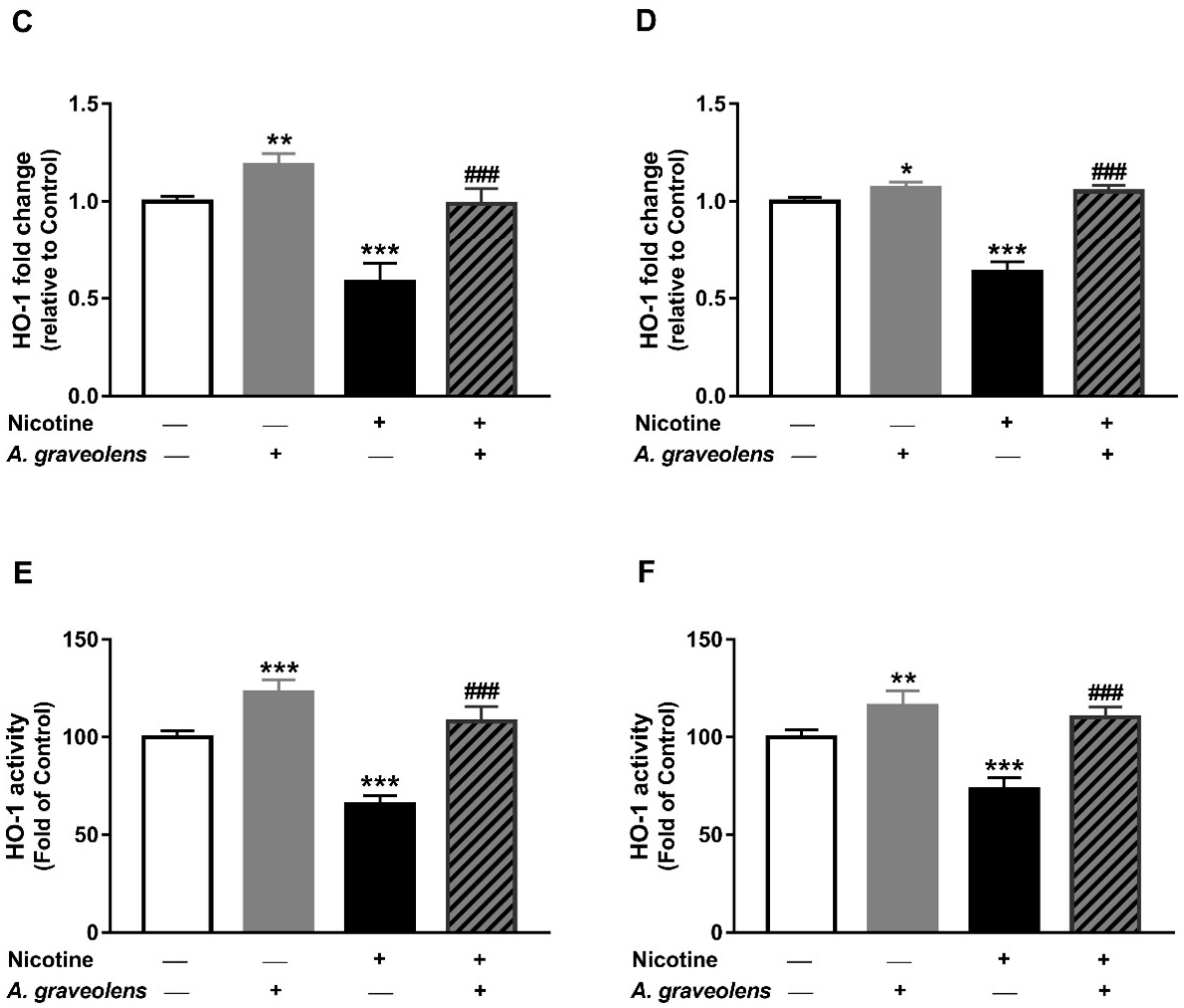

Figure 9. *A. graveolens* increased Nrf2 and HO-1 mRNA and HO-1 activity in liver and kidney of nicotine-exposed mice newborns. Data are mean ± SEM, $n = 8$. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus Control and ### $p < 0.001$ versus nicotine: (**A**) Liver Nrf2 mRNA; (**B**) Kidney Nrf2 mRNA; (**C**) Liver HO-1 mRNA; (**D**) Kidney HO-1 mRNA; (**E**) Liver HO-1 activity; (**F**) Kidney HO-1 activity.

4. Discussion

Preclinical studies have strongly demonstrated the adverse effects of nicotine exposure during critical developmental periods on different body systems [7]. Herein, we investigated the impact of perinatal nicotine exposure on the redox status and inflammatory response in liver and kidney of newborn mice and the protective effect of *A. graveolens*.

Perinatal nicotine exposure resulted in liver and kidney injury manifested by the significant increase in serum aminotransferases, creatinine and urea as well the histopathological alterations. Inflammatory cells infiltration, degenerative changes, blood vessel congestions and hemorrhage represented the main histological alterations in the liver and kidney of newborn mice exposed to nicotine. Owing to its major role in the transformation of nicotine, the liver is vulnerable to injury and altered function [33]. Following its absorption through the lungs, nicotine is primarily metabolized within the liver through the action of CYP2A6 [34]. The hepatic transformation of nicotine predisposes the liver to toxic, immunological and oncogenic adverse effects [34]. Accordingly, genotoxic effects and cell injury have been reported in the liver of rats exposed to smoking [35]. The exposure to nicotine can occur during fatal and early postnatal periods and different factors, in particular kidney function, can affect the rate of nicotine metabolism [36]. In this context, nicotine has been detected in the umbilical cord of newborns of smoking mothers and the elimination of

nicotine was prolonged when compared with the adults [37]. Besides liver injury, nicotine exposure resulted in kidney injury in the newborn mice. The impact of nicotine on kidney function has been recognized through in vitro and in vivo studies [38]. Nicotine strongly promotes the proliferation of human mesangial cells and the production of fibronectin at concentrations similar to its circulating peak concentration in active smokers [39].

A. graveolens extract ameliorated nicotine-induced liver and kidney injury in newborn mice. Oral supplementation of *A. graveolens* extract decreased serum aminotransferases, creatinine and urea and prevented liver and kidney tissue injury, demonstrating its hepato- and renoprotective effects. In support of our findings, the protective effect of *A. graveolens* against liver and kidney injuries induced by different agents has been reported in previous studies. In a rat model of CCl_4-induced toxicity, *A. graveolens* extract displayed a potential hepatoprotective activity where it ameliorated liver function markers and prevented LPO [18]. In addition, treatment with *A. graveolens* extract attenuated gentamicin-induced kidney injury in rats as reported by Srivastava et al. [19]. Given the role of oxidative stress and inflammation in mediating the toxic effects of nicotine [8,9], we assumed that the hepato- and renoprotective effect of *A. graveolens* extract was mediated via its antioxidant and anti-inflammatory activities. This notion is supported by the study of Jaimes et al. [39] showing the role of NADPH oxidase inhibitors in preventing the pro-proliferative and pro-fibrotic effects of nicotine in mesangial cells.

Numerous preclinical studies have demonstrated increased ROS generation following exposure to nicotine [9,12,40,41]. Our study revealed surplus ROS generation in the liver and kidney of newborn mice exposed perinatally to nicotine. Consequently, hepatic and renal MDA levels were increased, and cellular antioxidants were declined in nicotine-exposed newborns. In mesangial [39] and renal proximal tubule cells [42], nicotine activated NADPH oxidase and increased mitochondrial-mediated ROS production. Rats received nicotine administration exhibited an increase in ROS [42] and decreased circulating and tissue antioxidants [9,12,40,41]. The impact of nicotine on the liver has been reported in many studies [43–45]. It enhanced the progression of hepatic steatosis in mice [43], altered xenobiotic metabolism in offspring [44] and its maternal exposure provoked oxidative stress and steatosis in the liver of adult rat offspring [45]. In addition to oxidative stress, perinatal exposure to nicotine was associated with an inflammatory response in the newborn mice. In the present study, TNF-α, IL-6 and iNOS were remarkably upregulated in the liver and kidney of nicotine-exposed mice. Excessive ROS production coupled with diminished antioxidants promote inflammation by activating nuclear factor-kappaB (NF-κB) and the subsequent release of inflammatory cytokines, triggering mitochondrial damage and apoptosis [46].

Interestingly, *A. graveolens* extract suppressed ROS generation, LPO, and pro-inflammatory mediators and boosted cellular antioxidant defenses in the liver and kidney of nicotine-exposed newborns. These results indicate the effective potent anti-inflammatory and radical-scavenging efficacies of *A. graveolens*. These findings were supported by the in vitro radical scavenging assay where *A. graveolens* exerted concentration-dependent inhibition of DPPH radicals. *A. graveolens* prevented oxidative/nitrosative stress and downregulated the expression of iNOS and IL-6 in macrophages in vitro [47]. The ability of *A. graveolens* to suppress ROS and pro-inflammatory mediators could be directly connected to its rich content of polyphenols and flavonoids. Polyphenols are effective against liver and kidney injury associated with excessive production of ROS and the inflammatory response as we previously reported [10,48–51]. Flavonoids are effective radical-scavengers due to their ability to donate hydrogen, quench ROS and chelate metals. In addition, *A. graveolens* has been reported to contain a relatively high amount of alkaloids and moderate content anthocyanins which have antioxidant, anti-inflammatory and hepatoprotective properties [15,17].

Besides its radical-scavenging activity, the antioxidant and anti-inflammatory efficacy of *A. graveolens* could be attributed to the activation of Nrf2/HO-1 signaling. Here, nicotine-exposed newborns exhibited decreased hepatic and renal Nrf2 and HO-1 mRNA abundance and HO-1 activity. Although the impact of perinatal nicotine exposure on hepatic and

renal Nrf2/HO-1 signaling has not been adequately studied, previous literature supports the downregulation of this signaling pathway following treatment with nicotine. For instance, nicotine increased ROS production and insulin resistance and downregulated Nrf2 signaling in cardiomyocytes [52]. The neurobehavioral disorders in the adult rat cerebral cortex were associated with suppressed Nrf2 [53]. *A. graveolens* upregulated Nrf2 and HO-1 in the liver and kidney of both normal and nicotine-exposed mice offspring. Nrf2 is a redox-sensitive transcription factor that protects cells against the deleterious effects of oxidants and electrophiles by increasing the expression of antioxidant and defensive genes, including HO-1. Nrf2 is sequestered in the cytosol by Keap-1 under physiological conditions and this binding is interrupted upon exposure to ROS. The liberated Nrf2 transloctaes to the nucleus, binds to the antioxidant response element and elicits the expression of HO-1 and other genes [54]. The antioxidant enzymes expressed neutralize excess ROS and protect against oxidative cell injury. HO-1 maintains the redox homeostasis, prevents cell death, and attenuates the inflammatory response [55]. Therefore, Nrf2 activation by *A. graveolens* extract is involved, at least in part, in attenuating liver and kidney injury induced by perinatal exposure to nicotine. This study shows for the first time the protective effect of *A. graveolens* extract against perinatal nicotine-induced liver and kidney injury through activation of Nrf2/HO-1 signaling and attenuation of oxidative stress and inflammation. The lack of protein expression results of Nrf2 and HO-1 could be considered a limitation of this study; however, we determined HO-1 activity, and this confirmed the gene expression.

5. Conclusions

The present study introduces new information on the hepato- and renoprotective effects of *A. graveolens* extract against perinatal nicotine-induced tissue injury. *A. graveolens* prevented histopathological alterations and suppressed ROS, LPO and pro-inflammatory mediators in the liver and kidney of nicotine-exposed mice. In addition, *A. graveolens* upregulated hepatic and renal Nrf2/HO-1 signaling and enhanced antioxidant defenses, thereby prevented nicotine-induced oxidative stress and inflammation. Therefore, *A. graveolens* may be considered a potential candidate for attenuating smoking/nicotine-induced liver and kidney alterations in newborns, pending further studies to explore other mechanisms involved in its protective effects.

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