Protective effects of nanocurcumin against stress-induced deterioration in the intestine

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\textbf{ABSTRACT}

The therapeutic activities of curcumin have long been investigated in some chronic and inflammatory diseases. This study was designed to investigate the protective effects of nanocurcumin on intestinal barrier function, apoptosis, and oxidative stress in rats exposed to traffic noise. Forty rats were divided into four groups: two traffic noise-exposed groups of animals that received either vehicle (NOISE) or nanocurcumin (NCUR + NOISE) and two control groups that either remained intact (CON) or received nanocurcumin (NCUR). Nanocurcumin injection (15 mg/Kg/ip) and traffic noise exposure were administered daily for two weeks. The relative protein expression of intestinal tight junctions, occludin, and ZO-1 and Bax/Bcl-2 ratio was measured to evaluate barrier integrity and apoptosis in intestinal samples, respectively. Plasma D-lactate concentration was examined as a criterion of intestinal permeability. Corticosterone, superoxide dismutase (SOD) activity, glutathione (GSH), total antioxidant capacity (TAC), and nitrite were measured in serum. The noise exposure increased Bax/Bcl-2 ratio, corticosterone, and oxidative stress in the NOISE animals. Nanocurcumin treatment improved the Bax/Bcl-2 ratio and reduced corticosterone and oxidative stress in the NCUR + NOISE animals. The expression of tight junction proteins was decreased while the concentration of D-lactate was increased in the NOISE animals. Nanocurcumin did not efficiently impact the expression of tight junction proteins and the D-lactate level in the NCUR + NOISE group. Nanocurcumin administration displayed antioxidant and anti-apoptotic roles in the noise-exposed rats, however, it did not affect the intestinal barrier integrity. We concluded that reduced apoptosis in the intestine might be related to the antioxidant activity of nanocurcumin and its modulatory effects on the HPA axis in the nanocurcumin-treated animals.

\textbf{Introduction}

Stress is a lifestyle factor associated with the deterioration of the intestinal barrier through gut-brain interactions and is a recognized risk factor for the onset and reactivation of chronic disorders (Rodiño-Janeiro et al., 2015). Today, noise stress is one of the most important environmental issues whose health effects are ignored (Jones, 1983; Bo et al., 2013). Noise is defined as an unpleasant and annoying sound of more than 90 decibels (Jones, 1983; Bo et al., 2013). Noise stress may reduce the function of the intestinal barrier (Bijlsma et al., 2001). Intestinal barrier defect is associated with a wide range of diseases and thus represents a new therapeutic target. Endothelial and, thus, epithelial barrier functions have fundamental roles in protecting the gut against potentially invasive pathogens (Yu et al., 2012). Intestinal barrier functions may be essential to prevent the development of multiple organ dysfunction (Odenwald & Turner, 2017). This barrier function is maintained by a set of proteins that form the tight junctions, transmembrane protein (occludin), and cytoplasmic membrane proteins (ZO-1). These proteins are involved in regulating paracellular permeability (Vancamelbeke & Vermeire, 2017). In the gastrointestinal tract, bacterial fermentation and decomposition produce D-lactate (Marcos et al., 1991). D-lactate, which is not metabolized by the liver, is effluxed into the bloodstream when intestinal permeability increases (Grootjans et al., 2010). Therefore, the plasma level of D-lactate is a valuable predictor for evaluating intestinal permeability (Xun et al., 2021; Ficek et al., 2017).

It is shown that environmental stress induces apoptosis (Xie et al., 2019). Apoptosis is the process of programmed cell death regulated by genes, such as the Bcl-2 family, caspase family, C-myc oncogenes and tumor suppressor gene P53, and so on (Zhao, Li, et al., 2021). The Bcl-2 family of proteins can be divided into two categories based on function. One kind inhibits apoptosis, such as Bcl-2, Bcl-XL, Bcl-W, and Mcl-1, while the other promotes apoptosis, such as Bax,
Bcl-Xs, Bak, Bik/Nbk, and Bid (Lalier et al., 2022). In the process of apoptosis, Bax causes the release of cytochrome C and apoptosis-inducing factors and eventually leads to apoptosis (Lee et al., 2020). Meanwhile, Bcl-2 protein inhibits cytochrome C and apoptosis-inducing factors to prevent apoptosis (Li et al., 2013).

Environmental stress-induced apoptosis is associated with increased oxidative stress (Xie et al., 2019). Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the scavenger system (Song et al., 2020). We investigated oxidant/antioxidant balance by measuring serum nitric oxide (NO) glutathione (GSH), superoxide dismutase (SOD), and total antioxidant capacity (TAC).

Recent research has focused on the effects of natural antioxidant agents, such as curcumin, on bowel disease, mainly due to their safety profile and affordability (Lopresti, 2018). Curcumin, a naturally occurring polyphenolic compound, is known to have a wide range of therapeutic and pharmacological properties (Flora et al., 2013). In vivo and clinical studies have confirmed the antioxidant, anti-inflammatory, anti-tumor, analgesic, anti-arthritic, and immunoregulatory activities of curcuminoids relevant to the treatment of human diseases (Hewlings & Kalman, 2017). One of the effects of curcumin on the intestinal epithelium and immune system is to preserve the integrity of the intestinal barrier (Burge et al., 2019). However, the main obstacle to the clinical efficacy of curcumin is poor bioavailability due to its low aqueous solubility and rapid metabolism (Hassanzadeh et al., 2020). Therefore, efforts are devoted to developing curcumin formulations with greater bioavailability and systemic tissue distribution. In this study, curcumin was enhanced in bioavailability and stability by utilizing a novel nanomicelle formulation (Hatamipour et al., 2019). We designed this study to evaluate the protective effects of nanocurcumin on the intestinal barrier integrity, apoptosis, and oxidative stress status in the rats exposed to traffic noise.

Methods

Animals

Forty adult male Wistar rats weighing 200–250 g were used for the experiment. Experimental Animal Breeding Center of Tehran University of Medical Sciences provided rats. Rats were housed in a temperature-controlled room maintained at 22 ± 5 °C and relative humidity of 50% with a standard 12 h light/dark cycle and free access to food and water. The Ethical Committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1399.865) approved this research.

Experimental groups

Rats were divided into four groups (n = 10/group): two traffic noise-exposed groups that received either saline (NOISE) or nanocurcumin (NCUR + NOISE) and two control groups that either received saline (CON) or nanocurcumin (NCUR). The timeline of the research is shown in Figure 1.

Figure 1. Timeline of the experiments. Numbers represent days. CON: control; NCUR: nanocurcumin; NOISE: noise stress.

Noise exposure protocol

We recorded traffic noise using a standard recorder (Panasonic, RQ-L11, Japan) on a high-traffic square in Tehran, the capital city of Iran, and amplified by Sonar software (Cakewalk Inc., USA) to the level of 95 dB, which is comparable to the level of noise detected in some industrial workplaces. Rats were exposed to the noise in a metal-reflective chamber [60 cm (L) × 60 cm (w) × 90 cm (h)] equipped with two loudspeakers installed at the upper right and upper left corners. The sound intensity in the cage was consistently tracked by a precise sound level meter (Extech Instruments, USA). Rats were exposed to noise for 2 hours a day for two weeks (Alinaghipour, Salami, et al., 2022; Alinaghipour, Ashabi, et al., 2022).

Nanocurcumin administration

Nanocurcumin is a registered curcumin product (SinaCurcumin®, IRC: 1228225765; Exir Nano Sina, Tehran, Iran) in the form of capsules. Each capsule of nanocurcumin contained 40 mg curcumin in the form of nanomicelle dissolved in saline that was injected intraperitoneally (15 mg/kg) 30 min before the noise exposure. An equal volume of vehicles was injected in the CON and NOISE groups.

Western blotting

At the end of the study, the animals were deeply anesthetized using urethane (1.5 mg/Kg/ip) and duodenum samples were collected. Protein expression of Bax, Bcl2, occludin, and ZO-1 in the intestinal tissue was investigated by western blotting. Briefly, duodenum samples of different treatment groups homogenized in 1 mL of lysis buffer containing 50 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM PMSF, 0.5 mM Na3VO4.12H2O, 50 mM NaF and 1 mM benzamidine. The samples were centrifuged at 12,500 g for 15 min at 4 °C and the supernatant was separated (Zhou et al., 2010). The protein concentration in the supernatants was measured using Bradford’s method (Bradford, 1976). Thirty μg of lysate protein were loaded on SDS-12.5% polyacrylamide electrophoresis gel and then transferred to PVDF membrane (Chemicon Millipore Co. Temecula, USA). Blots were blocked in a 3% Electrochemiluminescence (ECL) advanced blocking reagent kit (Amersham Bioscience Co. Piscataway, USA) and incubated with primary antibodies (1/1000, Cell Signaling Technology Co. New York, USA) for 18 h. Blots were then incubated with secondary antibody (1/3000, Cell Signaling Technology Co. New York, USA) for 1 h and then detected by
chemiluminescence reagent kit (Amersham Bioscience Co. Piscataway, USA). Blots were stripped in stripping buffer (pH = 6.7) and then probed with anti-β-actin or GAPDH antibody (1/1000, Cell Signaling Technology Co. New York, USA). The picture of the whole membrane for western blot is provided in the supplementary material.

Biochemical analysis

Blood collection

At the end of the study, blood samples were collected from the left ventricle under anesthesia in two tubes to separate serum and plasma. D-lactate was measured in plasma and other factors in serum. For serum collection, the blood samples were centrifuged at 2000 rpm for 10 min and the supernatant was carefully removed and kept at −20°C until biochemical analysis. For plasma separation, blood samples were poured into tubes containing anti-coagulation and centrifuged at 2500 rpm for 7 min.

D-lactate

The concentration of plasma D-lactate was measured using a commercially available assay kit (KA0869, Abnova, Taiwan), as a circulating marker of damage to the intestinal mucosa.

Corticosterone

The serum concentration of corticosterone was measured using a commercially available corticosterone enzyme-linked immunosorbent assay (ELISA) kit (Zellbio, Germany) according to the manufacturer’s instructions.

SOD enzyme activity assay

To assess SOD activity, we used Nasdox kit (Navand Salamat, Iran) that is based on the inhibition of Pyrogallol autoxidation by SOD activity. In the presence of SOD, pyrogallol autoxidation is inhibited so that the activity of the enzyme can be measured indirectly. The assay system contained pentenic acid, catalase, and Tris-Cacodylate buffer at pH = 8.5.

GSH

The serum GSH content reacts with 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) reagent to form a compound that is absorbed in 412 nm. A total of 100 μL of serum was added to 500 μL of sodium phosphate buffer with pH = 8; next, 100 μL of DTNB was added. Optical densities were measured at 412 nm (blank) and the concentrations were calculated based on the standard samples provided by the manufacturer (Navand Salamat, Iran).

TAC

The TAC level of serum was determined by ferric ions reducing antioxidant power (FRAP) method according to TAC commercial kit (Navand Salamat, Iran). In this method, due to the presence of antioxidants, a reduction of ferric to ferrous ions at low pH occurs and a colored ferrous-tripyridyltriazine complex is formed which can be measured spectrophotometrically at 593 nm.

NO

The serum nitrite levels were measured as a proxy of NO using the Griess method (Navand Salamat, Iran). Using the protocol described by the manufacturer, all proteins were precipitated from the supernatants and aliquots of the remaining proteinless supernatant were reacted with the same volume of Griess reagent. After a 10 min incubation period, nitrite concentration was quantified spectrophotometrically at 570 nm (blank) concerning a standard curve plotted based on known-concentration standards provided by the manufacturer.

Statistical analysis

The normality of data was assessed using the Shapiro-Wilk test. Data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Statistical significance was set at p < .05.

Results

Bax/Bcl-2 protein expression

Two-way ANOVA showed a non-significant effect of the treatment (F(1,10) = 3.61, p > .05), a significant effect of noise (F(1,10) = 9.40, p < .05), and a significant effect of treatment and noise interaction (F(1,10) = 19.21, p < .01) on the Bax/Bcl-2 ratio in the duodenum samples among the different groups (Figure 2). The post hoc analysis revealed that the Bax/Bcl-2 ratio was higher in the NOISE group than in the CON group (p < .01). Bax/Bcl-2 ratio was significantly decreased in the NCUR + NOISE group compared to the NOISE group (p < .01). There was no significant difference between the NCUR and CON groups.

Tight junction protein expression

The relative protein expression of occludin and ZO-1 in the duodenum samples was determined using the band intensity ratio of these proteins to GAPDH band intensity in the same samples (Figure 3). Two-way ANOVA revealed a significant effect of treatment (F(1,8) = 15.36, p < .01), noise (F(1,8) = 83.63, p < .001), and treatment and noise interaction (F(1,8) = 19.76, p < .001) on the occludin protein expression in the duodenum samples among groups (Figure 3(A)). The post-test analysis revealed that occludin protein was lower in the NOISE compared to CON animals (p < .001). There was no significant difference in occludin protein expression between the NCUR + NOISE and NOISE groups.

Two-way ANOVA also revealed a significant effect of treatment (F(1,8) = 20.79, p < .01), noise (F(1,8) = 135.80, p < .001),
plasma concentration of D-lactate

Plasma D-lactate concentration was assessed as an index of intestinal permeability. Two-way ANOVA showed a non-significant effect of treatment ($F(1,40)=0.59, p>.05$), a significant effect of noise ($F(1,40)=8.10, p<.01$), and a non-significant effect of treatment and noise interaction ($F(1,40)=3.55, p>.05$) on the plasma concentration of D-lactate among the different groups (Figure 4). The post hoc analysis indicated that the noise exposure increased the D-lactate level in the NOISE compared to the CON group ($p<.01$).

**Plasma concentration of D-lactate**

Plasma D-lactate concentration was assessed as an index of intestinal permeability. Two-way ANOVA showed a non-significant effect of treatment ($F(1,40)=0.59, p>.05$), a significant effect of noise ($F(1,40)=8.10, p<.01$), and a non-significant effect of treatment and noise interaction ($F(1,40)=3.55, p>.05$) on the plasma concentration of D-lactate among the different groups (Figure 4). The post hoc analysis indicated that the noise exposure increased the D-lactate level in the NOISE compared to the CON group ($p<.01$).
Nanocurcumin did not change the concentration of D-lactate in the NCUR+NOISE animals compared to their NOISE counterpart. The CON and NCUR groups resembled intestinal permeability characteristics.

**Serum level of corticosterone**

Two-way ANOVA revealed a significant effect of treatment (F(1,16) = 2.26, p < .01), noise (F(1,16) = 26.35, p < .001), and a non-significant effect of treatment and noise interaction (F(1,16) = 0.35, p > .05) on the serum level of corticosterone among the different groups (Figure 5). The post hoc analysis showed that serum corticosterone level in the NOISE animals was significantly higher than in their CON counterparts (p < .01). Nanocurcumin treatment decreased the stress hormone levels in the NCUR + NOISE animals compared to the NOISE group (p < .05).

**SOD enzyme activity assay**

Two-way ANOVA revealed a non-significant effect of treatment (F(1,20) = 0.01, p > .05), a significant effect of noise (F(1,20) = 5.86, p < .05), and a significant effect of treatment and noise interaction (F(1,20) = 19.03, p < .001) on the serum level of SOD among the different groups (Figure 6(A)). The post hoc analysis showed that the SOD level in the NOISE animals was significantly lower than in their CON counterpart (p < .01). Nanocurcumin treatment increased the levels of SOD in the NCUR + NOISE animals compared to the NOISE group (p < .05).

**Serum level of GSH**

Two-way ANOVA revealed a significant effect of treatment (F(1,20) = 4.82, p < .05), noise (F(1,20) = 9.08, p < .01), and a non-significant effect of treatment and noise interaction (F(1,20) = 3.88, p > .05) on the serum level of GSH among the different groups (Figure 6(B)). The post hoc analysis showed that the GSH level was remarkably lower in the NOISE than in the CON group (p < .05). The GSH content was remarkably increased in the NCUR + NOISE compared to the NOISE animals (p < .05). There was no significant difference in the serum level of GSH between the NCUR + NOISE and CON groups (p > .05). The serum level of GSH was almost similar in the CON and NCUR rats.

**Serum level of TAC**

Two-way ANOVA revealed a non-significant effect of treatment (F(1,20) = 4.32, p > .05), noise (F(1,20) = 2.21, p > .05), and a significant effect of treatment and noise interaction (F(1,20) = 8.10, p < .01) on the serum level of TAC among the different groups (Figure 6(C)). The post-test analysis showed a lower concentration of TAC in the NOISE group than its CON counterpart (p < .05). Nanocurcumin treatment increased TAC in the NCUR + NOISE compared to the NOISE group (p < .05). No noticeable variation was evident between the measured TAC in the CON and NCUR + NOISE groups.

**Serum level of NO**

Two-way ANOVA revealed a non-significant effect of treatment (F(1,20) = 1.62, p > .05), noise (F(1,20) = 4.14, p > .05),

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*Figure 4. The plasma concentration of D-lactate in the experimental groups. **p < .01 is compared to the CON group. Data were analyzed using two-way ANOVA followed by Tukey's post hoc test. The values are represented as mean ± SEM (n = 10). CON: control; NCUR: nanocurcumin; NOISE: noise stress.*

*Figure 5. The serum corticosterone level in the experimental groups. †p < .05 and #p < .05 are compared to the CON and NOISE groups, respectively. Data were analyzed using a two-way ANOVA followed by Tukey's post hoc test. The values are represented as mean ± SEM (n = 5). CON: control; NCUR: nanocurcumin; NOISE: noise stress.*
and a significant effect of treatment and noise interaction ($F(1,20) = 7.50$, $p < .05$) on the serum level of NO among the different groups (Figure 6(D)). The post hoc analysis showed that the NO level in the NOISE and NCUR animals were significantly lower than in the CON group ($p < .05$).

**Discussion**

In this study, we evaluated the effect of noise stress on intestinal barrier integrity and apoptosis and that how much these phenomena respond to nanocurcumin treatment. The relevance of the oxidative/antioxidative factors with pathological alterations was also considered. Our findings showed that noise stress increased oxidative stress, D-lactate levels, and apoptosis and declined the expression of tight junction proteins in the duodenum. The nanocurcumin administration restored the detrimental effects of the noise stress on the oxidative stress status and apoptosis but displayed no significant effect on the intestinal barrier integrity.

**Nanocurcumin treatment ameliorates pro-apoptotic effects of noise exposure**

We found that Bax/Bcl-2 ratio was increased in the stressed animals, while the nanocurcumin treatment decreased the ratio. Whereas the Bax protein causes the release of cytochrome C and the apoptosis-inducing factor and ultimately leads to apoptosis, Bcl-2 protein inhibits cytochrome C and apoptosis-inducing factor to inhibit apoptosis (Li et al., 2013). Regulation of apoptosis depends not only on the expression level of Bcl-2 and Bax but also on their ratio. The increased ratio of Bax/Bcl-2 is an index of increased probability of apoptosis (Li et al., 2013). Consistent with our findings, Filho et al. showed that treatment with curcuminoids from *Curcuma longa L.* induces a slightly greater increase in the expression of Bcl-2 than in Bax in the epithelium of villi and crypts in mice intestine samples (Dos Santos Filho et al., 2016). Also, Qi et al. reported that curcumin pretreatment effectively protects trophoblast cells against oxidative stress-induced apoptosis by increasing the Bcl-2/Bax ratio (Qi et al., 2020). Accordingly, Xiang et al. reported that curcumin ameliorates copper-induced neurotoxicity by down-regulating Bax/Bcl-2 ratio (Xiang et al., 2021). Curcumin prevents apoptosis in intestinal epithelial cells and, thus, plays a role in relieving intestinal damage (Yucel et al., 2011). Our results demonstrated that the noise stress decreased the antioxidative factors SOD, GSH, and TAC. This, in turn, favors oxidative stress, which has been shown to induce apoptosis (Yang et al., 2011).
On the other hand, the nanocurcumin treatment increased the level of SOD, GSH, and TAC in the noise-exposed animals. One of the other possible mechanisms may be the increased release of corticosterone in noise-exposed rats (Zhao et al., 2021). Therefore, a possible mechanism by which nanocurcumin reduces apoptosis may be due to its modulatory effects on the HPA axis and oxidative stress. We found that both the noise exposure and nanocurcumin administration reduced the NO level in the animals. NO, as a free radical, appears to be a potential antioxidant. It participates in the termination of lipid peroxidation reactions. It can also be an oxidant, especially in indirect reactions with oxygen molecules or superoxide anions (Kowalczyk et al., 2005). Therefore, the precise determination of the role of nitric oxide in apoptosis and the effects of stress and nanocurcumin on its level need more investigations.

Although increased intestinal apoptosis is associated with the pathogenesis of gastrointestinal injury, little is understood about its role in intestinal epithelial permeability (Williams et al., 2015). A link between apoptosis and changes in epithelial permeability has been highlighted by recent studies in which enterocyte apoptosis was induced by drugs, immune factors, or microbes (Buret & Bhargava, 2014; Bischoff et al., 2014; Kapczuk et al., 2020; Zeng et al., 2015; Ahmad et al., 2017).

**Intestinal barrier integrity was not responsive to nanocurcumin treatment**

Accumulating data emphasizes the role of permeability of the intestinal barrier in health and disease. Intestinal barrier dysfunction causes the passage of noxious molecules, resulting in the excessive activation of mucosal immune cells and inflammation (Vancamelbeke & Vermeire, 2017). The present study showed that noise exposure declined the tight junction protein expression in the duodenum, demonstrating a deficiency in intestinal barrier function. In this regard, Bijlsma et al. reported that exposure to a 95 dB noise induces a two-fold increase in small intestinal permeability (Bijlsma et al., 2001). Also, Chi et al. confirmed that environmental noise stress diminishes tight junction protein expression in the intestine and hippocampus (Chi et al., 2021). The other types of stress are also reported to reduce tight junction protein expression. For instance, restraint stress induces intestinal mucosal injury by decreasing the expression of tight junction proteins ZO-1 and occludin (Lin et al., 2020). Loss of the occludin and ZO-1 proteins or reassembly of these proteins could lead to barrier dysfunction (Guo et al., 2019; Dodiya et al., 2020). There is ample evidence indicating that stress may damage intestinal barrier function, mainly through the systemic and peripheral release of corticotropin-releasing factors (Rodino-Janeiro et al., 2015; Ait-Belgnaoui et al., 2012; Vanuytsel et al., 2014). Moreover, noise stress-induced oxidative stress may disrupt the intestinal barrier integrity. Our results show no significant effect of nanocurcumin on the expression level of tight junction proteins. Consistently, Yan et al. demonstrated that curcumin could not up-regulate tight junction ZO-1 protein in pigs following intestinal damage (Yan et al., 2019). However, some findings suggest that curcumin protects intestinal epithelial cells against disruption of tight junction and barrier dysfunction in hydrogen peroxide-induced epithelial barrier disruption (Wang et al., 2012), experimental colitis (Ohno et al., 2017), and intestinal ischemia-reperfusion injury (Tian et al., 2016).

Plasma D-lactate, as the end product of intestinal bacteria, has been proposed as a circulating marker to assess the extent of damage and repair of the intestinal mucosa (Fukudome et al., 2014). When the intestinal mucosa is damaged, almost all D-lactate is released into the blood due to D-lactate dehydrogenase deficiency in mammals. Therefore, D-lactate in peripheral blood can indicate damage to the intestinal barrier (Ruh et al., 2000). Our results showed that noise exposure increased the plasma D-lactate concentration. We found that nanocurcumin treatment did not reduce plasma D-lactate concentration in the stressed animals. Contrary to our finding, Xun et al. reported that curcumin reduces plasma D-lactate levels and protects the intestinal mucosal barrier function in weaned piglets challenged with enterotoxigenic Escherichia coli (Xun et al., 2015). We found that nanocurcumin reduced oxidative stress and corticosterone levels in noise-exposed rats while failing to improve intestinal integrity. Presumably, other mechanisms may be involved in altering intestinal permeability. One propose might be that nanocurcumin probably does not affect intestinal permeability in the animals exposed to noise. Although our findings are not consistent with some other research (Wang et al., 2012; Ohno et al., 2017; Tian et al., 2016), where the changes in relative protein expression of intestinal tight junctions were consistent with the plasma D-lactate level in our study. Moreover, discrepancies observed between our findings and others may be due to the different stress paradigms employed.

**Conclusions**

Altogether, the noise stress activates the HPA axis, increases oxidative stress, and induces intestinal barrier permeability and apoptosis in the duodenum. Nanocurcumin treatment ameliorates the pro-apoptotic effects of noise exposure with no significant effect on the intestinal barrier integrity. The effects of nanocurcumin might be mediated by its modulatory effects on the HPA axis and its antioxidant properties. However, further investigation is required to answer the questions raised about the probable mechanisms involved.

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**Disclosure statement**

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