

*Osmangazi Journal of Medicine**e-ISSN: 2587-1579***Nesfatin-1: Does it Have Therapeutic Potential for Cyclophosphamide-Induced Interstitial Cystitis?**

Nesfatin-1: Siklofosfamid kaynaklı interstisyel sistit için terapötik potansiyeli var mı?

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Abstract: Bladder pain syndrome/interstitial cystitis (BPS/IC) is a clinical condition characterized by symptoms such as frequent urination, suprapubic or pelvic pain, and decreased urine output. Inflammation is a significant contributor to the pathophysiology of this disease. Recent research has also highlighted the potent anti-apoptotic and antioxidant properties of nesfatin-1(NES) which is a hypothalamic neuropeptide. This study aimed to investigate the anti-inflammatory, anti-apoptotic, and antioxidant effects of NES in a cyclophosphamide (CYP)-induced IC model in rats. In this study female Sprague Dawley rats (n=32) were randomly assigned to four groups: control, CYP, CYP+NES 5 µg/kg, and CYP+NES 10 µg/kg. BPS/IC was induced by administering a single intraperitoneal (i.p.) dose of 70 mg/kg of CYP every three days for 12 days. The effects of NES were assessed using behavioral tests, histological examination, histopathological analyses, and biochemical analyses of bladder tissue. NES at a dose of 5 µg/kg and 10 µg/kg significantly reduced bladder pain as measured by the von Frey test. It showed partially protective effects against CYP-induced BPS/IC as evidenced by reduced histological and histopathological damage to the bladder, by way of suppression of oxidative stress markers and a decrease in inflammatory burden. Notably, the 5 µg/kg dosage of NES proved to be more effective than the 10 µg/kg dosage. In summary, these preclinical findings suggest that NES holds promise as a therapeutic agent for the treatment of CYP-induced BPS/IC. Nonetheless, further preclinical and clinical investigations are warranted to substantiate its efficacy and safety.

Keywords: Bladder pain syndrome, Cyclophosphamide, Interstitial cystitis, Nesfatin-1, Rat.

Özet: Mesane ağrısı sendromu/interstisyel sistit (BPS/IC), sık idrara çıkma, suprapubik veya pelvik ağrı gibi belirtilerle karakterize edilen bir klinik durumdur. Enflamasyon, bu hastalığın patofizyolojisinde önemli bir rol oynar. Son zamanlarda yapılan araştırmalar, hipotalamik bir nöropeptid olan nesfatin-1 (NES)'in güçlü anti-apoptotik ve antioksidan özelliklerini de ortaya koymuştur. Bu çalışma, sıçanlarda siklofosfamid (CYP) ile indüklenen IC modelinde NES'in anti-enflamatuar, anti-apoptotik ve antioksidan etkilerini araştırmayı amaçlamıştır. Çalışmada dişi Sprague Dawley sıçanları (n=32) rastgele dört gruba ayrılmıştır: kontrol, CYP, CYP+NES 5 µg/kg ve CYP+NES 10 µg/kg. BPS/IC, 12 gün boyunca her üç günde bir 70 mg/kg CYP'nin tek bir intraperitoneal (i.p.) dozunun uygulanmasıyla indüklendi. NES'in etkileri, davranış testleri, histolojik inceleme, histopatolojik analizler ve mesane dokusunun biyokimyasal analizleri kullanılarak değerlendirildi. 5 µg/kg ve 10 µg/kg dozlarındaki NES, von Frey testi ile ölçülen mesane ağrısını önemli ölçüde azalttı. Mesanede histolojik ve histopatolojik hasarın azalması, oksidatif stres belirteçlerinin baskılanması ve inflamatuvar yükün azalmasıyla kanıtlandığı üzere, CYP kaynaklı BPS/IC'ye karşı kısmen koruyucu etkiler gösterdi. Özellikle 5 µg/kg'lık NES dozunun, 10 µg/kg'lık dozdan daha etkili olduğu ortaya çıktı. Özetle, prelinik bulgular, nesfatin-1'in CYP kaynaklı BPS/IC tedavisi için umut vaat eden bir terapötik ajan olduğunu göstermektedir. Bununla birlikte, etkinliğini ve güvenliğini doğrulamak için daha fazla prelinik ve klinik araştırmaların yapılması gerekmektedir.

Anahtar Kelimeler: İnterstisyel sistit, Mesane ağrısı sendromu, Nesfatin-1, Sıçan, Siklofosfamid.

Ethics Committee Approval: The study was approved by Local Ethics Committee for Animal Experiments of Kutahya Health Sciences University. Approval Number: 2022-04-01; Approval Date: 21.04.2022

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1. Introduction

Bladder pain syndrome/interstitial cystitis (BPS/IC) is a chronic condition that reduces quality of life, characterised by urinary urgency, increased frequency of urination, and a sensation of discomfort in the suprapubic or pelvic region. Epidemiological data indicate that nearly 80% of affected individuals are women (1, 2). This condition can severely impair quality of life. Proposed contributing factors include autoimmune mechanisms, infectious agents, epithelial barrier disruption, exposure to toxic substances, and mast cell infiltration (3,4,5). Nevertheless, the precise etiology and underlying pathophysiological mechanisms of BPS/IC have not yet been fully elucidated (6). Moreover, no highly effective therapeutic strategy currently exists for the majority of patients. Ongoing research primarily focuses on clarifying disease mechanisms and identifying novel, safe, and effective treatment approaches.

Cyclophosphamide (CYP) is a chemotherapeutic agent frequently used in the treatment of various malignant tumours. However, IC is a common side effect associated with treatment with this agent (7). This complication results from the formation of acrolein, a urotoxic metabolite produced during CYP metabolism. For this reason, CYP is commonly employed in preclinical studies to induce experimental IC models (8,9). Animals exposed to this agent exhibit increased micturition frequency and heightened bladder sensitivity, which are hallmark features of BPS/IC. In addition, CYP administration leads to bladder inflammation and tissue damage that closely resembles the pathological findings observed in patients with BPS/IC (10). CYP also induces abdominal mechanical hypersensitivity and bladder-associated pain. Recent studies have highlighted the role of intracellular inflammatory signalling pathways in the pathogenesis of IC, particularly those mediated by mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NF- κ B). NF- κ B acts as a key transcription factor that regulates the expression of numerous pro-inflammatory mediators, thereby contributing to the initiation and amplification of inflammatory responses within the bladder (11). Various stimuli, such as inflammatory cytokines and cellular stress, can activate and phosphorylate both the MAPK and NF- κ B pathways. The activation of these pathways leads to an increase in the expression of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α), and triggers the infection (12). Gene expression studies have shown that the MAPK/NF- κ B signalling pathway plays a role in

both the onset and progression of IC (12). These findings highlight the critical role of inflammatory processes and demonstrate that they represent a promising therapeutic target for IC.

NES is a bioactive peptide comprising 82 amino acids and has been identified as a cleavage product of the precursor protein nucleobindin-2 (NUCB2) (13). Beyond its initial identification, growing experimental evidence revealed that NES exerts broad cytoprotective effects, encompassing anti-inflammatory, antioxidant, and anti-apoptotic properties (14). It has been reported that it exerts a protective effect by regulating oxidative stress, inflammation and apoptosis triggered by reactive oxygen species (ROS) (14). It is thought that these effects are achieved through the enhancement of antioxidant defence mechanisms such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), and a reduction in the levels of lactate dehydrogenase, malondialdehyde (MDA) and myeloperoxidase (MPO). In addition to its antioxidant capacity, NES suppresses inflammatory cell recruitment by limiting neutrophil infiltration, reducing Iba-1-positive immune cell activation and preventing astrocytic activation (14). It is thought to modulate several inflammation-related pathways such as NF- κ B signaling pathways. Its anti-apoptotic effects are thought to involve preservation of mitochondrial membrane potential and modulation of caspase-dependent apoptotic signaling, reflected by increased Bcl-2/Bax ratio. Although NES was shown to exert anti-inflammatory, anti-apoptotic, and antioxidant effects in a variety of pathological conditions, its potential role in the treatment of BPS/IC has not yet been investigated. This gap in the literature is particularly noteworthy given the substantial burden of BPS/IC on quality of life, especially among female patients. NES could represent a promising therapeutic agent for IC, particularly in female patients. Building on these findings, our study aims to investigate the anti-inflammatory, anti-apoptotic, and antioxidant properties of NES in a CYP-induced BPS/IC rat model.

2. Materials and Methods

The study was conducted with the approval of the Local Ethics Committee for Animal Experiments at Kütahya Health Sciences University (Ethics approval number: 2022-04-01).

In this study, 32 female Sprague-Dawley rats (240–280 g) were used. The animals were housed under standardised environmental conditions, with a room

temperature of 24 ± 2 °C, relative humidity of 55 ± 15 per cent, and a 12-hour light/12-hour dark cycle. The animals were provided with standard laboratory feed and tap water ad libitum.

Following the acclimatisation period, the rats were divided into four random groups ($n = 8$).

Control group: Rats received 0.2 mL of saline (SF) via intraperitoneal (i.p.) injection as a single dose every three days for a total duration of 12 days.

CYP group: Rats were administered CYP at a dose of 70 mg/kg (500 mg Endoxan® inj. sol) by intraperitoneal injection at three-day intervals over a 12-day period (15).

N5 group: Rats received CYP in combination with NES at a dose of 5 µg/kg (Eurogentec/USA), administered i.p. once daily for 12 days (14).

N10 group: Rats received CYP in combination with NES at a dose of 10 µg/kg, administered i.p. once daily for 12 days (14).

Control animals were administered 0.2 mL of saline solution (SF) via i.p injection. In the CYP group, animals received saline intraperitoneally for the first three days, followed by a single i.p injection of cyclophosphamide (70 mg/kg) on day four (15).

This experimental timeline was completed on the day twelve.

In the treatment groups, NES was given at doses of 5 and 10 µg/kg following the same protocol (14). No animals were lost in the CYP groups.

All pharmacological interventions were performed at 09:00 AM. Cyclophosphamide injections were administered 30 minutes after NES or saline treatment. Pain-related behavioral assessments were conducted prior to treatment initiation and subsequently on the third day following each drug administration.

Throughout the 12-day experimental period, pain behavior was evaluated on four separate occasions. Twenty-four hours after completion of the treatment protocol, animals were weighed and anesthetized using ketamine (90 mg/kg, i.p.) in combination with xylazine (10 mg/kg, i.p.). Euthanasia was performed via intracardiac blood collection.

Following euthanasia, the bladders were removed and weighed. Each bladder was divided into two parts: one part for histopathological assessment, whilst the remaining part was immediately stored at -80 °C for biochemical analyses. The general experimental workflow is summarised in Figure 1.

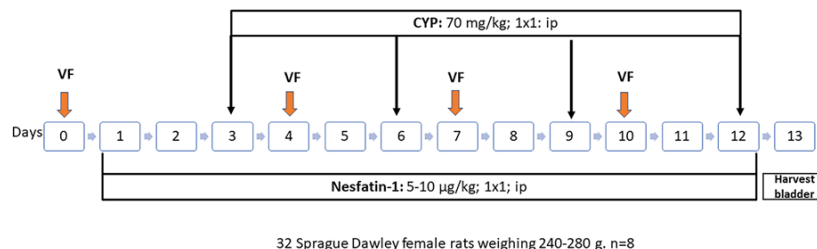


Figure 1. Experimental protocol, VF: Von frey

Von Frey Test

Mechanical sensitivity in the suprapubic region was assessed using the von Frey filament test, which is widely accepted for the evaluation of mechanical allodynia and withdrawal thresholds. Rats were individually placed in two-compartment cages equipped with wire mesh floors. Animals were allowed a 15-minute habituation period prior to testing. A single rigid von Frey filament was applied

perpendicularly to the suprapubic area, with pressure gradually increased from 0 g to 20 g over a 10-second interval. Behavioral responses, including licking or scratching of the stimulated area, rearing, or sudden movement, were considered positive responses indicative of sensitivity (16). The applied force was automatically recorded to ensure the response of the rat. The minimum value at which

animals showed a behavioral response was set as the threshold. Two days before the test, each rat was habituated the plastic compartments to prevent the animals from becoming stressed during the experiment.

Histopathological Evaluation:

Immediately after excision, bladder tissues were fixed in 10% neutral buffered formalin. Routine histological procedures were performed to process the tissues, which were then embedded in paraffin blocks. Thin sections were cut from these blocks and stained with hematoxylin and eosin (H&E). Apoptotic activity was assessed using caspase-3 immunostaining, while antioxidant immunoreactivity was evaluated using staining for SOD, glutathione peroxidase (GPX), and CAT. Acrolein presence within the bladder wall was assessed via specific acrolein staining. The histopathological scoring of the bladder was performed according to the Gray criteria (17), where grade 0 represents normal epithelium, grade 1 indicates moderate submucosal edema, moderate hemorrhage, and limited ulceration, and grade 2 reflects severe cell infiltration, fibrin deposition, pronounced hemorrhage, and extensive ulceration (17).

For immunohistochemical analyses of GPX, SOD, CAT, and caspase-3, three randomly selected microscopic fields were examined at 40× magnification per section. Immunopositive cells were quantified and expressed as percentages.

Biochemical Evaluation:

Investigations of bladder homogenates:

Bladder Preparation:

Bladder tissues were homogenized in a solution consisting of 1 mL of 5% Triton X-100 and 1 mL of phosphate-buffered saline (PBS) at a 1:1 ratio. The homogenates were centrifuged at 1000 g for 10 minutes, and the resulting supernatants were collected for biochemical analysis.

Measurements

The levels of IL-1 β , IL-6, and NF- κ B were quantified using commercially available assay kits, following the manufacturers' protocols. Protein concentrations in bladder homogenates were determined using a Maestrogen Maestronano spectrophotometer.

Statistical Analysis

Statistical analyses were performed using SPSS version 21.0. Data obtained from pain assessments were analysed using two-way repeated-measures analysis of variance (ANOVA). The Tukey post-hoc test was applied for multiple comparisons. Biochemical parameters were analysed using one-way ANOVA, and the Dunnett post-hoc test was applied for comparisons. Histopathological scores were evaluated using the Kruskal–Wallis test, followed by Dunn's multiple comparison test. All results are expressed as mean $\pm$ standard error of the mean (SEM), and $P < 0.05$ was considered statistically significant.

3.Results

Von Frey Test

Assessment of mechanical sensitivity using the von Frey test revealed a progressive decrease in pain threshold in the CYP-treated group, which became evident from day 8 and reached statistical significance on the day 12 when compared with days 4 and 8 ($P < .05$). These findings confirmed the successful induction of cyclophosphamide-induced cystitis.

In contrast, significant increases in pain thresholds were observed in both the N5 and N10 treatment groups on the day 8 ($P < .001$), and this improvement persisted through day 12 ($P < .001$). When treatment groups were compared, the N10 group exhibited a lower pain threshold than the N5 group ($P < .05$) (Figure 2).

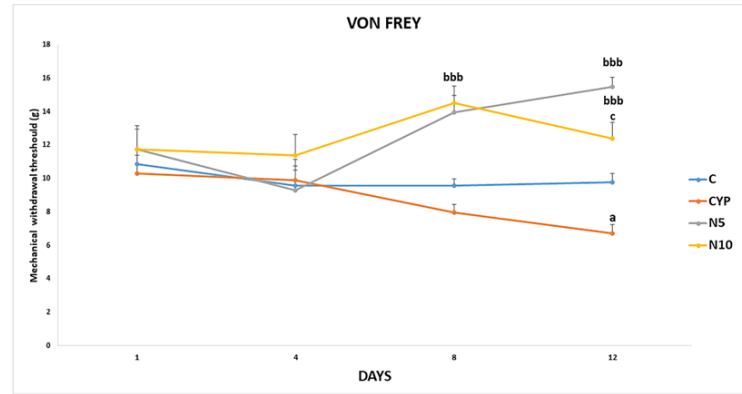


Figure 2. The effect of NES on CYP-induced bladder pain on Von Frey test.

C, control; CYP, cyclophosphamide; N5, NES 5 µg/kg; N10, NES 10 µg/kg. Data are given as mean ± SEM. (a): Different from Day 1, ^a $P < .05$; (b): Different from Day 2, ^{bbb} $P < .001$; (c): Different from N5, ^c $P < .05$.

Histopathological Examinations

Compared with the control group, H&E-stained bladder sections from the CYP group demonstrated pronounced mucosal hyperemia, edema, bladder wall thickening, and inflammatory cell infiltration ($P < .001$). These pathological alterations were not observed in the N5 group, while mild histopathological changes were detected in the N10

group ($P < .01$). Acrolein staining revealed no positive staining in the control group, whereas intense acrolein-positive areas were evident in the CYP group. Among the treatment groups, the lowest degree of acrolein staining was observed in the N5 group (Figure 3A-B; Table 1).

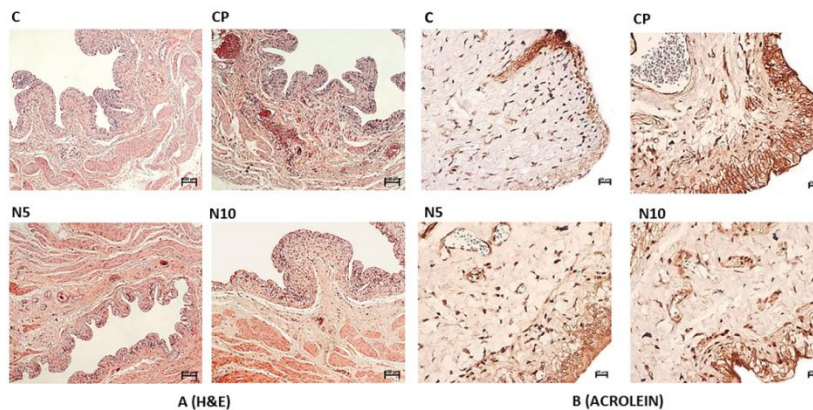


Figure 3. The effect of NES on CYP-induced IC in H&E and acrolein staining. $\times 40$

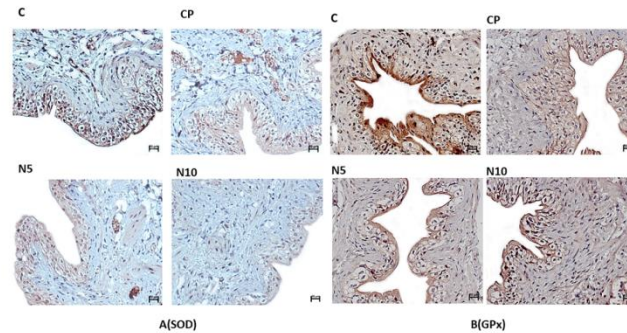
C, control; CYP, cyclophosphamide; N5, NES 5 µg/kg; N10, NES 10 µg/kg.

Table 1. The effect of NES on CYP-Induced IC in Histological and Immunohistochemical Parameters.

n:8	Histological Score	SOD (positive cell count)	GPX (positive cell count)	CAT (positive cell count)	Caspase Immunoreactivity	3
Control	0.1250 ± 0.12	48.83 ± 0.57	32.46 ± 1.21	53.68 ± 1.71	23.8 ± 1.55	
CYP	1.75 ± 0.16 ^{aaa}	28.09 ± 0.46 ^{aaa}	15.56 ± 0.83 ^{aaa}	32.21 ± 0.89 ^{aaa}	62.11 ± 1.06 ^{aaa}	
N5	1.13 ± 0.12	31.36 ± 0.40 ^{aaabb}	26.59 ± 1.01 ^{aabbb}	39.60 ± 0.94 ^{aaabb}	48.13 ± 1.11 ^{aaabbb}	
N10	1.38 ± 0.18 ^{aa}	40.78 ± 0.54 ^{aaabbbccc}	21.69 ± 0.45 ^{aaabbbcc}	49.78 ± 1.57 ^{bbbccc}	55.55 ± 0.91 ^{aaabbbcc}	

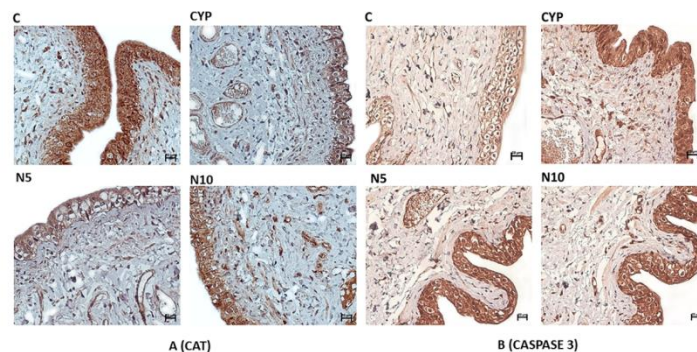
C, Control; CYP, Cyclophosphamide; N5, NES 5µg/kg; N10, NES 10 µg/kg. Data are given as mean SEM.
(a), different from control, ^{ad}P < .01; ^{aaa}P < .001. (b): Different from CYP, ^{bb}P < .01; ^{bbb}P < .001. (c): Different from N5, ^{cc}P < .01; ^{ccc}P < .001.

For SOD and GPX immunostaining, pathological features consistent with IC—such as urothelial desquamation, increased inflammatory cell infiltration within the lamina propria, and hemorrhage were observed in the CYP group ($P < .001$). SOD immunoreactivity was significantly higher in the N10 group compared with the N5 group ($P < .001$) (Figure 4 A-B; Table 1).

**Figure 4.** The effect of NES on CYP-induced IC in SOD and GPX staining. x40

C, control; CYP, cyclophosphamide; N5, NES 5µg/kg; N10, NES 10 µg/kg.

CAT immunoreactivity was significantly reduced in the CYP group compared with controls ($P < .001$). However, CAT immunoreactivity increased in the N5 and N10 treatment groups ($P < .01$) and the closest increase to control values was observed in the N10 group ($P < .001$) (Figure 5A; Table 1).

**Figure 5.** The effect of NES on CYP-induced IC in CAT and Caspase 3 staining x40.

C, control; CYP, cyclophosphamide; N5, NES 5µg/kg; N10, NES 10 µg/kg.

Caspase-3 immunostaining showed a significant increase in apoptotic cell counts in the CYP group ($P < .001$). In contrast, a marked reduction in apoptotic cells was observed in the N5 group compared with the CYP group ($P < .001$). Caspase-3-positive cell counts in the N10 group differed significantly from those in the N5 group ($P < .01$) (Figures 5B; Table 1).

Biochemical Examinations

IL-6 levels were significantly elevated in the CYP-treated group ($P < .05$). However, this increased level in the CYP group decreased in the N5 group compared to the CYP group ($P < .05$). When the N10 group was analyzed, there was a decrease compared to the CYP group ($P < .01$) (Figure 6A). For the mean values of IL-1 β and NF- κ B levels, there was an increase in the CYP group compared to the control group and a decrease in the N5 and N10 groups. However, there was no statistical significance between these values (Figure 6B-C).

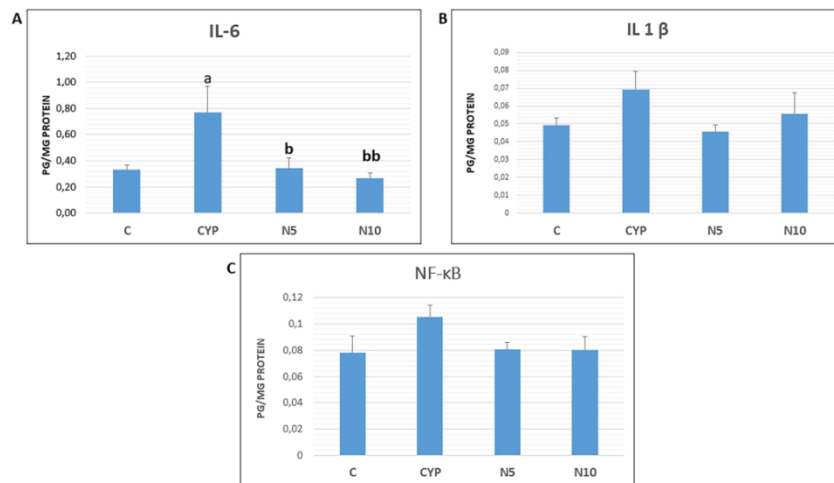


Figure 6. The effect of NES on CYP-induced IC in biochemical parameters.

C, control; CYP, cyclophosphamide; N5, NES 5 μ g/kg; N10, NES 10 μ g/kg. data are given as mean SEM.

(a): Different from control, ^a $P < .05$. (b): Different from CYP, ^b $P < .05$; ^{bb} $P < .01$.

4. Discussion

In this study, we hypothesized that NES could serve as a potential therapeutic agent for interstitial cystitis, a disorder that predominantly affects women. Accordingly, we aimed to examine how NES impacts bladder pain symptoms associated with IC induced by CYP in rats. We also aimed to compare its anti-inflammatory, anti-apoptotic, and antioxidant effects, as well as the impact of different dosing intervals on this condition. Behavioral assessment using the von Frey test demonstrated a significant decrease in mechanical withdrawal thresholds in the CYP-treated group on days 8 and 12, reflecting enhanced pain sensitivity and confirming successful establishment of the IC model. In contrast, treatment with NES led to a marked increase in pain thresholds, indicating an attenuation of bladder-associated hypersensitivity. These findings suggest that NES may exert an antinociceptive effect in CYP-induced IC.

NES was reported to modulate somatosensory processing through interaction with an as-yet-unidentified G protein-coupled receptor, influencing

nociceptive signaling via protein kinase C-dependent mechanisms in cultured rat sensory neurons (18). In clinical settings, reduced circulating NES levels were observed in female patients with fibromyalgia syndrome, suggesting a potential contribution of NES deficiency to disease-related pathophysiological alterations, although no direct association with pain severity was identified (19). Similarly, markedly decreased NES concentrations were reported in patients with acute myocardial infarction, where inflammation was emphasized to be a central pathological mechanism (20). In view of these findings, our preclinical study investigated the effect of NES on pain behavior in a CYP-induced BPS/IC rat model. Although our results suggest a potential effect, further research is needed to draw definitive conclusions regarding the therapeutic potential of NES.

Histopathological evaluation using H&E staining demonstrated prominent mucosal hyperemia, edema, bladder wall thickening, and inflammatory cell infiltration in the CYP-treated group, consistent with

cystitis-related tissue injury. Notably, these alterations were not observed in the N5 treatment group, suggesting a protective effect of NES on bladder morphology. Comparable histological improvements following NES administration were reported in a renal ischemia–reperfusion injury model, where NES attenuated tissue damage as evidenced by H&E staining (21).

Previous reports indicate that the side effects of CYP are attributed to its active metabolite, acrolein (22). Acrolein is excreted in urine (23,24), making the bladder the organ most exposed to this metabolite. CYP treatment frequently results in epithelial edema, ulceration, hemorrhage, and leukocyte infiltration in the bladder (25). In our acrolein staining analysis, we observed notably dark staining in the bladder tissue of the CYP group, whereas the N5 group exhibited minimal acrolein staining. This finding suggests that cystitis was induced in the CYP group. However, no previous study was found that investigates the effect of NES in the BPS/IC model.

In our study, treatment with CYP induced oxidative stress, consistent with previous research that has demonstrated the generation of ROS following CYP treatment, which leads to oxidative damage in bladder urothelium (26). Given its pro-oxidant properties, CYP contributes to oxidative stress by reducing antioxidant enzyme activity, thereby increasing lipid peroxidation in the body. The pro-inflammatory effects of CYP intoxication may also disrupt the overall redox balance in bladder tissues. Statistical analysis revealed a decrease in SOD levels in the CYP groups, while these levels were elevated in the treatment groups. In addition, we observed a reduction in GPX levels and an increase in CAT immunoreactivity in the treatment groups. NES was previously shown to inhibit oxidative stress (27). In addition, Jiang et al. (21) found similar results to our study in relation to SOD and CAT immunoreactivity. Musuroglu Keloğlu et al. (28) examined the effects of NES in an experimental epilepsy model and reported that NES increased GPx immunoreactivity. Furthermore, acrolein was reported to diminish GPX concentration (28).

Caspases are cysteine proteases that are crucial for protein cleavage. Caspase-3 is a key protein that plays a crucial role in both extracellular and intracellular apoptosis (29). Apoptosis is a critical process for normal cellular development, and its disruption is linked to the development of several diseases (30). Previous research reported that caspase-3, an apoptosis marker, is upregulated in experimental models of IC induced by CYP (2). In

this study, an increase in caspase-3 expression in the CYP groups was found, which is consistent with the literature. This expression decreased in the treatment groups. Although levels in the treatment groups were higher than those in the control group, they were significantly lower than those in the CYP group. According to our findings, NES did not fully restore caspase immunoreactivity. Nevertheless, these findings suggest that NES could lower caspase-3 expression and display anti-apoptotic effects. According to the literature, Jin et al. (31) reported that NES decreased caspase-3 immunoreactivity in rats with spinal cord injury. It was also reported in other studies that NES decreases caspase 3 immunoreactivity (32,33).

In our study, an increase in IL-6 levels was observed in the CYP group, while significant decreases were observed in N5 and N10 groups. The mean values for NF- κ B and IL-1 β increased in the CYP group, while the treatment groups exhibited values closer to the control group. No statistical significance was found for these markers. However, previous studies have shown that CYP increases the expression of inflammatory markers in the bladder and leads to histopathological changes, such as mucosal haemorrhage and oedema, in experimental IC models (26,34). The NF- κ B family of transcription factors is essential for regulation of the inflammatory response. NF- κ B typically forms both homo- and heterodimers in which the p50 and p65 subunits bind to the inhibitory protein I κ B α . Inflammatory conditions activate I κ B α kinase, leading to disruption of this complex through phosphorylation of NF- κ B (p65). NF- κ B is thought to contribute to the progression of IC. In a previous study, the effects of chlorogenic acid (CGA) on rats with CYP-induced BPS/IC were investigated, with a focus on the interaction between CGA and the MAPK/NF- κ B signalling pathway (2). Based on their findings, the researchers proposed that the MAPK/NF- κ B pathway plays a significant role in alleviating inflammation and apoptosis in CYP-induced BPS/IC. In our study, we could not demonstrate the interaction between NES and the MAPK/NF- κ B signaling pathway due to various limitations. Our research in this field is still ongoing. NES has not been previously studied in the BPS/IC model. However, in a study by Panditrao Lahane et al. (32) on the renoprotective effect of NES in adenine-induced chronic kidney injury, it was found that NES reduced the expression of inflammation markers including NF- κ B, IL-1 β and IL-6. As a result, NES has a potential effect in improving renal function in adenine-induced chronic kidney injury by preventing oxidative stress, alleviating inflammation markers, and inhibiting apoptosis and

fibrosis. Although there was no statistical significance for the NF- κ B and IL-1 β values in our study, it is thought that NES will reduce inflammation markers based on previous studies and our data.

We examined the dose-dependent effects of NES in the IC/BPS model. For this purpose, we used doses of 5 and 10 μ g/kg. When the results were evaluated, the 5 μ g/kg dose was more effective on pain behavior and antiapoptotic effects. However, both doses had similar effects in terms of anti-inflammatory and antioxidant effects. In a study conducted by Tang et al. (14) in 2012, the effects of NES on traumatic rat brain were examined and 3 different doses of 5-10-20 μ g/kg were tested in the study. The researchers found no effect at a dose of 5 μ g/kg given for 5 days. In our study, we used NES for 12 days. This period may be crucial in terms of the time it takes for low-dose NES to reach effective plasma concentration. A similar result to our results can be seen in a recent study by Panditrao Lahane et al. (32) The researchers reported that 5 μ g/kg NES given for 30 days had antioxidant, anti-apoptotic,

anti-inflammatory and antifibrotic effects on chronic kidney damage induced by adenine. However, the dose-response and time relationships of NES still need to be studied in detail.

5. Conclusion

NES is under ongoing investigation as a potential therapeutic agent for various diseases. Current findings about this molecule are mixed, with both supportive and inconclusive results reported in the literature. These diverse perspectives contribute to a more comprehensive evaluation of its therapeutic potential. Based on our findings, NES appears to offer partial protection against CYP-induced BPS/IC. However, as these results are derived from a preclinical model, further extensive preclinical and clinical studies are necessary to determine its suitability for therapeutic use.

Limitation

The fact that the MAPK/NF κ B pathway was not investigated is among the limitations of this study. We plan to investigate this pathway in future studies.

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