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RESEARCH

Modulatory effects of Hypericum perforatum extract on sciatic nerve injury-induced peripheral neuropathy: an experimental study on mice

Hypericum perforatum ekstresinin siyatik sinir hasarı ile indüklenen periferik nöropati üzerindeki düzenleyici etkisi: fareler üzerinde deneysel bir çalışma

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Abstract

Purpose: The effect of Hypericum perforatum (HP), which is a medicinal plant, on sciatic nerve injury-induced peripheral neuropathy has been less studied so far. The current experimental study aimed to investigate the neuroprotective and antinociceptive effects of Hypericum perforatum (HP) extract on sciatic nerve injury-induced peripheral neuropathy in mice.

Materials and Methods: In the present study, 18 Balb/C albino mice were allocated equally into three groups. The first group was determined as controls, and no procedure was performed on these mice. Neuropathy was generated by the partial sciatic nerve ligation method on mice allocated to the second and third groups. Mice in the third group received HP extract at a dose of 70 mg/kg per day for fourteen days. Nociception (cold allodynia) was evaluated using the cold plate test at the end of the experimental period. Tumor necrosis factor –αlpha (TNF-α) and interleukin-6 (IL-6) in plasma; inducible nitric oxide synthase (iNOS), phospholipase A2, cyclooxygenase-2 (COX-2), nuclear factor-kappa B (NF-ϰB), caspase-3, Bcl-2, and Bax levels in sciatic nerve were measured by enzyme-linked immunosorbent assay test.

Results: Cold plate latencies (sec) of the neuropathy + HP, neuropathy, and control groups were 8.33 ± 0.67, 5.17 ± 0.60, and 13 ± 0.73, respectively. Plasma TNF-α, IL-6 levels, and sciatic nerve iNOS, COX-2, NF-ϰB, caspase-3, and Bax levels were significantly decreased after HP supplementation. Bcl-2 levels of the neuropathy + HP, neuropathy, and control groups were 9.92 ± 0.71, 5.37 ± 0.53, and 13.65 ± 0.68, respectively.

Conclusion: HP has improved oxidative, inflammatory, and apoptotic responses, as well as cytokine levels in plasma and sciatic nerves of mice. It has been concluded that HP provided neuroprotective, anti-inflammatory, and antinociceptive effects in experimental mice with sciatic

Öz

Amaç: Tıbbi bir bitki olan Hypericum perforatum'un (HP) siyatik sinir hasarına bağlı periferik nöropati üzerindeki etkisi şimdiye kadar az oranda çalışılmıştır. Bu çalışmada, Hypericum perforatum (HP), ekstresinin farelerde siyatik sinir hasarına bağlı periferik nöropati üzerindeki nöroprotektif ve antinosiseptif etkilerini araştırmayı amaçlandı.

Gereç ve Yöntem: Bu çalışmada, 18 Balb/C albino fare eşit olarak üç gruba ayrıldı. Birinci grup kontrol olarak belirlendi ve bu farelere herhangi bir işlem yapılmadı. İkinci ve üçüncü gruplara ayrılan farelerde kısmi siyatik sinir ligasyonu yöntemiyle nöropati oluşturuldu. Üçüncü gruptaki farelere, on dört gün boyunca günde 70 mg/kg HP ekstresi verildi. Nosisepsiyon (soğuk allodini), deneysel süreç sonunda soğuk plaka testi kullanılarak değerlendirildi. Plazmada, tümör nekroz faktörü –αlpha (TNF-α) ve interlökin-6 (IL-6); siyatik sinirde ise indüklenebilir nitrik oksit sentaz (iNOS), fosfolipaz A2, siklooksijenaz-2 (COX-2), nükleer faktör-kappa B (NF-ϰB), kaspaz-3, Bcl-2 ve Bax düzeyleri enzim bağlı immunosorbant testi ile değerlendirildi.

Bulgular: Nöropati + HP, nöropati ve kontrol gruplarının soğuk plaka latansları (sn) sırasıyla 8,33 ± 0,67, 5,17 ± 0,60 ve 13 ± 0,73 idi. Plazma TNF-α, IL-6 seviyeleri ve siyatik sinir iNOS, COX-2, NF-ϰB, kaspaz-3 ve Bax seviyeleri, HP takviyesinden sonra önemli ölçüde azaldı. Nöropati + HP, nöropati ve kontrol gruplarının Bcl-2 düzeyleri sırasıyla 9,92 ± 0,71, 5,37 ± 0,53 ve 13,65 ± 0,68 idi.

Sonuç: HP, farelerin plazma ve siyatik sinirlerindeki sitokin seviyelerinin yanı sıra oksidatif, inflamatuvar ve apoptotik yanıtları iyileştirmiştir. HP'nin deneysel fare siyatik sinir hasarı modellerinde nöroprotektif, anti-inflamatuvar ve antinosiseptif etkiler sağladığı sonucuna varılmış olup, nöropatik ağrı yönetimiyle ilgili gelecekteki çalışmalara rehberlik edeceği düşünülmektedir.

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nerve injury models, which is suggested to guide future studies on neuropathic pain management.
Keywords: Cytokine, Hypericum perforatum, neuroinflammation, neuropathic pain, sciatic nerve injury

Anahtar kelimeler: Sitokin, Hypericum perforatum, nöroinflamasyon, nöropatik ağrı, siyatik sinir hasarı

INTRODUCTION

Diseases or lesions either in the central or peripheral somatosensory nervous system may cause neuropathic pain that affects 7-10% of the general population[1,2]. A better comprehension of neuropathic pain and underlying pathophysiological and molecular mechanisms will provide a more efficacious and targeted mechanism-based treatment approach. A growing number of animal and human studies have shown that the immune system exerts an important role in mediating neuropathic pain. It is a well-known phenomenon that the plasma and neuronal tissue proinflammatory cytokine levels, inflammatory and oxidative mediators and also multiple nerve metabolism changes occur after neuronal injury[3-5]. Activation of inflammatory and oxidative pathways accompanies apoptotic changes, which in turn enhance peripheral nerve damage[6]. These changes trigger parallel, interdependent processes, including neuroimmune interactions at both the peripheral and central levels. These contribute to the emergence of clinically significant pain states such as cold allodynia and hyperalgesia. Neuropathic pain is a challenging condition often resistant to current treatment options. Inhibition of inflammatory and immune pathways enables tremendous interest in managing neuropathic pain[7].

Hypericum perforatum (HP), widely known as St. John's wort, has a wealthy historical background. This medicinal plant has been used for the management of medical conditions, including psychiatric disorders. It has an excellent safety profile, confirmed by extensive clinical trials[8]. Moreover, several studies have shown that HP has anti-inflammatory effects by inhibiting of cytokines, cyclooxygenase (COX), phospholipase A_2 (PLA_2), lipoxygenase, and nuclear factor-kappa B (NF-ϰB)[9-12]. It also has anti-oxidative effects and regulatory effects on cell proliferation and apoptosis[13,14]. In addition to these critical roles, recent studies have also demonstrated the antinociceptive and analgesic effects of HP extract, which corroborate the traditional use of the plant in painful conditions. Preclinical animal studies demonstrated its ability to relieve acute and chronic hyperalgesia by modulation of central and peripheral pain pathways[15].

There are a few studies concerning the neuroprotective effect of HP extract through inhibiting inflammatory, oxidative, and apoptotic pathways in sciatic nerve injury-induced peripheral neuropathy in animal models[6]. The novelty of the study is its comprehensive evaluation of these diverse molecules of inflammation, proliferation and oxidation in mice with peripheral neuropathy. With a better and more detailed understanding of the interaction between the apoptotic system-immune system and the pathophysiology of neuropathic pain, yields a development of novel target-specific treatment strategies. The effects of HP extract on inflammatory mediators, oxidative stress, apoptotic and anti-apoptotic molecules, as well as plasma cytokine levels in experimental animals and humans with sciatic nerve injury, are yet to be clarified. In light of the knowledge mentioned above, the present study aimed to assess the neuroprotective and antinociceptive potential of HP extract in a sciatic nerve injury-induced model of peripheral neuropathy in mice and to investigate some underlying mechanisms in detail.

MATERIALS AND METHODS

Study design and animals

An experimental study design was applied. The working environment was the laboratory of the Cukurova University Faculty of Medicine, Department of Pharmacology. All applications were carried out by certified pharmacology specialists with extensive experience in studies on experimental animals. This experimental study protocol was approved by the Experimental Animal Local Ethical Committee of Cukurova University (Date of approval: June 20, 2017, Number: 6/1). 18 Balb/C albino male mice over eight weeks old, weighing 25-30 grams, were provided by Cukurova University Medical Sciences Experimental Research and Application Center. Mice were randomly assigned to standard cages, with six animals per cage, and all animals' data were analyzed. Randomization was performed using computer-generated random permuted block-by a researcher who did not

contribute to data analyses. The study was conducted strictly following the principles of the National Research Council Guide for the Care and Use of Laboratory Animals[16].

Preparation of Hypericum perforatum extract

HP was grown in Pozantı province in Türkiye and harvested in August. Above-ground parts of the plant were dried at room temperature for one week and ground into powder particles by an electric blender. Ten grams of plant powder were soaked in 100 ml of 80% ethanol. After the extract maceration procedure, the concentrated solution was filtered and dried by a rotary evaporator (IKA RV 10 basic, IKA company, Germany) at 40 °C. The dried extract was solvated in distilled water under sterile conditions and kept without light exposure at − 20 °C. Structural component ratios in HP extract differ due to genetic variations within the species, plant growing conditions, collection time, storage conditions, exposure to light, and the preparation method. Bioanalytical techniques have shown that despite the diversity, an average of 20% of the plant extract consists of bioactive compounds[17].

Partial sciatic nerve ligation surgery

In this study, the partial sciatic nerve ligation model is among the partial denervation models first described by Seltzer and Shir in rats and by Malmberg and Basbaum in mice, and was applied to induce neuropathic pain in mice[18,19]. Before the surgical procedure, mice were anesthetized with 150 mg/kg ketamine (Pfizer Pharma GMBH, Germany) and 5-10 mg/kg xylazine hydrochloride (Alfasan International, Holland) intraperitoneally.

Experimental design

Mice (n= 18) were randomly and equally assigned to the following three experimental groups:

Control Group: Any surgical procedure was not performed. HP extract was not given. The same volume of distilled water as the HP extract was given by gastric gavage for 14 days.

Neuropathy Group: Neuropathy was induced in mice by the partial sciatic nerve ligation method. HP extract was not given to the animals. The Same volume of distilled water as the HP extract was given by gastric gavage for 14 days.

Neuropathy + HP Group: Mice in this group were exposed to the same surgical procedure as the neuropathy group. HP extract was administered by gastric gavage at a dose of 70 mg/kg daily for 14 days after surgery. The extract dose was chosen from a previous study about cigarette smoke-induced lung inflammation and the anti-inflammatory activity of the extract on mice[20].

In accordance with the protocol described above, the experiment continued for 14 days post-surgery. At the end of day 14, the cold plate latencies of all mice were measured by a cold plate test. Subsequently, eighteen animals were sacrificed. To analyze the plasma levels of tumor necrosis factor –αlpha (TNF-α) and interleukin-6 (IL-6), blood samples were taken from inside the heart by opening the thorax of the mice. Samples were brought into tubes containing anticoagulants, and cells were removed from the plasma by centrifugation at 1000 g for 10 min at 4°C. Sciatic nerve samples were stored in Eppendorf tubes at -20°C to determine the levels of nitric oxide synthase (iNOS), COX-2, PLA_2, NF-ϰB, Bax, Bcl-2, and caspase-3.

Assessment of cold allodynia

In the current study, a cold allodynia test with a cold plate device (Ugo Basile, Gemonio, VA, Italy) was used to evaluate the pain due to mononeuropathy induced by the partial sciatic nerve ligation method in mice on day 14 at the end of the experiment. The cold plate device consists of a circular metal plate with a transparent cylinder of plastic glass around it. It is arranged in a way that does not allow the experimental animal to move away from the environment and is maintained at 4 ± 0.1 °C. The automatic timer controlled by the pedal connected to the device determines the time elapsed until the experimental animal left on the plate exhibits one of the specific pain behaviors. One of these behaviors is mononeuropathy-induced paw waving or standing up on the hind paws [21]. In the current study, the time elapsed until exhibiting any of these behaviors was expressed in seconds (sec) and was defined as cold plate latency.

Homogenization of samples and ELISA test

To homogenize tissue samples, three ml/g Radio-Immunoprecipitation Assay buffer (Santa Cruz Biotechnology, Santa Cruz, CA), 30 µl

phenylmethanesulfonyl fluoride, 30 µl sodium vanadate, 30 µl protease inhibitor were added to the frozen tissue samples that are stored in Eppendorf tubes. Homogenates were obtained by shredding the tissues on ice with an ultrasonic fragmentation device (Bandelin Sonopuls Ultrasonic Homogenizers UW 2070, Germany 2008). Homogenates were centrifuged at 10,000 RPM for 10 minutes with a centrifuge device (NUVE NF 800, Ankara, Turkey). The underlying precipitates (pellets) were discarded. The supernatants were taken for use in enzyme-linked immunosorbent assay (ELISA) tests.

The Bradford method was used to determine protein content in homogenized tissues using bovine serum albumin as its standard [22]. Standards were prepared at concentrations of 1, 2, 3, 5, 7, 8, and 10 µg/ml using bovine serum albumin (1µg/ml). Ten µl of each sample was taken and completed to 100 µl with distilled water. After mixing with the vortex, one milliliter of Bradford solution was added to the standards and samples. Then absorbances at the 595-nanometer wavelength were measured manually in the spectrophotometer (Rayto Life Reader, Shenzhen, China). Protein quantification (µg/µl) was done according to the standard curve drawn in Prism software (La Jolla, CA).

ELISA kits were used to assess the expression of COX-2, iNOS, PLA2 (USCN Life Science, Wuhan, China) enzymes, and IL-6, TNF-α, NF-ϰB, Bax, Bcl-2 (USCN Life Science, Wuhan, China), and caspase-3 (RayBiotech, GA, USA) proteins. An ELISA microplate reader and an ELISA microplate washer (Rayto Life and Analytical Sciences, Shenzhen, China) were used for analysis. The dilutions in the kit were added sequentially according to the manufacturer's instructions. Then, the optical densities of the wells were measured by a microplate reader at 450 nm. The amounts of enzymes and proteins were determined.

Statistical analysis

The sample size was determined using the resource equation method. The resource equation approach sets the acceptable range of the error degrees of freedom (DF) that calculate the minimum and maximum numbers of animals required in animal research by reformulating the error DF formulas in an analysis of variance (ANOVA). In the current study, 15 to 21 animals (5 and 7 animals per group) were required to keep the DF within the range of 10 to 20 (an acceptable range). Accordingly, the sample size was determined to be 6 mice per group for a total of 18 animals [23].

The GraphPad Prism® (San Diego, CA, USA) statistical software was used to analyze the data. All the values were expressed as means ± standard deviation (SD) for six animals per group (n = 6). The behavior data and the ELISA assay results were analyzed by one-way ANOVA and corrected for multiple comparisons (post hoc Bonferroni corrections). *P* values below 0.05 were accepted as "statistically significant."

RESULTS

Results of cold plate tests

The analyses of cold plate tests showed that the mean (sec) values of cold plate latencies of the neuropathy + HP, neuropathy, and control groups were 8.33 ± 0.67, 5.17 ± 0.60, and 13 ± 0.73, respectively. Accordingly, there was a statistically significant decrease in cold plate latency in the neuropathy group compared to the control group ($P<0.001$). In the neuropathy + HP group, cold plate latencies were statistically increased compared to the neuropathy group ($P<0.001$) (Figure 1).

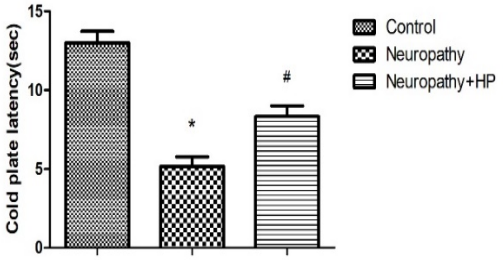

Figure 1. Effect of HP extract on nociception

Values are expressed as mean ± SD. N = 6 for each group. HP: Hypericum perforatum

* *P*< 0.001 neuropathy group vs control group (*effect size* =10.8)

\# *P*< 0.001 neuropathy + HP group vs neuropathy group *(effect size* = 4.59)

Effect of Hypericum perforatum extracts on plasma cytokine levels

The mean (pg/ml) ± SD values for plasma TNF-α levels were 44.83 ± 2.30 in the neuropathy + HP group, 63.83 ± 2.94 in the neuropathy group, and 38.33 ± 2.17 in the control group. Mean (pg/ml) ± SD values for plasma IL-6 levels were 104.2 ± 3.03 in the neuropathy + HP group, 124.2 ± 6.22 in the

neuropathy group, and 93.33 ± 1.71 in the control group. Accordingly, the plasma TNF-α and IL-6 levels were significantly higher in the neuropathy group than in the control group (P<0.001). The increase of TNF-α and IL-6 levels in plasma after sciatic nerve surgery (neuropathy group) was decreased by HP extract supplementation (P<0.001) (Figure 2, Figure 3).

Effect of Hypericum perforatum extract on oxidation and inflammation in the sciatic nerve

The results of analyses of COX-2, iNOS, PLA_2 enzymes, and NF-ϰB protein concentrations in the sciatic nerve samples of each experimental group (n = 18) were provided in Table 1. The sciatic nerve COX-2, iNOS, PLA_2 enzymes, and NF-ϰB protein concentrations were significantly higher in the neuropathy group (n= 6) than in the control groups (n = 6) (P<0.001). And also, all of these molecule concentrations except PLA_2 decreased significantly in the neuropathy + HP group compared to the neuropathy group (n = 6) (P<0.001). The PLA_2 enzyme levels in sciatic nerve samples decreased after HP supplementation. However, there was no statistically significant difference between the neuropathy and the neuropathy + HP group regarding the PLA_2 enzyme levels of the sciatic nerve (P= 0.103).

Effects of Hypericum perforatum extract on proliferation and apoptosis in the sciatic nerve

The mean ± SD values of Bax, caspase-3, and Bcl-2 protein concentrations in the sciatic nerve samples of each experimental group were displayed in Table 2. Accordingly, Bax and caspase-3 levels in the sciatic nerve samples were significantly higher in the neuropathy group than in the control group (P<0.001). The increase of these apoptotic proteins after sciatic nerve surgery (neuropathy group, n = 6) was decreased by HP extract supplementation (neuropathy + HP group, n = 6) (P<0.001). Bcl-2 levels in the sciatic nerve samples were significantly lower in the neuropathy group than in the control group (P<0.001). Also, Bcl-2 levels were significantly higher in the neuropathy + HP group than in the neuropathy group (P<0.001).

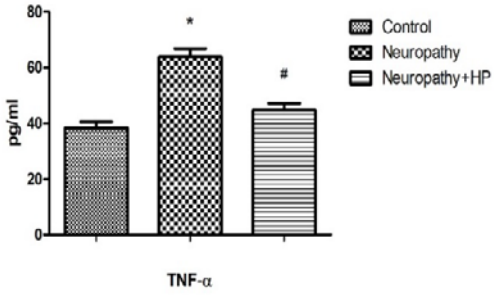

Figure 2. Effect of HP extract on plasma TNF-α levels. The increase of TNF-α levels in plasma after sciatic nerve surgery (neuropathy group) was decreased by HP extract supplementation (neuropathy + HP group). TNF- α: tumor necrosis factor –αlpha, HP: Hypericum perforatum

Values are expressed as mean ± SD. N = 6 for each group.
* P< 0.001 neuropathy group vs control group (effect size = 9.11), # P< 0.001 neuropathy + HP group vs neuropathy group (effect size = 6.64)

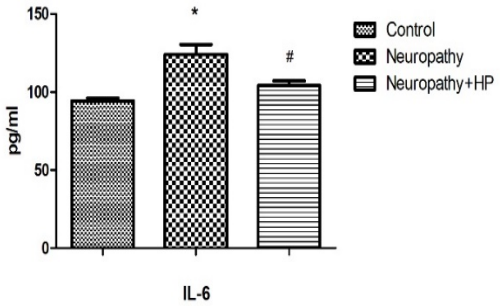

Figure 3. Effect of HP extract on plasma IL-6 levels. The increase of IL-6 levels in plasma after sciatic nerve surgery (neuropathy group) was decreased by HP extract supplementation (neuropathy + HP group). IL-6: interleukin-6, HP: Hypericum perforatum.

Values are expressed as mean ± SD. n = 6 for each group.
* P< 0.05 neuropathy group vs control group (effect size = 6.25), # P< 0.05 neuropathy + HP group vs neuropathy group (effect size = 3.77)

Table 1. Effect of HP extract on oxidation and inflammation in the sciatic nerve

	Neuropathy + HP (n= 6)	Neuropathy group (n= 6)	Control group (n= 6)
COX-2 (pg/ml)	13851 ± 536.4 # d=3.69	15879 ± 476.5 * d=4.88	13368 ± 472.4
Phospholipase A_2 (pg/ml)	2494 ± 137.5	2791 ± 80.79 * d=24.05	1094 ± 44.16
iNOS (IU/ml)	10794 ± 636 # d=3.12	15596 ± 1907 * d=8	3850 ± 178
NF-ϰB (ng/ml)	6.08 ± 0.61 # d=4	8.77 ± 0.63 * d=6.09	4.48 ± 0.67

Note: Data were expressed as mean± SD.
# P< 0.001 neuropathy + HP group vs neuropathy group
* P< 0.001 neuropathy group vs control group
Abbreviations: d: effect size, COX-2: cyclooxygenase-2, HP: Hypericum perforatum, iNOS: inducible nitric oxide synthase, NF-ϰB: nuclear factor kappa B

Table 2. Effect of HP extract on proliferation and apoptosis in the sciatic nerve

	Neuropathy + HP group (n= 6)	Neuropathy group (n= 6)	Control group (n= 6)
Bax (ng/ml)	6.28 ± 0.74 # d= 3.04	8.79 ± 0.79 * d=6.85	2.93 ± 0.32
Caspase-3 (ng/ml)	12.33 ± 0.88 # d=5.35	16.17 ± 1.35* d=6.10	7.25 ± 0.44
Bcl-2 (pg/ml)	9.92 ± 0.71 # d=6.70	5.37 ± 0.53 * d=13.09	13.65 ± 0.68

Note: Data were expressed as mean ± SD.
# P< 0.001 neuropathy + HP group vs neuropathy group
* P< 0.001 neuropathy group vs control group
Abbreviations: d: effect size, HP: Hypericum perforatum

DISCUSSION

The present study revealed that significant inflammatory and oxidative damage developed in both plasma and sciatic nerves, as indicated by increases in plasma TNF-α, IL-6 levels and sciatic nerve COX-2, iNOS, PLA_2, and NF-ϰB levels after partial sciatic nerve ligation in mice. In addition, activation of apoptotic pathways was observed with increases in the levels of Bax, and caspase-3, but a decrease in the level of Bcl-2 in the sciatic nerve. The major findings of the current study are as follows: 1) Supplementation of HP extract significantly decreased the level of neuroinflammatory and apoptotic factors that were increased by sciatic nerve injury in mice. 2) Supplementation of HP extract reduced the severity of cold allodynia, as demonstrated by an increase in cold plate latency.

Cold allodynia, an exaggerated response to normally non-painful cool temperatures, is a characteristic and elusive feature of clinical neuropathic pain. It is generally observed in experimental neuropathic pain models. Detecting cold allodynia is essential in the studies related to pain behavior[21]. Cold allodynia usually occurs within one week after partial sciatic nerve ligation and lasts up to 6 weeks[24]. In the current study, cold allodynia was demonstrated by increased cold plate latency on day 14 in the neuropathy group.

Recently, "neuroinflammation" has been the most studied topic, which is related to the etiology of neuropathic pain and the emergence of clinically significant pain states with cold allodynia and hyperalgesia. Inflammatory mediators can modify peripheral neuroimmune interactions and neuroinflammatory processes. Increasing evidence supports the hypothesis that the inflammatory microenvironment and the release of mediators, rather than the nerve injury, are essential for developing neuropathic pain [25]. In particular, TNF-α, IL-1, IL-6, caspase signaling pathways, Bax/ Bcl-2, iNOS and NF-ϰB have been associated with the development of neuropathic pain in various animal

models [3,26-28]. The present study showed COX-2, iNOS, PLA_2, NF-κB, Bax, and caspase-3 in the sciatic nerve, and TNF-α and IL-6 cytokines in plasma were significantly higher in the neuropathy group than in the control group. These findings supported previous studies about the role of neuroinflammation and apoptotic pathways in the pathogenesis of neuropathic pain.

There is clear evidence that HP alleviates inflammatory damage in various cell types and tissues. It suppresses the activities of 5-lipoxygenase, PLA_2, and COX, crucial enzymes in forming pro-inflammatory eicosanoids from cell membrane phospholipids and arachidonic acid[11,29]. On the other hand, oxidative stress and inflammation are interdependent and interconnected processes co-existing in the inflamed milieu. Stimulation of iNOS by inflammatory cytokines or other inflammatory agents causes the release of significant amounts of nitric oxide (NO). It reaches high concentrations in the site of inflammation[30]. Several studies indicated that NO regulates COX activity in normal and inflamed tissues. Raso et al. reported the inhibitory effect of HP on both COX-2 and iNOS expression[10,31]. Besides, HP strengthens its regulatory effect on oxidative and inflammatory pathways by suppressing cytokine release[32]. Although many studies have demonstrated the suppressive effect of HP on cytokine levels in the plasma and various tissues in inflammatory conditions and diseases, few studies examine whether this effect exists in the peripheral neuropathy model. Uslusoy et al. reported that HP reduced the plasma levels of TNF-α, IL-1β, and IL-2 cytokines in rats with sciatic nerve injury-induced neuropathy[6]. HP also exerts its protective effects by concomittant inhibition of multiple phosphorylation cascades along divergent cytokine signaling pathways and consequent limitation of apoptotic and inflammatory gene expression. NF-κB is a crucial transcription factor in several physiological processes such as inflammation, immune regulation, proliferation/apoptosis, and pain modulation[32-34]. Our study demonstrated the anti-oxidative and anti-inflammatory effects of HP with the decrease in NF-κB, COX-2, iNOS, and PLA_2 in the sciatic nerve, and TNF-α and IL-6 cytokines in plasma in sciatic-nerve injury-induced peripheral neuropathy.

Apoptosis is a sequential order of cell death that regularly occurs to ensure a homeostatic balance between cell formation and cell death rate. HP and its active compounds show apoptotic and antiproliferative effects in both hematological and solid malignancies with many different and synergistic mechanisms, including suppression of Bcl-2 expression and stimulation of Bax and caspase expression[35,14]. On the other hand, recent studies indicated that HP exerts neuroprotective effects in experimental spinal cord and sciatic nerve damage by suppressing caspase-3 and caspase-9 activity in the dorsal root ganglia and sciatic nerve[36,37]. In this study, the suppressive effect of HP on apoptotic pathways has been demonstrated by a decrease in caspase-3 and Bax levels and an increase in Bcl-2 levels in mice sciatic nerve samples. This is the first study in the literature demonstrating the effect of HP on both apoptotic caspase-3, Bax, and anti-apoptotic Bcl-2 levels of sciatic nerve samples in the sciatic nerve injury-induced peripheral neuropathy model. Additionally, several studies have demonstrated the efficacy of HP in pain management through different physiological mechanisms[38]. The current study revealed that HP extract alleviates cold allodynia and inhibits neuroinflammatory and oxidative pathways that have the potential to modulate pain in sciatic nerve injury-induced neuropathy in mice. It is thought that the above-mentioned possible mechanisms play a role in the occurrence of the analgesic effect of HP extract. Mechanisms underlying the analgesic effect of HP in sciatic nerve injury-induced peripheral neuropathy should be studied further.

There are several limitations to be discussed. Firstly, the study failed to investigate the effect of HP extract on other pain behaviors observed in peripheral neuropathy models and its inability to detail its antinociceptive effect on sciatic nerve damage. Secondly, although the present study provides essential data on the neuroprotective effects of HP extract on sciatic nerve injury-induced peripheral neuropathy in mice, oxidative pathways and possible pain mechanisms involved in the pathogenesis have not been studied in detail. Moreover, it would be much more valuable to run a regression analysis and evaluate the association of biochemical markers with neuropathy as well as the effect of HP on neuropathy.

In conclusion, this study demonstrated that HP extract supplementation improves oxidative, inflammatory, and apoptotic pathways and cytokine changes in plasma and sciatic nerve in an experimental peripheral neuropathy model, which delays the progression of sciatic nerve damage and

complications. These findings shed light on the neuroprotective effect of HP. This is the first experimental study that demonstrates the efficacy of HP on apoptosis, oxidative damage, cytokine production, inflammation, and nociception in PSLL-induced peripheral neuropathy in mice by examining all of these parameters together. We have suggested that future preclinical and clinical studies on the effects of HP will be promising homeopathic therapeutic option for neuropathic pain states, especially those originating from the sciatic nerve which is commonly seen in daily clinical practice.

Author Contributions: Concept/Design : AS, HMK, ES, EK; Data acquisition: AS, HMK, EK, ES; Data analysis and interpretation: AS, HMK, EK, ES; Drafting manuscript: AS, EK; Critical revision of manuscript: AS, HMK, EK, ES; Final approval and accountability: AS, HMK, ES, EK; Technical or material support: AS, HMK; Supervision: AS, HMK, EK, ES; Securing funding (if available): n/a.
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Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors declare that they have no conflict of interest.
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Declarations: This study was presented as an oral presentation at the Turkish League Against Rheumatology (TLAR) Congress 2020 and received the 1st runner-up award.

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