doi.org/10.36721/PJPS.2023.36.1.REG.149-157.1

New insights into the cytoprotective mechanism of nano curcumin on neuronal damage in a vincristine-induced neuropathic pain rat model

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Abstract: Vincristine is a frequently used antineoplastic drug for treating a variety of malignancies, although its usage has been restricted by the painful neuropathy it induces. A more profound comprehension of the pathogenesis of vincristine-induced painful neuropathy (VIPN) is crucial for developing efficient preventive and therapeutic approaches. With its well-established anti-inflammatory and immunomodulatory actions, curcumin (nanoemulsion form) was utilized in our work as a protective and therapeutic tool for VIPN. This study aims to clarify the mechanisms behind curcumin's neuroprotective effects against vincristine-induced neurotoxicity. For painful neuropathy induction, rats were injected with vincristine sulfate (150µg/kg/i.p.: once every two days) for five injections. For treatment, pregabalin (30 mg/kg/p.o.), curcumin (30mg/kg/p.o.) and curcumin nanoemulsion (30mg/kg/p.o.) were administered daily for 14 consecutive days. Our results showed that curcumin nanoemulsion significantly reduced cold allodynia and thermal hyperalgesia. It also increased the sciatic nerve levels of [CAT, SOD, and IL-10] and the spinal cord PPAR-γ. Additionally, immunostaining of the sciatic nerve revealed a reduction in NF-κB expression and an increase in HSP70 expression. These findings suggest that curcumin has neuroprotective effects against VIPN, which might be attributed to its interference with the PPAR-γ, HSP70 and IL-10 signaling pathways.

Keywords: Curcumin, mechanism, neuropathic, vincristine.

INTRODUCTION

Up to 30-40 % of patients receiving chemotherapeutic agents like vinca alkaloids, platinum drugs and taxanes suffer from neuropathic pain termed chemotherapy-induced painful neuropathy (CIPN) (Kaley and Deangelis, 2009). Vincristine is one of the frequently used antineoplastic agents for treating various types of lymphomas, leukemias, and sarcomas; unfortunately, its induction of painful neuropathy termed vincristine-induced painful neuropathy (VIPN) limited its use in a duration dose-dependent manner (Sisignano *et al*., 2014). A better understanding of the etiology and pathophysiology of VIPN is mandatory to provide efficient preventive and treatment approaches (GZ Li *et al*., 2020). Opioids and nonsteroidal anti-inflammatory medications (NSAIDs), typical analgesics, are ineffective in treating severe neuropathy in cancer patients. Anticonvulsants and tricyclic antidepressants may also aid in managing neuropathic pain, although their usage is limited due to their side effects (Dworkin *et al*., 2010).

Curcumin, one of the natural medicines being investigated recently, has piqued the specialists' interests due to its powerful therapeutic effects. Curcumin's specific properties are [antioxidant, anti-inflammatory, immunomodulatory and anti-apoptotic(Guo *et al*., 2020)], denoting that its neuroprotective activity may be due to a combination of these actions (Guo *et al*., 2020). It exerts its immunomodulatory effect by either down-regulation of pro-inflammatory cytokines such as (Interleukins (IL 1, 2, 6, 8), nitric oxide, and inducible nitric oxide synthetase iNOS) and upregulation of IL-10 production that neutralizes the inflammatory circumstances (Abdollahi *et al.*, 2017). This immunomodulation is mediated via the inhibition of the immune cells' activation and function, resulting in repression of tumor necrosis factor-alpha (TNF-α) production via down-regulation of cyclooxygenase 2 (COX-2) and (iNOS) expression (Kwilasz *et al.*, 2015). These effects may contribute to its antinociceptive effects in neuropathic pain animal models (Wilkerson *et al.*, 2012).

Peroxisome proliferator-activated receptors (PPARs) are a group of proteins normally activated by their ligands inside the cell nuclei to regulate metabolism and homeostasis (Korbecki *et al*., 2019). Peroxisome proliferator-activated receptor-gamma (PPAR-γ) has been established as a significant player in the immune response due to its ability to inhibit inflammatory mediators production (Korbecki *et al*., 2019).

Moreover, several papers have concluded that curcumin's anti-inflammatory effect is due to PPAR-γ activation with unclear mechanisms (Sanjay *et al*., 2021). Nonetheless, it is fair to speculate that curcumin induced anti-inflammatory impact via the upregulation of PPAR-γ, which is linked to the nuclear factor kappa-B (NF-κB) pathway (Mazidi *et al*., 2016).

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Heat shock proteins (HSPS) are an abundant protein family specifically induced upon cells' exposure to various stress sources, including heat and oxidative stress (Shevtsov *et al*., 2020). HSP chaperones regulate cell division, apoptosis, NF-κB signaling, and other stress-associated pathways, leading to cytoprotection against diverse heat shock and infection-induced inflammatory pathways (Giffard *et al*., 2008).Numerous studies have found that curcumin has a cytoprotective impact on multiple cell types and tissues via upregulation of HSP70 (Seo *et al*., 2018).

The current study attempted to explore curcumin's cytoprotective mechanisms in vincristine-induced neuropathic pain; consequently, these mechanisms may serve as valuable therapeutic targets for managing VIPN. However, the main limiting factor of using curcumin as a therapeutic agent is its exceedingly low oral bioavailability (Aller, 2019); therefore, we developed a new nanoemulsion formulation to enhance its bioavailability.

MATERIALS AND METHODS

Chemicals and drug solutions preparation

Vincristine sulfate was obtained from (EMIC® Pharmaceutical, Egypt); pregabalin was obtained from (Pfizer® Pharma, Egypt); Curcumin powder was purchased from (TITAN Biotech), Spectrophotometric assay kits for Superoxide Dismutase (SOD, Catalog No. EC1.15.1.1), Catalase (CAT, Catalog No. EC 1.11.1.6) were purchased from (Bioassay Systems, USA), Enzyme-linked immunosorbent assay (ELISA) kits for PPAR-γ (Catalog No. CSB E08624r), Interleukin-10 (IL-10, Catalog No. CSBE04595r) was purchased from (CUSABIO Houston, USA). Anti NF-κB antibody (Catalog No. ABIN668961), Anti-HSP70 antibody (Catalog No. ABIN361707) were purchased from (antibodies-online). All the other used reagents were of analytical degree.

Curcumin and pregabalin were suspended in 0.3%W/V carboxymethylcellulose (CMC) solution and vincristine was diluted with sterile phosphate-buffered saline (PBS) just before administration.

Nanoemulsion preparation

Curcumin nanoemulsion was prepared by the phase inversion method (Ren et al., 2019). In this method, Tween 80 and PEG 400 were mixed with the following concentrations [PEG 400 (25% V/V), Oleic acid (8.3% V/V), Distilled water (16.7% V/V), Curcumin powder (23.3% W/V), and Tween 80 (50% V/V)] at temperature 60ºC using hot plate magnetic stirrer. The prepared nanoemulsion's particle size and zeta potential were measured after 4X dilution and prob sonication by Malvern Mastersizer laser diffraction particle analyzer (Zetasizer Ver. 7.13).

Experimental animals

A total of 36 adult male Sprague Dawley albino rats weighing (130-150g) were used for this study (purchased from a private Egyptian animal house). The animals were housed under a steady 12h/12h light/dark cycle at (23±1°C). With unrestricted access to food and water, they were acclimated to the researcher's handling during the week before the experiment. The ethics committee of the Faculty of Pharmacy, South Valley University, Egypt, authorized the study's design and execution under accepted ethical standards (Approval P.1025).

Induction of the peripheral neuropathy

For induction of painful peripheral neuropathy, rats were injected with vincristine sulfate (150µg/kg; once every two days) intraperitoneally for five injections, as described by (Authier *et al*., 2003).

Experimental design

The animals were distributed into six groups, each group with six rats. Group I served as the normal control, with rats receiving orally (0.3% w/v carboxymethylcellulose (CMC)) once daily for 14 days. Group II represented the induction group, in which rats were given a dose of vincristine intraperitoneally (150µg g/kg) every two days for five injections; for treatment, rats in groups III, IV, V and VI were given orally (the dose volume were specified for each rat according to its body weight) daily: A dose of pregabalin (30mg/kg), curcumin (30mg/kg), curcumin nanoemulsion (30mg/kg), and nanoemulsion vehicle [PEG, oleic acid, tween 80, DW], respectively for 14 consecutive days in addition to a dose of vincristine intraperitoneally (150µg g/kg) every two days for five injections.

Behavioral tests, such as tail immersion and hot plate, were accomplished on specific days, namely days 0, 7, 10 and 14. At the end of the experiment (day 14), all animals were sacrificed under deep ether anesthesia, and tissue samples (sciatic nerve and spinal cord) were collected and used for biochemical analysis. According to the Bradford method (1976), protein concentration was assessed using bovine serum albumin as a standard (Pedrol and Tamayo, 2001).

Behavioral assessment of vincristine-induced neuropathy

Hot plate test (thermal hyperalgesia)

The animals were placed in a glass cylinder on a heated metal plate at a stable temperature of (55±1°C). The latency of nociceptive responses, such as jumping, shaking one of the paws, or licking, was recorded. The maximum duration allowed for standing on the plate was 40 seconds to prevent injury to animals' paws (Bannon and Malmberg, 2007).

Tail immersion Test (cold allodynia)

In this test, the rat's tail was immersed nearly 1 cm below the surface of cold water at a noxious temperature (4°C)

up to the tail was withdrawn; the immersion duration was recorded in seconds (Necker and Hellon, 1978).

Biochemical parameters

The isolated parts of the sciatic nerve and spinal cord were immediately processed to measure (SOD, catalase, IL-10), and PPAR-γ, respectively. The tissue homogenates were prepared using cold, sterile PBS (10% w/v).

PPAR-γ level measurement

Rat PPAR-γ ELISA kit was applied to determine the PPAR-γ levels in spinal cord tissue homogenate.

Interleukin-10 level measurement

Rat IL-10 ELISA kit was applied to assess the IL-10 levels in sciatic nerve tissue homogenate.

Antioxidant markers (Super oxide dismutase (SOD), catalase (CAT))

Manufacturer's instructions (Bio Assay Systems Co, CA, USA) were applied to determine the super oxide dismutase and catalase activity in the sciatic nerve homogenate.

Histological examination

Sciatic nerve samples were kept in 10% buffered formalin (fixative solution). For routine hematoxylin and eosin staining, cut paraffin-embedded sections at 3-5 mm thickness on glass and charged slides (Sudoh *et al*., 1988). The light microscope was used to examine the processed sections to detect axonal degeneration.

Immunohistochemistry

Immunohistochemical identification of NF-κB and HSP70 was performed using the peroxidase-anti peroxidase (PAP) method on formalin-fixed, paraffin-embedded sciatic nerve tissue sections (Hsu *et al*., 1981).

STATISTICAL ANALYSIS

The results in this work have been stated as mean ± SEM. For the biochemical estimations, the results were statistically analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison test. Two-way ANOVA (treatment versus duration) followed by Bonferroni's post hoc test was used for data analyses of the behavioral tests. Significance was considered when probability values (P<0.05). GraphPad Prism® was used for statistical calculations (Version 8.0.2, GraphPad Software, USA, www.graphpad.com).

RESULTS

Particle size and zeta potential of the curcumin nanoemulsion

As shown in fig. 1 A, B, the nanoemulsion's particle size and size distribution (uniformity) were evaluated by Mastersizer Malvern. Curcumin nanoemulsion showed a particle size of 273.7±8.1 nm, a surface charge of -2.77± 0.91 mv and a polydispersity index (PdI) of 0.439±0.01, indicating its uniform particle size distribution.

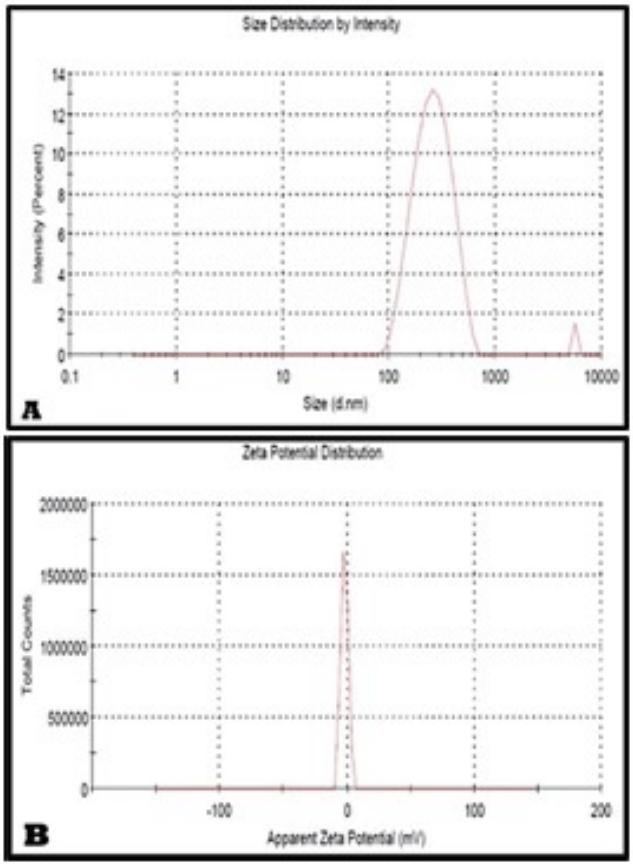

Fig. 1: A: Particle size of the curcumin nanoemulsion. B: Zeta potential of the curcumin nanoemulsion.

Effect of curcumin on thermal hyperalgesia

As shown in fig. 2, the normal control group did not show any change in withdrawal latency at any time point of the study compared to day zero (P>0.999)**.** Thermal hyperalgesia was significantly increased (P<0.0001) after vincristine administration, as demonstrated by increased jumping off the hot plate surface and hind paw licking on days 7, 10 and 14 compared to the normal control. Pregabalin and curcumin nanoemulsion treatments significantly increased the pain threshold compared to the induction group on days 7 and 10 (P<0.05) and (P<0.0001) on day 14. However, the administration of native curcumin demonstrated a significant (P=0.0001) increase in the pain threshold on day 14 only. Vehicle administration did not show any significant ($P>0.999$) change in vincristine-induced thermal hyperalgesia at any time during the experiment.

Effect of curcumin on cold allodynia

As demonstrated in fig. 3, the normal control group did not exhibit any change in withdrawal latency at any time point of the study compared to day zero (P>0.999).

Table 1: Effect of curcumin on sciatic nerve homogenate SOD, Catalase, and IL-10

Treatment	SOD (U/mg protein)	Catalase (U/mg protein)	IL-10 (Pg./mg protein)
Normal control	1.25 ± 0.14	1.63 ± 0.15	10.27 ± 0.77
VCR	0.09 ± 0.01*	0.13 ± 0.0*	0.72 ± 0.07*
VCR+ PREG	0.55 ± 0.08*#	0.72 ± 0.12*#	3.46± 0.53*#
VCR +CUR	1.01 ± 0.06#	1.23 ±0.07#	6.75 ± 0.44*#
VCR+ NANO CUR	2.17± 0.10*#	2.52 ±0.11*#	14.51± 0.67*#
VCR +Vehicle	0.27 ± 0.09*	0.40±0.10*	1.99± 0.26*

The data are represented as mean ± SEM (n = 6). One-way ANOVA followed by post-hoc Tukey's multiple comparison test. (*) indicates significance at (P<0.05) in comparison with the normal control, and (#) indicates significance at (P<0.05) compared to the induction group. VCR: Vincristine, PREG: Pregabalin, CUR: Curcumin.

Table 2: Effect of curcumin on sciatic nerve histopathological changes

Histopathological changes	Normal	VCR	VCR+ PREG	VCR + CUR	VCR+ NANO CUR	VCR + Vehicle
Endoneurium vacuolar degeneration	-	+++	++	++	+	+++
Perineurium inflammation	-	++	-	-	-	++
Endoneurium inflammation	-	++	-	-	-	++
Vascular dilatation	-	++	-	-	-	++

Effect of curcumin on sciatic nerve histopathological changes, +++ indicates severe, ++ indicates moderate, + indicates mild, - indicates nil. VCR: Vincristine, PREG: Pregabalin, CUR: Curcumin.

Cold allodynia developed significantly (P<0.0001) in vincristine-receiving rats, as indicated by a decrease in the tail withdrawal latency on days 7, 10 and 14 compared to the normal control. Pregabalin and curcumin nanoemulsion administration significantly decreased the vincristine-induced cold allodynia compared to the induction group on day 7 (P<0.05) and on days 10 and 14(P<0.0001). At the same time, the administration of the native curcumin or vehicle did not show any significant (P=0.689) and (P>0.999) change in the pain threshold at any time of the study compared to the induction group, respectively.

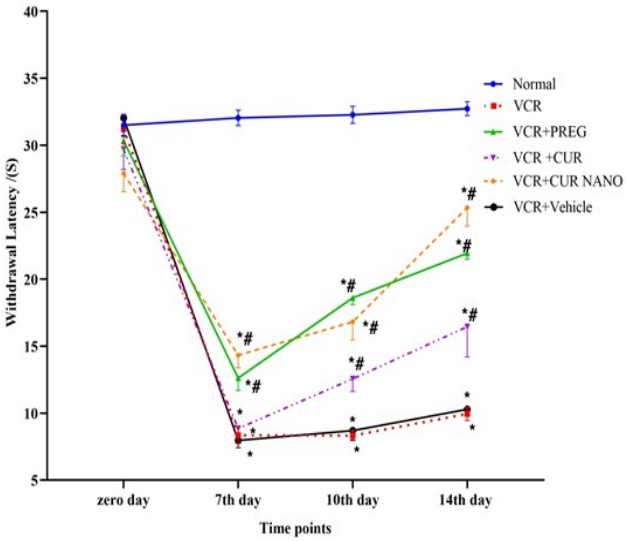

Fig. 2: Effect of curcumin on thermal hyperalgesia in vincristine-induced neuropathy. Two-way ANOVA followed post-hoc Bonferroni's comparison test. The data are represented as mean ± SEM (n=6). (*) indicates significance at P<0.05 compared to the normal control group and (#) indicates significance at P<0.05 compared to the induction group. VCR: Vincristine, PREG: Pregabalin, CUR: Curcumin.

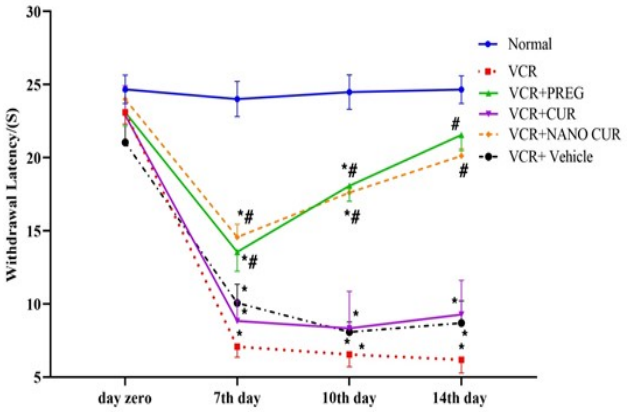

Fig. 3: Effect of curcumin on cold allodynia in vincristine-induced neuropathy. Two-way ANOVA followed post-hoc Bonferroni's comparison test. The data are represented as mean ± SEM (n=6). (*) indicates significance at P<0.05 compared to the normal control group and (#) indicates significance at P<0.05 compared to the induction group. VCR: Vincristine, PREG: Pregabalin, CUR: Curcumin.

Effect of curcumin on spinal cord PPAR-γ

As shown in fig. 4, vincristine administration significantly (P<0.05) depleted the spinal cord PPAR-γ levels compared to the normal control. Pregabalin and curcumin

nanoemulsion-treated rats showed a significant (P<0.05) increase in the PPAR-γ levels compared to induction group animals. On the other hand, native curcumin and vehicle-treated groups exhibited a non-significant (P>0.05) elevation of the spinal cord PPAR-γ content.

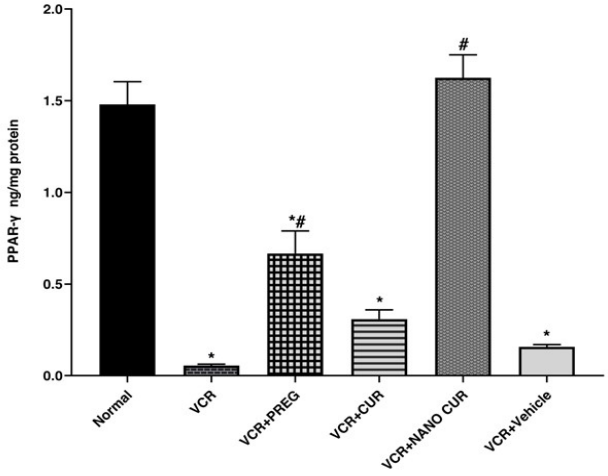

Fig. 4: Effect of curcumin on spinal cord PPAR-γ levels. Vincristine-induced neuropathic pain rat model. The data are represented as mean ± SEM (n=6). One-way ANOVA followed by post-hoc Tukey's multiple comparison test. (*) indicates significance at P<0.05 compared to the normal control, (#) indicates significance at P<0.05 compared to the induction group. VCR: Vincristine, PREG: Pregabalin, CUR: Curcumin.

Effect of curcumin on sciatic nerve IL-10

Vincristine administration significantly (P<0.05) diminished the sciatic nerve IL-10 levels compared to the normal control. Pregabalin, native curcumin, and curcumin nanoemulsion-treated rats showed a significant (P<0.05) increase in IL-10 levels compared to induction group rats. At the same time, vehicle-treated animals exhibited a non-significant elevation (P>0.05) in the IL-10 levels compared with those treated with vincristine, as shown in table 1.

Effect of curcumin on antioxidant markers (SOD, Catalase)

Vincristine administration significantly (P<0.05) depleted the SOD and catalase levels compared to normal control animals. Pregabalin, native curcumin, and curcumin nanoemulsion-treated rats demonstrated a significant increase (P<0.05) in their levels in the sciatic nerve compared to induction group rats. In contrast, vehicle-treated animals showed a non-significant increase (P>0.05) in antioxidant indicators levels compared to those treated with vincristine, as shown in table 1.

Effect of curcumin on sciatic nerve NF-κB

Immunostaining of the sciatic nerve samples with NF-κB antibodies revealed a significant increase in the nerve NF-κB expression after vincristine induction of the neuropathy compared to the normal control animals. Different drug treatments decreased the factor expression to various degrees, as shown in fig. 5 A, B. At the same time, vehicle-treated rats did not show any change compared with those in the induction group.

Effect of curcumin on sciatic nerve HSP70

The immunostaining of the sciatic nerve samples with HSP70 antibodies revealed a significant reduction in the nerve HSP70 expression after vincristine induction of the neuropathy compared to the normal control animals. Different drug treatments increased the factor expression to various degrees, as shown in fig. 6 A, B. At the same time, vehicle-treated rats did not show any change compared with those in the induction group.

Effect of curcumin on sciatic nerve histology

The microscopic examination of sciatic nerve samples from vincristine-treated animals showed severe changes compared to those from normal control rats. Vincristine-treated animals showed a severe dilatation of vascular spaces with (edema and inflammatory cells infiltration in perineurial tissues) and degenerated axons. The native curcumin-treated animals exhibited moderate vacuolar degeneration in nerve fibers. In contrast, sciatic nerve samples from pregabalin and curcumin nanoemulsion-treated animals showed a normal histological structure of endoneurium with myelinated axon nerve fibers, as shown in fig. 7 and table 2.

DISCUSSION

Vincristine has been extensively used to treat several malignancies. Unfortunately, the prevalence of acute painful neuropathy severely restricts its therapeutic use. Multiple studies have demonstrated that VIPN pathophysiology is complex, implying that a single mechanism does not cause it (Triarico *et al.*, 2021).

In the current work, we endeavored to explore the potential role of cytoprotective mediators such as PPAR-γ, IL-10 and HSP70 as promising drug targets for managing VIPN. Their protective effect is studied using curcumin in the nanoemulsion form for pain alleviation of VIPN in a rat model. Pregabalin: A selective calcium channel alpha 2-delta (α2-δ) subunit antagonist, has been used as a standard treatment of vincristine-induced hyperalgesia in this work (Thiagarajan *et al*., 2014). As previously reported, vincristine-injected rats developed neuropathy, as evidenced by a substantially lower pain threshold in hyperalgesic and allodynic models (A. L. Li *et al*., 2021). In our study, vincristine-treated animals exhibited a significant rise in thermal hyperalgesia and cold allodynia. Additionally, a histopathologic examination of the sciatic nerve showed severe edema with inflammatory cells infiltration in perineurial tissues and degenerated axons indicating nerve injury and painful neuropathic pain development.

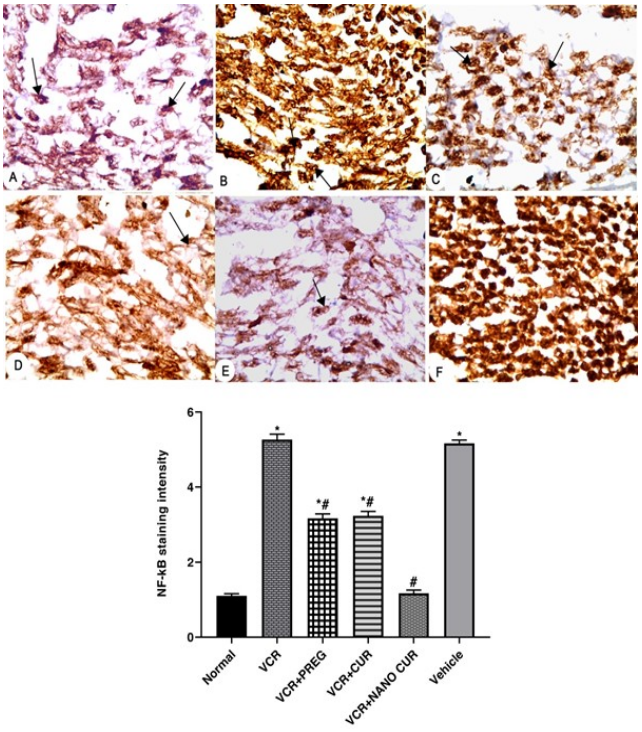

Fig. 5: Effect of curcumin on sciatic nerve NF-κB expression. Estimated by immunohistochemical analysis (× 400). Light Photomicrographs showing sciatic nerve A, B, C, D, E, and f sections of the sciatic nerve of normal control, vincristine control, pregabalin, curcumin, curcumin nanoemulsion and vehicle-treated groups, respectively. Arrowheads refer to immune positive cells. The data are represented as mean ± SEM (n=6). One-way ANOVA followed by post-hoc Tukey's multiple comparison test. (*) indicates significance at $P<0.05$ compared to the normal control, and (#) indicates significance at $P<0.05$ compared to the induction group. VCR: Vincristine, PREG: Pregabalin, CUR: Curcumin.

Moreover, our findings showed that curcumin in the nanoemulsion form was able to decrease the pain threshold resulting from VIPN in our model, as indicated by the reduced thermal hyperalgesia and cold allodynia in the behavioral tests, the increased antioxidant enzymes (SOD and catalase) levels and upregulation of neuroinflammation moderators PPAR-γ, IL-10 and HSP70 which in turn decrease NF-κB expression. However, animal treatment with the native form of curcumin (30mg/kg) improved the mentioned parameters, but it is not the same as the drug in the nanoform. The statistical analysis of the results revealed a significant difference between the nanoform and the native form of curcumin.

Similarly, Numerous studies described the beneficial therapeutic effects of curcumin in many neuropathic pain animal models, such as paclitaxel, via inhibiting the oxidative stress-mediated activation of NF-κB (Ashrafizadeh *et al*., 2020) and cisplatin by modulation of nuclear factor erythroid 2-related factor 2 (Nrf2) and NF-κB transcription factors which are vital regulators of oxidative stress and inflammation (Rezaee *et al*., 2017). One previous study showed that a high dose of native curcumin (60 mg/kg) alleviated the VIPN in a mice model via decreasing the oxidative stress mediators and increasing the antioxidant enzymes like GSH, GPx, SOD and catalase (Babu, Prasanth and Balaji, 2015).

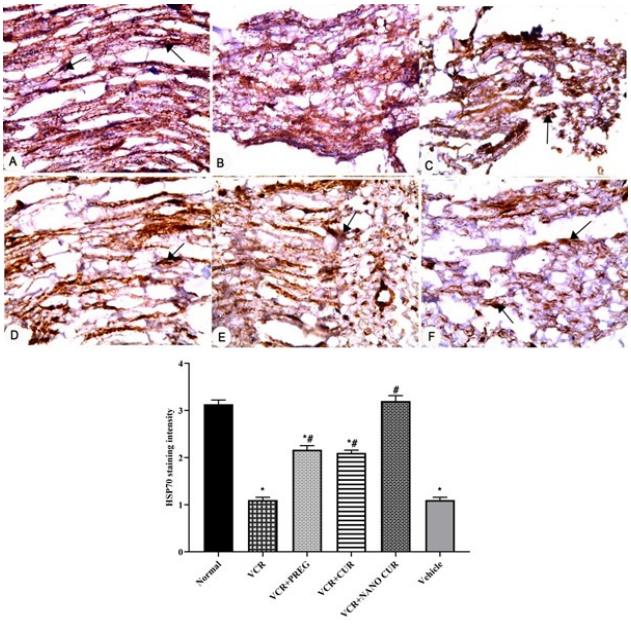

Fig. 6: Effect of curcumin on sciatic nerve HSP70 expression. Estimated by immunohistochemical analysis (× 400). Light photomicrographs showing sciatic nerve A, B, C, D, E, and f sections of the sciatic nerve of normal control, vincristine control, pregabalin, curcumin, curcumin nanoemulsion, and vehicle-treated groups, respectively. Arrowheads refer to immune positive cells. The data are represented as mean ± SEM (n=6). One-way ANOVA followed by post-hoc Tukey's multiple comparison test. (*) indicates significance at $P<0.05$ compared to the normal control, and (#) indicates significance at $P<0.05$ compared to the induction group. VCR: Vincristine, PREG: Pregabalin, CUR: Curcumin.

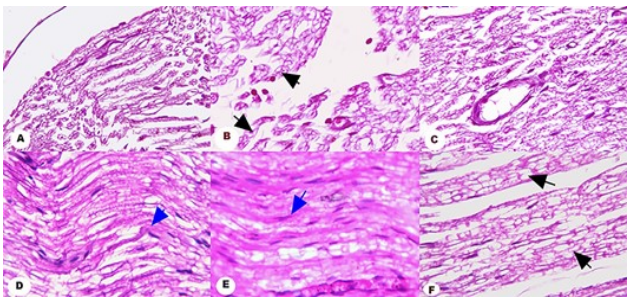

Fig. 7: Effect of curcumin on the histopathological changes in vincristine-induced neuropathy. Light photomicrographs showing sciatic nerve A, B, C, D, E, and f sections of the sciatic nerve of normal control, vincristine control, pregabalin, curcumin, curcumin

nanoemulsion, and vehicle-treated groups, respectively. (Black arrow) shows focal areas of demyelination and degeneration of the nerve fibers. (Blue arrow) marking a decrease in demyelination (arrows) (x100).

On the other hand, many research papers demonstrated that the protective effect of curcumin is mediated by the upregulation of PPAR-γ in many disorders such as hepatic fibrosis (Mazidi *et al*., 2016), cigarette smoke-induced inflammation (Q. Li *et al*., 2019). There are no previous studies on the protective role of PPAR-γ in VIPN. Here, in our model of VIPN, curcumin significantly up regulated the spinal cord PPAR-γ levels and decreased the sciatic nerve NF-κB expression levels giving evidence that PPAR-γ may have a role in managing VIPN via the NF-κB suppression pathway.

Moreover, numerous studies reported that curcumin possesses a cytoprotective effect on various cell types and tissues via the upregulation of HSP70 (Seo *et al.*, 2018). Curcumin inhibited cell death in rat hippocampus neurons by upregulating HSP70 expression (Qin *et al*., 2012). Also, curcumin inhibited ischemia/reperfusion-induced liver injury in rats via increasing HSP70 expression (Shen *et al*., 2007). Unfortunately, no previous studies investigated the protective role of HSP70 in VIPN models. Therefore, we attempted to explore this role using curcumin's effect on HSP70. We found that curcumin upregulated the sciatic nerve HSP70 expression significantly compared to the vincristine-treated animals, indicating that HSP70 may be a promising protective tool against VIPN.

The neuroprotective impact of IL-10 has been widely investigated in various neuropathic pain models (Fonseca *et al.*, 2020). These studies reported that IL-10 was down-regulated in severe nerve injury (Zhang *et al*., 2020) and VIPN (Xie *et al*., 2020). Consistently, our results showed that curcumin could upregulate IL-10 after nerve injury induced by vincristine administration, confirming the protective role of IL-10 against the induced VIPN

Along with these findings, the present study postulates that curcumin's cytoprotective effect alleviating the neuropathic pain induced by vincristine is due to combined immunomodulation and antioxidant effects. Also, these results suggest that PPAR-γ, HSP70 and IL-10 may be critical regulators for managing neuropathic pain induced by chemotherapy.

CONCLUSION

The current results demonstrate that improving curcumin's bioavailability produced the required therapeutic and protective effects on the induced neuropathic pain by vincristine. The protective mechanism is probably mediated via the up regulation of the protective mediators (PPAR-γ, HSP70, and IL-10). These results pave the way for more studies on these mediators as future drug targets for Chemotherapy-induced neuropathic pain.

ACKNOWLEDGMENT

We are grateful to Prof. Zaid A., Department of Pathology, Faculty of Veterinary Medicine, Sadat City University, for his honest help interpreting histopathological and immunohistochemistry examination results.

REFERENCES

Abdollahi E, Amir, Momtazi A, Thomas, Johnston P and Sahebkar A (2017). Therapeutic effects of curcumin in inflammatory and immune-mediated diseases: A nature-made jack-of-all-trades?. *J. Cell Physiol.,* **233**(2): 830-848

Aller LL (2019). What about bioavailability of oral curcumin? *CMAJ.* **191**(15): E427.

Aschkenasy G, Bromberg Z, Raj N, Deutschman CS and Weiss YG (2011). Enhanced Hsp70 expression protects against acute lung injury by modulating apoptotic pathways. *PLoS One* **6**(11): e26956.

Ashrafizadeh M, Zarrabi A, Hashemi F, Moghadam ER, Hashemi F, Entezari M, Hushmandi K, Mohammadinejad R and Najafi M (2020). Curcumin in cancer therapy: A novel adjunct for combination chemotherapy with paclitaxel and alleviation of its adverse effects. *Life Sci.* **256**: 117984.

Authier N, Gillet JP, Fialip J, Eschalier A and Coudore F (2003). A New Animal Model of Vincristine-Induced Nociceptive Peripheral Neuropathy. *Neurotoxicol.* **24**(6): 797-805.

Babu A, Prasanth KG and Balaji B (2015). Effect of curcumin in mice model of vincristine-induced neuropathy. *Pharm. Biol.* **53**(6): 838-848.

Bannon AW and Malmberg AB (2007). Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. *Curr. Protoc. Neurosci.* **8**: 8-9.

Bromberg Z, Deutschman CS and Weiss YG (2005). Heat shock protein 70 and the acute respiratory distress syndrome. *J. Anesth.* **19**(3): 236-242.

Dworkin RH, Malone DC, Panarites CJ, Armstrong EP and Pham S V (2010). Impact of postherpetic neuralgia and painful diabetic peripheral neuropathy on health care costs. *J. pain* **11**(4): 360-368.

Fonseca MM, Davoli-Ferreira M, Santa-Cecília F, Guimarães RM, Oliveira FFB, Kusuda R, Ferreira DW, Alves-Filho JC, Cunha FQ and Cunha TM (2020). IL-27 Counteracts Neuropathic Pain Development Through Induction of IL-10. *Front. Immunol.* **10**(January): 1-14.

Giffard RG, Han R-Q, Emery JF, Duan M and Pittet JF (2008). Regulation of apoptotic and inflammatory cell signaling in cerebral ischemia: the complex roles of heat shock protein 70. *Anesthesiol.* **109**(2): 339-348.

Guo J, Cao X, Hu X, Li S and Wang J (2020). The anti-apoptotic, antioxidant and anti-inflammatory effects of curcumin on acrylamide-induced neurotoxicity in rats. *BMC Pharmacol. Toxicol.* **21**(1): 62.

Hsu SM, Raine L and Fanger H (1981). The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase technics. *Am. J. Clin. Pathol.* **75**(6): 816-821.

Kaley TJ and Deangelis LM (2009). Therapy of chemotherapy-induced peripheral neuropathy. *Br. J. Haematol.* **145**(1): 3-14.

Kampinga HH (2006). Chaperones in preventing protein denaturation in living cells and protecting against cellular stress. *Handb. Exp. Pharmacol.,* **172**: 1-42.

Karimian MS, Pirro M, Majeed M and Sahebkar A (2017). Curcumin as a natural regulator of monocyte chemoattractant protein-1. *Cytokine Growth Factor Rev.* **33**: 55-63.

Korbecki J, Bobiński R and Dutka M (2019). Self-regulation of the inflammatory response by peroxisome proliferator-activated receptors. *Inflamm. Res.* **68**(6): 443-458.

Kwilasz AJ, Grace PM, Serbedzija P, Maier SF and Watkins LR (2015). The therapeutic potential of interleukin-10 in neuroimmune diseases. *Neuropharmacol.* **96**(Pt A): 55-69.

Li AL, Crystal JD, Lai YY, Sajdyk TJ, Renbarger JL and Hohmann AG (2021). An adolescent rat model of vincristine-induced peripheral neuropathy. *Neurobiol. pain (Cambridge, Mass.)* **10**: 100077.

Li G-Z, Hu Y-H, Li D-Y, Zhang Y, Guo H-L, Li Y-M, Chen F and Xu J (2020). Vincristine-induced peripheral neuropathy: A mini-review. *Neurotoxicol.* **81**: 161-171.

Li Q, Sun J, Mohammadtursun N, Wu J, Dong J and Li L (2019). Curcumin inhibits cigarette smoke-induced inflammation via modulating the PPARγ-NF-κB signaling pathway. *Food Funct.* **10**(12): 7983-7994.

Mazidi M, Karimi E, Meydani M, Ghayour-Mobarhan M and Ferns GA (2016). Potential effects of curcumin on peroxisome proliferator-activated receptor-γ *in vitro* and *in vivo* . *World J. Methodol,* **6**(1): 112.

Milligan ED, Sloane EM, Langer SJ, Hughes TS, Jekich BM, Frank MG, Mahoney JH, Levkoff LH, Maier SF, Cruz PE, Flotte TR, Johnson KW, Mahoney MM, Chavez RA, Leinwand LA and Watkins LR (2006). Repeated intrathecal injections of plasmid DNA encoding interleukin-10 produce prolonged reversal of neuropathic pain. *Pain* **126**(1-3): 294-308.

Necker R and Hellon RF (1978). Noxious thermal input from the rat tail: Modulation by descending inhibitory influences. *Pain* **4**(3): 231-242.

Pedrol N and Tamayo P (2001). Protein Content Quantification by Bradford Method. 283-295.

Qin XY, Lv JH, Cui J, Fang X and Zhang Y (2012). Curcumin protects against staurosporine toxicity in rat neurons. *Neurosci. Bull*. **28**(5): 606-610.

Ren B, Luo S, Tian X, Jiang Z, Zou G, Xu F, Yin T, Huang Y and Liu J (2018). Curcumin inhibits liver cancer by inhibiting DAMP molecule HSP70 and TLR4 signaling. *Oncol. Rep.* **40**(2): 895-901.

Ren G, Sun Z, Wang Z, Zheng X, Xu Z and Sun D (2019). Nanoemulsion formation by the phase inversion temperature method using polyoxypropylene surfactants. *J. Colloid Interface Sci.* **540**: 177-184.

Rezaee R, Momtazi AA, Monemi A and Sahebkar A (2017). Curcumin: A potentially powerful tool to reverse cisplatin-induced toxicity. *Pharmacol. Res.* **117**: 218-227.

Sabat R, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K and Geginat J (2010). Biology of interleukin-10. *Cytokine Growth Factor Rev.* **21**(5): 331-344.

Sanjay, Sharma A and Lee H-J (2021). Role of Phytoconstituents as PPAR Agonists: Implications for Neurodegenerative Disorders. *Biomedicines* **9**(12): 1914.

Seo SU, Min KJ, Woo SM, Seo JH and Kwon TK (2018). HSP70 Acetylation Prevents Combined mTORC1/2 Inhibitor and Curcumin Treatment-Induced Apoptosis. *Molecules* **23**(11): 2755.

Shen SQ, Zhang Y, Xiang JJ and Xiong CL (2007). Protective effect of curcumin against liver warm ischemia/reperfusion injury in rat model is associated with regulation of heat shock protein and antioxidant enzymes. *World J. Gastroenterol.* **13**(13): 1953-1961.

Shevtsov M, Balogi Z, Khachatryan W, Gao H, Vígh L and Multhoff G (2020). Membrane-Associated Heat Shock Proteins in Oncology: From Basic Research to New Theranostic Targets. *Cells.* **9**(5): 1263.

Sisignano M, Baron R, Scholich K and Geisslinger G (2014). Mechanism-based treatment for chemotherapy-induced peripheral neuropathic pain. *Nat. Rev. Neurol.* **10**(12): 694-707.

Sood A, Mathew R and Trachtman H (2001). Cytoprotective effect of curcumin in human proximal tubule epithelial cells exposed to shiga toxin. *Biochem. Biophys. Res. Commun.* **283**(1): 36-41.

Sudoh T, Kangawa K, Minamino N and Matsuo H (1988). A new natriuretic peptide in porcine brain. *Nature* **332**(6159): 78-81.

Teiten MH, Reuter S, Schmucker S, Dicato M and Diederich M (2009). Induction of heat shock response by curcumin in human leukemia cells. *Cancer Lett.* **279**(2): 145-154.

Thiagarajan VRK, Shanmugam P, Krishnan UM and Muthuraman A (2014). Ameliorative effect of Vernonia cinerea in vincristine-induced painful neuropathy in rats. *Toxicol. Ind. Health* **30**(9): 794-805.

Triarico S, Romano A, Attinà G, Capozza MA, Maurizi P, Mastrangelo S and Ruggiero A (2021). Vincristine-Induced Peripheral Neuropathy (VIPN) in Pediatric Tumors: Mechanisms, Risk Factors, Strategies of Prevention and Treatment. *Int. J. Mol. Sci.* **22**(8): 4112.

Vanderwall AG, Noor S, Sun MS, Sanchez JE, Yang XO, Jantzie LL, Mellios N and Milligan ED (2018). Effects of spinal non-viral interleukin-10 gene therapy formulated with D-mannose in neuropathic interleukin-10 deficient mice: Behavioral characterization, mRNA and protein analysis in pain relevant tissues. *Brain Behav. Immun.* **69**: 91-112.

Westerheide SD and Morimoto RI (2005). Heat shock response modulators as therapeutic tools for diseases of protein conformation. *J. Biol. Chem.* **280**(39): 33097-33100.

Wilkerson JL, Gentry KR, Dengler EC, Wallace JA, Kerwin AA, Armijo LM, Kuhn MN, Thakur GA, Makriyannis A and Milligan ED (2012). Intrathecal cannabilactone CB(2)R agonist, AM1710, controls pathological pain and restores basal cytokine levels. *Pain* **153**(5): 1091-1106.

Xie H, Chen Y, Du K, Wu W and Feng X (2020). Puerarin alleviates vincristine-induced neuropathic pain and neuroinflammation via inhibition of nuclear factor-κB and activation of the TGF-β/Smad pathway in rats. *Int. Immunopharmacol.* **89**(Pt B): 107060.

Zhang X, Zhang Y, Cai W, Liu Y, Liu H, Zhang Z and Su Z (2020). MicroRNA-128-3p Alleviates Neuropathic Pain Through Targeting ZEB1. *Neurosci. Lett.* **729**: 134946.