# Cardamonin inhibits nitric oxide production modulated through NMDA receptor in LPS-Induced SH-SY5Y cell *in vitro* model

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## ABSTRACT

**Background:** Cardamonin is a naturally occurring chalcone from the *Alpinia species*. It is known to possess antioxidant and anti-inflammatory properties. Our previous studies have shown that cardamonin has antihyperalgesic and antiallodynic effects on CCI-induced neuropathic pain in mice. Although the evidence of the association between cardamonin and neuropathic pain has been reported in animal studies, specific targets using *in vitro* models are still lacking. **Objectives/Methods:** This study aims to investigate the effect of cardamonin on nitric oxide production using the LPS-induced neuropathic pain-like SH-SY5Y in vitro model through NMDA receptor expression. **Results:** Cardamonin administration in differentiated SH-SY5Y cells significantly reduced nitric oxide production assessed using Griess reagent. Western blot analysis demonstrated a significant reduction in GluN2B receptor expression in the cardamonin treated SH-SY5Y cells compared to the vehicle treated group. **Conclusions:** These data suggest that cardamonin reduces nitric oxide production modulated through NMDA GluN2B receptor subunit. Our results provides preliminary data to support the *in vivo* studies using cardamonin and may contribute to further understanding the mechanisms of action of cardamonin.

***Keywords:*** *Cardamonin; NMDA receptor; SHSY-5Y cells; neuropathic pain*

## INTRODUCTION

Neuropathic pain is caused by a lesion or disease of the somatosensory system as defined by the International Association for the Study of Pain (IASP). Alteration of the somatosensory system disrupts the normal sensory transmission and subsequently give rise to neuropathic pain. The changes may come in several forms including injury of neuronal cells leading to the changes in growth-factor expression, hypersensitization of intact uninjured nociceptors and generation of spontaneous activities resulting in persistent pain (Campbell & Meyer, 2006). Neuropathic pain affects both central and peripheral nervous systems and are commonly linked to some of the common chronic diseases such as Parkinson's, multiple sclerosis, diabetic neuropathy and post-herpetic neuralgia.

Individuals with these diseases usually suffer from hyperalgesia and allodynia. Hyperalgesia is referred to an exaggerating pain to a painful stimulus while allodynia is pain arising from an innocuous stimlus (Gilron et al., 2015). IASP has reported that based on general population studies, 7-8% of adults are currently suffering from chronic pain with neuropathic characteristics (Van Hecke et al., 2014). The numerous adverse effects arising from the use of current medications have attracted neuropathic pain related researches. For example, ketamine and pregabalin, the two common drugs used to manage neuropathic pain symptoms are known to cause unwanted side effects such as hallucination (Niesters et al., 2014) and drowsiness (Bansal et al., 2009).

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In neuropathic pain conditions, the N-Methyl-D-Aspartate (NMDA), a glutamate receptor has been extensively studied. The NMDA receptor is responsible for the binding of glutamate at the postsynaptic neuron (Li et al., 2011). NMDA is required in the normal physiology of the body, however, an increase of NMDA receptor activation lead to the potentiation of numerous signaling cascade leading to peripheral and central sensitization (Petrenko et al., 2003). Injuries and lesions to the nerve leads to the activation of glutamate receptor specifically NMDA and triggers the nitric oxide (NO) pathway through the conversion of L-arginine into L-citrulline mediated through the $Ca^{2+}$ influx (Luo & Cizkova, 2000). Production of NO will further catalyze soluble guanylyl cyclase (sGC) which in turn generates cyclic guanosine monophosphate (cGMP) a secondary messenger causing pain hypersensitization (Maruyama et al., 2012; Ahlawat et al., 2014).

Neuropathic pain is also mediated through neuroinflammation at peripheral and central nervous systems leading to the initiation and maintenance of persistence pain (Ellis & Bennett, 2013). Neuroinflammation triggers the pro-inflammatory mediators to be released during neuronal injury including interleukin 1β and 6, tumor necrosis factor α (TNF α) and NO and thereby induce neuronal hypersensitization (Boje et al., 2003; Üçeyler et al., 2007; Miyamoto et al., 2009; Lurie, 2018).

SY-SY5Y is a human neuroblastoma cell line that possess a neuronal phenotype that has been widely used to study neurodegenerative diseases (Zhao, 2009; Kovalevich & Langford, 2013). The differentiated SH-SY5Y cells have been shown to be almost similar in morphology, biochemical and electrophysiology to the living neuron in the human brain (Xie et al., 2010). Besides, upon the differentiation of the cells, SH-SY5Y was shown to express similar receptor activation and signaling pathways as in neuropathic pain (Inada et al., 1998; Renauld & Spengler, 2002; Andaloussi-Lilja et al., 2009). On the other hand, lipopolysaccharide (LPS), a gram negative bacteria induced neuroinflammation is an established model to induced neuropathic pain condition in numerous *in vivo* and *in vitro* study (Clark et al., 2010; Yoon et al., 2012; K. Sharma et al., 2018). Induction of LPS to SHSY5Y was shown to increase the release of pro-inflammatory mediators and increase glutamate receptor channel expression which is important in maintenance of neuropathic pain (Mengke et al., 2016; Chia et al., 2020). Therefore, LPS-induced SH-SY5Y cell model is a reliable model to be used in this study to mimic the neuropathic pain-like condition *in vitro*.

In this study, cardamonin, a type of naturally occurring chalcone that is isolated primarily from fruits or seeds of *Alpinia* species (Rao et al., 1976) was used to evaluate its potential to treat neuropathic pain in LPS-induced neuropathic pain-like condition *in-vitro* model of SH-SY5Y human neuroblastoma cells. A previous study by (Lee et al., 2012) reported that cardamonin exhibits anti-inflammatory and other numerous pharmacological properties such as anti-cancer (Park et al., 2013) and anti-oxidant (Bajgai et al., 2011). Our previous study also demonstrated that cardamonin was able to alleviate hyperalgesia and allodynia in CCI-induced neuropathic pain mice (Sambasevam et al., 2017). However, a study on how cardamonin affects defined molecules or proteins in alleviating neuropathic pain is still lacking. This *in-vitro* study was conducted to further support our *in vivo* findings. Therefore, this study aims to investigate the effect of cardamonin in LPS-induced neuropathic pain-like condition *in vitro* model of SH-SY5Y cells modulated through the NMDA receptor and NO pathway.

## MATERIALS AND METHODS

### Materials

Cardamonin or 2',4'-dihydroxy-6'-methoxychalcone was purchased from Calbiochem (US), RIPA buffer, Protease inhibitor and Chemiluminescent reagent kit. Dimethyl sulfoxide and Griess reagent were purchased from Sigma Aldrich (St. Louis, US). Dulbecco's modified essential medium/Ham's Nutrient Mixture (DMEM:F12), 1% Penicillin-Streptomycin solution, Trypsin, and 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium Bromide (MTT) from Nacalai Tesque (Tokyo, Japan). Inactivated fetal bovine albumin (FBS) and Non-Essential Amino Acid (NEAA) were purchase from Gibco-BRL (Grand Island, NY). Lipopolysaccharide (LPS) and BCA reagent kit were purchased from Merck (Darmstadt, Germany) and Thermo Fisher (Massachusetts, US) respectively.

### Compound preparation

100 μg/mL of cardamonin stock was freshly prepared in 100% DMSO and further diluted with media containing DMEM/Nutrient Mixture F-12 (DMEM:F12), 1% Penicillin-Streptomycin solution and 15% heat-inactivated fetal bovine albumin (FBS) to a concentration of interest; 2.5 μg/mL, 5.0 μg/mL and 10.0 μg/mL (Hatziieremia et al., 2006; Kim et al., 2010; El-Naga, 2014). The final concentration of DMSO was kept constant at 0.1% and it does not affect the cell viability (Pascoal et al., 2014).

### Cell culture

SH-SY5Y human neuroblastoma cell lines were obtained from ATCC, thawed and cultured using growth medium mixture; DMEM:F12 which contains 4.5g/l glucose with 2mM of L-glutamate and sodium pyruvate supplemented with 15% heat-inactivated FBS, 1% of Penicillin-Streptomycin mixed solution and 1% NEAA maintained at 37˚C humidified incubator containing 5% $CO_2$ were seeded in a 96-well plate with cell density of $1X10^4$ cells/well. The cells were induced with 10μM of all-*trans* retinoic acid for 5 days to induce differentiation in differentiation media (DMEM:F12, supplemented with 2.5% fetal bovine serum (FBS) and 1% of Penicillin-Streptomycin mixed solution) (Chia et al., 2020; Mohammed Izham et al., 2018).

### Cell viability

MTT assay was used the measure the viability of SH-SY5Y cells. The media on the cell were discarded. 50 μL of serum-free media and 50 μL of MTT solution were added in each group and incubated for 3 hours at 37°C. After incubation, 150 μL of MTT solvent was added into each well. The plate was wrapped in foil and shaken for 15 minutes. Then, the absorbance was read at 590nm. The percentage of cell viability was compared to the non-treated group, and the percentage of viable cells were calculated.

### LPS induction and treatment groups

LPS was used to induce neuronal sensitization to mimic neuropathic pain like condition *in vitro*. Following differentiation, the cells were induced with 1 μg/mL of freshly prepared LPS (except normal control group) for 12 hours maintained in 37°C and 5% $CO_2$ incubator (Chia et al., 2020). 12 hours post LPS induction, three different concentrations of cardamonin (2.5 μg/mL, 5.0 μg/mL and 10.0 μg/mL), 16 μg/mL of L-NAME as positive control and vehicle (0.1% DMSO) were added to the LPS-induce cell culture for 24 hours.

### NO measurements

NO concentration was assessed using Griess' reagent (Bryan & Grisham, 2007). After 24 hours treatment, 100 μL of cell culture media with an equal volume of Griess' reagent were added to each group and incubated for 15 minutes at room temperature. The incubation was done in a light-protected environment and absorbance was read at 540 nm using a microplate reader. The amount of nitrite in the media was calculated from sodium nitrite (NaNO2) standard curve.

### Western blot

Further evaluation on NMDA protein expression was done using western blot. 24 hours post treatment, the media was removed and the cells were washed with pre-chilled PBS. 200μl of RIPA and protease inhibitor mixture were added before scrapping the cells in the plate. The

samples were then centrifuged at 10,000 rpm for 10 minutes and the supernatants were used to measure the protein using bicinchoninic acid assay (BCA). Equal amount of 10µg of proteins were separated in 4-20% of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred into polyvinylidene di-fluoride (PVDF) membranes. The membranes were then blocked using 5% skimmed milk in TBST for 1 hour and washed for 3 times with 5 minutes interval. Then, the membranes were incubated with primary antibody (GluN2b, 1:1000; Cell Signaling Technology, US; β-actin, 1:5000; Abcam Group, UK) overnight followed by secondary antibody (anti-goat IgG, 1:5000; Abcam Group, UK) for one hour with continuous agitation. The membranes were visualized using ECL solution (Advansta, USA) and detected by ChemiDoc™ imaging system. The band were measured and analysed using NIH ImageJ software.

**Statistical analysis**

All results were presented as mean ± S.E.M. Data was statistically analysed using one-way analysis variance (ANOVA) followed by post-hoc Tukey test using GraphPad Prism software, v8.0 (GraphPad San Diego, CA). Results were considered significant when $p<0.05$.

## RESULTS

**Cell viability**

The effect of cardamonin on SH-SY5Y cell viability was measured using MTT assay. 12 hours post-treatment of three different cardamonin doses produced an increase in cell viability compared to the non-treated cells. Figure 1 shows a significant increase in SH-SY5Y cell viability when treated with 5ug/mL and 10ug/mL of cardamonin with significant level of $p<0.05$ and $p<0.001$ respectively. On the other hand, the lowest dose of cardamonin 2.5ug/mL did not significantly increase or decrease the SH-SY5Y cell viability. Thus, we suggest that all the cardamonin doses do not cause toxicity to the cells.

**Nitric oxide production**

The effect of cardamonin on nitric oxide production in LPS-induced SH-SY5Y human neuroblastoma cells was evaluated by measuring total nitrate concentration as presented in Figure 2. The cells (except normal, N group) were administered with 1 µg/mL LPS for 24 hours. Following that, the cells were treated with respective doses of cardamonin for 24 hours before the total nitrite concentration was measured using Griess reagent. LPS and vehicle treated groups showed the highest total nitrate concentration compared to the normal SH-SY5Y cell. Different concentrations of cardamonin at 2.5 µg/mL, 5 µg/mL and 10 µg/mL significantly reduced the total nitrate concentrations at $p<0.001$ compared to LPS only and vehicle treated groups. The highest dose of cardamonin 10ug/mL showed highest total nitrate concentration reduction. Based on this study and previously used dose of cardamonin, 10 µg/mL will be used for further evaluation on protein expression.

**NMDA GluN2B protein expression**

NMDA protein expression specifically the N2B subtype in cells was evaluated using western blotting. Figure 3 shows a significant reduction with $p<0.05$ on NMDA GluN2B expression in cardamonin treated cells compared to LPS only group. Cardamonin treated cells also managed to further reduce the NMDA GluN2B protein expression compared to the vehicle treated group however no significant difference was found between the two groups.

## DISCUSSION

Our previous studies have demonstrated that cardamonin possesses antihyperalgesic and antiallodynic effects in CCI-induced neuropathic pain mice (Sambasevam et al., 2017). Even though numerous *in vivo* studies have been conducted to understand the mechanisms of neuropathic pain, the exact pathway remains unclear. One of the main reason is due to the multiple factors involved in the progression of neuropathic pain, types of immune cells activated, site of injury and type of injury involved (Colburn et al., 1999; Obata et al., 2006; Calvo et al., 2012; Inoue & Tsuda, 2018).

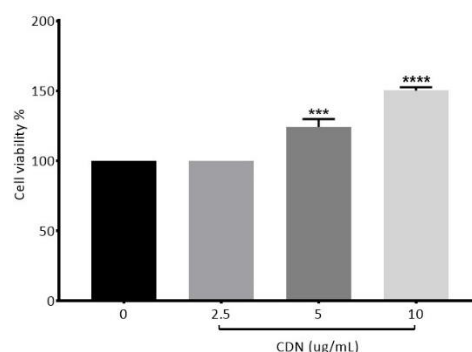


**Figure 1:** Cell viability of SH-SY5Y human neuroblastoma cells were determine using MTT assay. Data were presented in mean ± SEM with n=4. Significant difference was measured through one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P level was set at *$p<0.05$/****$p<0.001$ compared to non-treated group. CDN (Cardamonin).

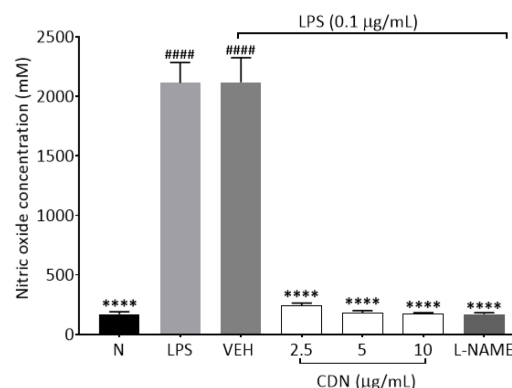


**Figure 2:** Total nitrate concentration in SH-SY5Y human neuroblastoma cells was determined using Griess assay. Data were presented in mean ± SEM with n=3. Significant difference was measured through one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P level was set at ****$p<0.001$ compared to LPS group and ####$p<0.001$ compared to normal group. N (Normal); VEH (Vehicle, 0.1% DMSO); CDN (Cardamonin).

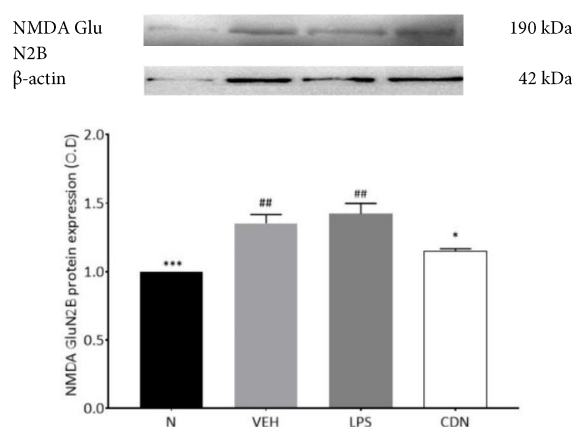


**Figure 3:** NMDA GluN2B receptor expression in LPS-induce SHSY5Y cells samples of normal, vehicle, LPS only and cardmonin-treated groups were evaluated using western blotting. Data were presented in mean ± SEM with n=4. *$p<0.05$/****$p<0.001$ as compared to LPS only group and ##$p<0.01$ as compared to normal group. N (Normal); VEH (Vehicle, 0.1% DMSO); CDN (Cardamonin, 10 µg/ml).

Studies using specific *in-vitro* models may be beneficial in finding specific pathways and may help to provide more effective treatment in the future for neuropathic pain. This study aimed to investigate the effect of cardamonin in LPS-induced SH-SY5Y cells a neuropathic pain-like condition *in- vitro* model.

As described earlier, neuropathic pain arises due to a lesion or injury at the somatosensory system (Campbell & Meyer, 2006). Several mechanisms have been postulated to involve in neuropathic pain disease progression including inflammation (Scholz & Woolf, 2007; Baron et al., 2010). Nerve injury or lesion of the neuron leads to the infiltration of pro-inflammatory mediators to cause pain sensitization (Moalem & Tracey, 2006; Thacker et al., 2007). Excessive inflammation that occurs in peripheral and central nervous systems promotes the neuroimmune activation and sensitizes the primary efferent neurons that contribute to the initiation and maintenance of pain (Moalem & Tracey, 2006; Ellis & Bennett, 2013).

Several types of pro-inflammatory mediators that are released during inflammation that mediates neuropathic pain sensitization including IL1β, IL6, TNF-α and NO (Milligan et al., 2003; J. Sharma et al., 2007; Leung & Cahill, 2010; Clark et al., 2013). Compared to the other pro-inflammatory mediators, nitric oxide has been studied widely in the pathophysiology of neuropathic pain (Luo & Cizkova, 2000). This is due to the dual function of NO that is able to promote and inhibit nociception depending on the availability and downstream pathway activation in peripheral and central nervous system (Sousa & Prado, 2001; Cury et al., 2011). Nitric oxide production is known to be enhanced in neuropathic pain models through the increase of nitric oxide synthase activity at the injured nerve (Levy & Zochodne, 2004; Naik et al., 2006). Therefore, inducing nitric oxide production in cells can be used to mimic the neuropathic pain-like condition in *in-vitro* studies.

In this experiment, lipopolysaccharide (LPS) has been used to induce nitric oxide synthesis in human neuroblastoma cells (SH-SY5Y) to mimic the *in-vitro* model of neuropathic pain-like condition. LPS is a structure of gram-negative bacteria that is important in immunomodulatory effects (Thomson & Moland, 2000). High concentration of LPS in the cells is able to enhance the release proinflammatory mediators to trigger the inflammatory response causing the development of neuropathic pain (Caroff & Karibian, 2003; Tanga et al., 2005; Lin et al., 2008; Xu et al., 2013). According to the data reported by (Chen et al., 2001), LPS treatment in raw cells were able to enhance the production of nitric oxide mediated through nitric oxide synthase activity. In line with this, exposure of LPS in SH-SY5Y cells also enhance total nitrate concentration when compared to non-treated LPS cells. In the present study, our data shows that cardamonin reduced nitric oxide levels in the LPS-induced SHSY5Y cells model indicating an anti-inflammatory effects on the neuronal cells.

Unlike the other inflammatory mediators, NO is produced through the activation of nitric oxide synthase (NOS) enzyme consisting of three different isoforms including neuronal NOS, inducible NOS and endothelial NOS. All nitric oxide synthases are $Ca^{2+}$-calmodulin-dependent enzymes constitutively produce in the body except for inducible NOS which is depend on $Ca^{2+}$ influx (Ortiz-Ortiz et al., 2009; Förstermann & Sessa, 2011; Joca et al., 2019). Transmission of $Ca^{2+}$ into the postsynaptic neuron is facilitated through the glutamate channel, N-Methyl-D-Aspartate (NMDA) receptor. Nerve injury to the neurons enhance glutamate release at the presynaptic membrane and subsequently activate NMDA receptors, thereby increasing the $Ca^{2+}$ influx which then triggers the nitric oxide cascade in the neuron (Cury et al., 2011; Lüscher & Malenka, 2012; Mukherjee et al., 2014).

NMDA receptor is further subdivided into different subunit including GluN1, GluN2, and GluN3 (Cull-Candy et al., 2001). Among the NMDA subunit, NR2B subtypes are known to be responsible for pain transmission through the activation of the downstream pathway that leads to pain sensitization (Qiu et al., 2017). Our study demonstrated that GluN2B expression increased in SH-SY5Y cells upon LPS exposure. In addition, our result also reveals that cardamonin treatment in LPS induced group reduces NMDA GluN2B receptor subunit expression in SH-SY5Y cells which in turn reduces the nitric oxide production. Therefore, the current data suggests pretreatment of LPS and co-administration of cardamonin after LPS exposure is able to reduce neuropathic pain-like neuroinflammation condition in the *in-vitro* model used.

## CONCLUSION

In summary, the present study shows that LPS successfully induced neuroinflammation in the SH-SY5Y used and is able to mimic neuropathic pain-like condition in an *in-vitro* model. This process is modulated through nitric oxide and NMDA GluN2B receptor. Cardamonin was shown to reduce the GluN2b expression and eventually reduce nitric oxide production in SH-SY5Y cells. Thus, this study provides an insight into the role of cardamonin in SH-SY5Y cells for the development of a more specific treatment for neuropathic pain in future.

## CONFLICT OF INTEREST

All authors declare no conflict of interest.

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## REFERENCES

Ahlawat, A., Rana, A., Goyal, N., & Sharma, S. (2014). Potential role of nitric oxide synthase isoforms in pathophysiology of neuropathic pain. Inflammopharmacology, 22(5), 269-278. https://doi.org/10.1007/s10787-014-0213-0

Andaloussi-Lilja, J. E., Lundqvist, J., & Forsby, A. (2009). TRPV1 expression and activity during retinoic acid-induced neuronal differentiation. Neurochemistry international, 55(8), 768-774. https://doi.org/10.1016/j.neuint.2009.07.011

Attal, N., Lanteri-Minet, M., Laurent, B., Fermanian, J., & Bouhassira, D. (2011). The specific disease burden of neuropathic pain: results of a French nationwide survey. Pain, 152(12), 2836-2843. https://doi.org/10.1016/j.pain.2011.09.014

Bajgai, S. P., Prachyawarakorn, V., Mahidol, C., Ruchirawat, S., & Kittakoop, P. (2011). Hybrid flavan-chalcones, aromatase and lipoxygenase inhibitors, from Desmos cochinchinensis. Phytochemistry, 72(16), 2062-2067. https://doi.org/10.1016/j.phytochem.2011.07.002

Bansal, D., Bhansali, A., Hota, D., Chakrabarti, A., & Dutta, P. (2009). Amitriptyline vs. pregabalin in painful diabetic neuropathy: a randomized double blind clinical trial. Diabetic medicine, 26(10), 1019-1026. https://doi.org/10.1111/j.1464-5491.2009.02806.x

Baron, R., Binder, A., & Wasner, G. (2010). Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. The Lancet Neurology, 9(8), 807-819. https://doi.org/10.1016/S1474-4422(10)70143-5

Boje, K. M., Jaworowicz, D., & Raybon, J. J. (2003). Neuroinflammatory role of prostaglandins during experimental meningitis: evidence suggestive of an in vivo relationship between nitric oxide and prostaglandins. Journal of Pharmacology and Experimental Therapeutics, 304(1), 319-325. https://doi.org/10.1124/jpet.102.041533

Bryan, N. S., & Grisham, M. B. (2007). Methods to detect nitric oxide and its metabolites in biological samples. Free Radical Biology and Medicine, 43(5), 645-657. https://doi.org/10.1016/j.freeradbiomed.2007.04.026

Calvo, M., Dawes, J. M., & Bennett, D. L. (2012). The role of the immune system in the generation of neuropathic pain. The Lancet Neurology, 11(7), 629-642. https://doi.org/10.1016/S1474-4422(12)70134-5

Campbell, J. N., & Meyer, R. A. (2006). Mechanisms of neuropathic pain. Neuron, 52(1), 77-92. https://doi.org/10.1016/j.neuron.2006.09.021

Caroff, M., & Karibian, D. (2003). Structure of bacterial lipopolysaccharides. Carbohydrate research, 338(23), 2431-2447.

https://doi.org/10.1016/j.carres.2003.07.010

Chen, Y.-C., Shen, S.-C., Chen, L.-G., Lee, T. J., & Yang, L.-L. (2001). Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expressions induced by nitric oxide synthase inhibitors and lipopolysaccharide. Biochemical pharmacology, 61(11), 1417-1427. https://doi.org/10.1016/S0006-2952(01)00594-9

Chia, J. S. M., Izham, N. A. M., Farouk, A. A. O., Sulaiman, M. R., Mustafa, S., Hutchinson, M. R., & Perimal, E. K. (2020). Zerumbone Modulates α2A-Adrenergic, TRPV1, and NMDA NR2B Receptors Plasticity in CCI-Induced Neuropathic Pain In Vivo and LPS-Induced SH-SY5Y Neuroblastoma In Vitro Models. Frontiers in Pharmacology, 11(92). https://doi.org/10.3389/fphar.2020.00092

Clark, A. K., Old, E. A., & Malcangio, M. (2013). Neuropathic pain and cytokines: current perspectives. Journal of pain research, 6, 803. https://doi.org/10.2147/JPR.S53660

Clark, A. K., Staniland, A. A., Marchand, F., Kaan, T. K., McMahon, S. B., & Malcangio, M. (2010). P2X7-dependent release of interleukin-1β and nociception in the spinal cord following lipopolysaccharide. Journal of Neuroscience, 30(2), 573-582. https://doi.org/10.1523/JNEUROSCI.3295-09.2010

Colburn, R., Rickman, A., & DeLeo, J. (1999). The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. Experimental neurology, 157(2), 289-304. https://doi.org/10.1006/exnr.1999.7065

Cull-Candy, S., Brickley, S., & Farrant, M. (2001). NMDA receptor subunits: diversity, development and disease. Current opinion in neurobiology, 11(3), 327-335. https://doi.org/10.1016/S0959-4388(00)00215-4

Cury, Y., Picolo, G., Gutierrez, V. P., & Ferreira, S. H. (2011). Pain and analgesia: the dual effect of nitric oxide in the nociceptive system. Nitric oxide, 25(3), 243-254. https://doi.org/10.1016/j.niox.2011.06.004

El-Naga, R. N. (2014). Pre-treatment with cardamonin protects against cisplatin-induced nephrotoxicity in rats: impact on NOX-1, inflammation and apoptosis. Toxicology and applied pharmacology, 274(1), 87-95. https://doi.org/10.1016/j.taap.2013.10.031

Ellis, A., & Bennett, D. (2013). Neuroinflammation and the generation of neuropathic pain. British journal of anaesthesia, 111(1), 26-37. https://doi.org/10.1093/bja/aet128

Förstermann, U., & Sessa, W. C. (2011). Nitric oxide synthases: regulation and function. European heart journal, 33(7), 829-837. https://doi.org/10.1093/eurheartj/ehr304

Gilron, I., Baron, R., & Jensen, T. (2015). Neuropathic pain: principles of diagnosis and treatment. Paper presented at the Mayo Clinic Proceedings. https://doi.org/10.1016/j.mayocp.2015.01.018

Hatziieremia, S., Gray, A., Ferro, V., Paul, A., & Plevin, R. (2006). The effects of cardamonin on lipopolysaccharide-induced inflammatory protein production and MAP kinase and NFκB signalling pathways in monocytes/macrophages. British journal of pharmacology, 149(2), 188-198. https://doi.org/10.1038/sj.bjp.0706856

Inada, H., Shindo, H., Tawata, M., & Onaya, T. (1998). cAMP regulates nitric oxide production and ouabain sensitive Na+, K+-ATPase activity in SH-SY5Y human neuroblastoma cells. Diabetologia, 41(12), 1451-1458. https://doi.org/10.1007/s001250051091

Inoue, K., & Tsuda, M. (2018). Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nature Reviews Neuroscience, 19(3), 138. https://doi.org/10.1038/nrn.2018.2

Joca, S. R., Sartim, A. G., Roncalho, A. L., Diniz, C. F., & Wegener, G. (2019). Nitric oxide signalling and antidepressant action revisited. Cell and tissue research, 1-14. https://doi.org/10.1007/s00441-018-02987-4

Kim, Y.-J., Ko, H., Park, J.-S., Han, I.-H., Amor, E. C., Lee, J. W., & Yang, H. O. (2010). Dimethyl cardamonin inhibits lipopolysaccharide-induced inflammatory factors through blocking NF-κB p65 activation. International immunopharmacology, 10(9), 1127-1134. https://doi.org/10.1016/j.intimp.2010.06.017

Kovalevich, J., & Langford, D. (2013). Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology Neuronal cell culture (pp. 9-21): Springer. https://doi.org/10.1007/978-1-62703-640-5_2

Lee, M.-Y., Seo, C.-S., Lee, J.-A., Shin, I.-S., Kim, S.-J., Ha, H., & Shin, H.-K. (2012). Alpinia katsumadai H AYATA Seed Extract Inhibit LPS-Induced Inflammation by Induction of Heme Oxygenase-1 in RAW264. 7 Cells. Inflammation, 35(2), 746-757. https://doi.org/10.1007/s10753-011-9370-0

Leung, L., & Cahill, C. M. (2010). TNF-α and neuropathic pain-a review. Journal of neuroinflammation, 7(1), 27. https://doi.org/10.1186/1742-2094-7-27

Levy, D., & Zochodne, D. W. (2004). NO pain: potential roles of nitric oxide in neuropathic pain. Pain Practice, 4(1), 11-18. https://doi.org/10.1111/j.1533-2500.2004.04002.x

Li, J.-H., Vicknasingam, B., Cheung, Y.-w., Zhou, W., Nurhidayat, A. W., Des Jarlais, D. C., & Schottenfeld, R. (2011). To use or not to use: an update on licit and illicit ketamine use. Substance abuse and rehabilitation, 2, 11. https://doi.org/10.2147/SAR.S15458

Lin, W., Wu, R. T., Wu, T., Khor, T.-O., Wang, H., & Kong, A.-N. (2008). Sulforaphane suppressed LPS-induced inflammation in mouse peritoneal macrophages through Nrf2 dependent pathway. Biochemical pharmacology, 76(8), 967-973. https://doi.org/10.1016/j.bcp.2008.07.036

Luo, Z. D., & Cizkova, D. (2000). The role of nitric oxide in nociception. Current review of pain, 4(6), 459-466. https://doi.org/10.1007/s11916-000-0070-y

Lurie, D. I. (2018). An integrative approach to neuroinflammation in psychiatric disorders and neuropathic pain. Journal of experimental neuroscience, 12, 1179069518793639. https://doi.org/10.1177/1179069518793639

Lüscher, C., & Malenka, R. C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harbor perspectives in biology, 4(6), a005710. https://doi.org/10.1101/cshperspect.a005710

Maruyama, K., Okamoto, T., & Shimaoka, M. (2012). Integrins and nitric oxide in the regulation of glia cells: potential roles in pathological pain. J Anesth Clin Res, 4(292), 2. https://doi.org/10.4172/2155-6148.S7-008

Mengke, N. S., Hu, B., Han, Q. P., Deng, Y. Y., Fang, M., Xie, D., Li, A., & Zeng, H. K. (2016). Rapamycin inhibits lipopolysaccharide-induced neuroinflammation in vitro and in vivo. Molecular medicine reports, 14(6), 4957-4966. https://doi.org/10.3892/mmr.2016.5883

Milligan, E. D., Twining, C., Chacur, M., Biedenkapp, J., O'Connor, K., Poole, S., Tracey, K., Martin, D., Maier, S. F., & Watkins, L. R. (2003). Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. Journal of Neuroscience, 23(3), 1026-1040. https://doi.org/10.1523/JNEUROSCI.23-03-01026.2003

Miyamoto, T., Dubin, A. E., Petrus, M. J., & Patapoutian, A. (2009). TRPV1 and TRPA1 mediate peripheral nitric oxide-induced nociception in mice. PloS one, 4(10). https://doi.org/10.1371/journal.pone.0007596

Moalem, G., & Tracey, D. J. (2006). Immune and inflammatory mechanisms in neuropathic pain. Brain research reviews, 51(2), 240-264. https://doi.org/10.1016/j.brainresrev.2005.11.004

Mohammed Izham, N. A., Chia, J. S. M., Vidyadaran, S., Sulaiman, M. R., Bharatham, B. H., & Perimal, E. K. (2018). The Effect of DMEM and DMEM:F12 Culture Media on the Growth of SH-SY5Y Cells. Life Sciences, Medicine and Biomedicine, 2(3). https://doi.org/10.28916/lsmb.2.3.2018.23

Mukherjee, P., Cinelli, M. A., Kang, S., & Silverman, R. B. (2014). Development of nitric oxide synthase inhibitors for neurodegeneration and neuropathic pain. Chemical Society Reviews, 43(19), 6814-6838. https://doi.org/10.1039/C3CS60467E

Naik, A. K., Tandan, S. K., Kumar, D., & Dudhgaonkar, S. P. (2006). Nitric oxide and its modulators in chronic constriction injury-induced neuropathic pain in rats. European journal of pharmacology, 530(1-2), 59-69. https://doi.org/10.1016/j.ejphar.2005.11.029

Niesters, M., Martini, C., & Dahan, A. (2014). Ketamine for chronic pain: risks and benefits. British journal of clinical pharmacology, 77(2), 357-367. https://doi.org/10.1111/bcp.12094

Obata, K., Yamanaka, H., Kobayashi, K., Dai, Y., Mizushima, T., Katsura, H., Fukuoka, T., Tokunaga, A., & Noguchi, K. (2006). The effect of site and type of nerve injury on the expression of brain-derived neurotrophic factor in the dorsal root ganglion and on neuropathic pain behavior. Neuroscience, 137(3), 961-970. https://doi.org/10.1016/j.neuroscience.2005.10.015

Ortiz-Ortiz, M. A., Morán, J. M., González-Polo, R. A., Niso-Santano, M., Soler, G., Bravo-San Pedro, J. M., & Fuentes, J. M. (2009). Nitric oxide-mediated toxicity in paraquat-exposed SH-SY5Y cells: a protective role of 7-nitroindazole. Neurotoxicity research, 16(2), 160-173. https://doi.org/10.1007/s12640-009-9065-6

Park, S., Gwak, J., Han, S. J., & Oh, S. (2013). Cardamonin suppresses the proliferation of colon cancer cells by promoting β-catenin degradation. Biological and Pharmaceutical Bulletin, b13-00158. https://doi.org/10.1248/bpb.b13-00158

Pascoal, A. C. R. F., Ehrenfried, C. A., Lopez, B. G.-C., De Araujo, T. M., Pascoal,

V., Gilioli, R., Anhê, G. F., Ruiz, A. L. T. G., Carvalho, J. E. d., & Stefanello, M. É. A. (2014). Antiproliferative activity and induction of apoptosis in PC-3 cells by the chalcone cardamonin from Campomanesia adamantium Myrtaceae) in a bioactivity-guided study. Molecules, 19(2), 1843-1855. https://doi.org/10.3390/molecules19021843

Petrenko, A. B., Yamakura, T., Baba, H., & Shimoji, K. (2003). The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review. Anesthesia & Analgesia, 97(4), 1108-1116. https://doi.org/10.1213/01.ANE.0000081061.12235.55

Qiu, Q., Sun, L., Wang, X. M., Lo, A. C., Wong, K. L., Gu, P., Wong, S. C. S., & Cheung, C. W. (2017). Propofol produces preventive analgesia via GluN2B-containing NMDA receptor/ERK1/2 signaling pathway in a rat model of inflammatory pain. Molecular pain, 13, 1744806917737462. https://doi.org/10.1177/1744806917737462

Rao, C. B., Rao, T. N., & Suryaprakasam, S. (1976). Cardamonin and alpinetin from the seeds of Amomum subulatum. Planta Medica, 29(04), 391-392. https://doi.org/10.1055/s-0028-1097682

Renauld, A., & Spengler, R. (2002). Tumor necrosis factor expressed by primary hippocampal neurons and SH-SY5Y cells is regulated by α2-adrenergic receptor activation. Journal of neuroscience research, 67(2), 264-274. https://doi.org/10.1002/jnr.10101

Sambasevam, Y., Farouk, A. A. O., Mohamad, T. A. S. T., Sulaiman, M. R., Bharatham, B. H., & Perimal, E. K. (2017). Cardamonin attenuates hyperalgesia and allodynia in a mouse model of chronic constriction injury-induced neuropathic pain: Possible involvement of the opioid system. European journal of pharmacology, 796, 32-38. https://doi.org/10.1016/j.ejphar.2016.12.020

Scholz, J., & Woolf, C. J. (2007). The neuropathic pain triad: neurons, immune cells and glia. Nature neuroscience, 10(11), 1361. https://doi.org/10.1038/nn1992

Sharma, J., Al-Omran, A., & Parvathy, S. (2007). Role of nitric oxide in inflammatory diseases. Inflammopharmacology, 15(6), 252-259. https://doi.org/10.1007/s10787-007-0013-x

Sharma, K., Sharma, D., Sharma, M., Sharma, N., Bidve, P., Prajapati, N., Kalia, K., & Tiwari, V. (2018). Astaxanthin ameliorates behavioral and biochemical alterations in in-vitro and in-vivo model of neuropathic pain. Neuroscience letters, 674, 162-170. https://doi.org/10.1016/j.neulet.2018.03.030

Sousa, A. M., & Prado, W. A. (2001). The dual effect of a nitric oxide donor in nociception. Brain research, 897(1-2), 9-19. https://doi.org/10.1016/S0006-8993(01)01995-3

Tanga, F. Y., Nutile-McMenemy, N., & DeLeo, J. A. (2005). The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proceedings of the National Academy of Sciences, 102(16), 5856-5861. https://doi.org/10.1073/pnas.0501634102

Thacker, M. A., Clark, A. K., Marchand, F., & McMahon, S. B. (2007). Pathophysiology of peripheral neuropathic pain: immune cells and molecules. Anesthesia & Analgesia, 105(3), 838-847. https://doi.org/10.1213/01.ane.0000275190.42912.37

Thomson, K. S., & Moland, E. S. (2000). Version 2000: the new β-lactamases of Gram-negative bacteria at the dawn of the new millennium. Microbes and Infection, 2(10), 1225-1235. https://doi.org/10.1016/S1286-4579(00)01276-4

Üçeyler, N., Rogausch, J. P., Toyka, K. V., & Sommer, C. (2007). Differential expression of cytokines in painful and painless neuropathies. Neurology, 69(1), 42-49. https://doi.org/10.1212/01.wnl.0000265062.92340.a5

Van Hecke, O., Austin, S. K., Khan, R. A., Smith, B., & Torrance, N. (2014). Neuropathic pain in the general population: a systematic review of epidemiological studies. PAIN®, 155(4), 654-662. https://doi.org/10.1016/j.pain.2013.11.013

Xie, H.-r., Hu, L.-s., & Li, G.-y. (2010). SH-SY5Y human neuroblastoma cell line: in vitrocell model of dopaminergic neurons in Parkinson's disease. Chinese medical journal, 123(8), 1086-1092.

Xu, Z.-Z., Berta, T., & Ji, R.-R. (2013). Resolvin E1 inhibits neuropathic pain and spinal cord microglial activation following peripheral nerve injury. Journal of neuroimmune pharmacology, 8(1), 37-41. https://doi.org/10.1007/s11481-012-9394-8

Yoon, S.-Y., Patel, D., & Dougherty, P. M. (2012). Minocycline blocks lipopolysaccharide induced hyperalgesia by suppression of microglia but not astrocytes. Neuroscience, 221, 214-224. https://doi.org/10.1016/j.neuroscience.2012.06.024

Zhao, B. (2009). Natural antioxidants protect neurons in Alzheimer's disease and Parkinson's disease. Neurochemical Research, 34(4), 630-638. https://doi.org/10.1007/s11064-008-9900-9

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