Extracellular Vesicles from Human Umbilical Cord Mesenchymal Stem Cells Loaded with Cannabidiol Alleviate Paclitaxel Induced Peripheral Neuropathy

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Abstract

PURPOSE: Chronic paclitaxel (PTX) treatment causes excruciating pain in cancer patients, limiting its use in cancer chemotherapy. Herein, neuroprotective potential of synthetic cannabidiol (CBD) and CBD formulated in extracellular vesicles (CBD-EVs) isolated from human umbilical cord derived mesenchymal stem cells were studied against PTX-induced neuropathic pain (PIPN) in C57BL/6J mice

METHODS: PTX (8 mg/kg, i.p,) was injected every other day (four doses) to induce neuropathy in C57BL/6J mice. CBD and CBD-EVs was administered (5 mg/kg, i.p) for 6 weeks with twice a week frequency. At the end of study, behavior of the animals towards pain perception were measured using Hargreaves plantar apparatus, hot and cold tail immersion test, vonfrey aesthesiometer and randalsellito apparatus. Dorsal root ganglions (DRGs) were isolated from animals for molecular studies. Further, invitro studies were conducted in DRG primary cultures to study the mitochondrial effects of CBD and CBD-EVs against PTX insult.

RESULTS: EVs and CBD-EVs particle size, surface roughness, nanomechanical attributes, stability, and release studies were investigated. CBD-EVs treatment significantly improved mechanical and thermal hypersensitivity (P<0.001) as compared to EVs or CBD alone. PTX-treated mice's dorsal root ganglions and spinal homogenates had mitochondrial dysfunction which was significantly improved by CBD and CBD-EVs by regulating the AMPK pathway (P<0.001). Blocking studies with 5HT1A receptors and AMPK demonstrated that CBD had no effect on PIPN neurobehavioral or mitochondrial function.

CONCLUSION: Our results suggest that CBD-EVs can be a novel therapeutic option for the treatment of PIPN and CBD treatment activates AMPK axis in regulating PIPN.

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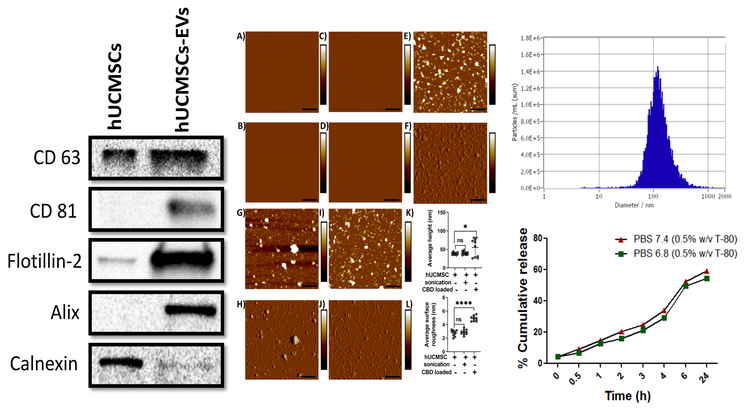
Introduction

- Paclitaxel (PTX) is the most potent drug in the taxanes group and is used to treat aggressive and metastatic breast, , ovarian, pancreatic, non-small-cell lung and Kaposi's sarcoma cancers
- The incidence of neuropathy from PTX is one of the major clinical challenges which has a very high prevalence ranging from 11 to 87%
- It can lead to development of sensory dominant neuropathy manifesting clinical outcomes like paraesthesias, dysesthesia, altered proprioception , numbness, and loss of sensitivity in the fingers and finger tips
- Current therapies for PTX induced neuropathic pain are partially effective and the underpinning mechanisms for developing PTX induced neuropathic pain are not fully understood. Therefore, there is an imperative need to develop a therapy which does not impede anti-tumor efficacy but effectively controls PTX induced neuropathic pain (PIPN).
- Cannabidiol (CBD) a major non-psychoactive components of Cannabis sativa has an important role in regulating the pain associated with different conditions and also has been shown to be effective in managing breast cancer, psoriasis, human epithelial carcinoma, colon cancer, inflammatory bowel disease, glaucoma, platelet aggregation and also acts as antidote for psychoactive cannabinoids
- CBD has poor solubility and susceptible to degradation via the action of light and temperature in solution form and undergoes extensive first pass metabolism Thus, a properly formulated version of CBD can play a crucial role in enhancing its physiochemical stability and therapeutic efficacy
- Recently we have established isolation and characterization procedures for extracellular vesicles (EVs) derived from mesenchymal stem cells/stromal cells (hUCMSCs).
- EVs proved to be efficient in reducing neuropathic pain by their paracrine secretions. hUCMSCs-derived EVs shuttles bioactive components like proteins, lipids, mRNA, miRNA, lncRNA, circRNA, and DNA that participate in Schwann cell regeneration, macrophage activation, and reconstruction of vascular networks to repair the nerve damage.
- Presently, no study has been conducted to understand the role of CBD loaded in hUCMSCs - derived EVs in PIPN. In this study, we have evaluated the effect of CBD and CBD-EVs formulation on oxidative stress and mitochondrial function via targeting endocannabinoid and non endocannabinoid receptors and AMPK pathway.
- To accomplish these objectives, we have isolated and characterized the EVs from hUCMSCs. Further, we investigated the pharmacological effects of CBD and CBD-EVs administration on pathophysiological indices of neuropathy in PTX treated mice and cultured primary DRG neurons (isolated from rat spinal region (L1-L5). We used 5HT1A and CB1 blockers and Compound C (AMPK inhibitor) to validate the pharmacological mechanism of CBD in offering neuroprotection

characterization of CBD loaded hUCMSC-EVs

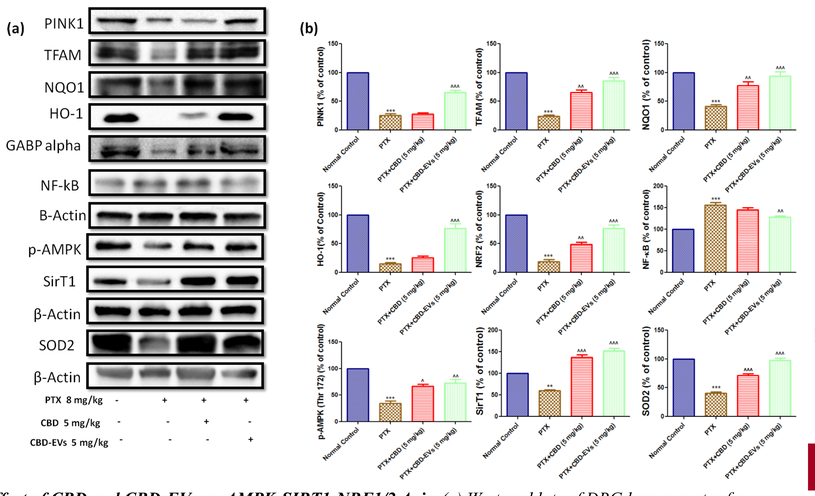


Neurobehavioral changes

Parameter	Normal Control	PTX	PTX+EVs	PTX+CBD (5 mg/kg)	PTX+CBD-EVs (5mg/kg)
Paw withdrawal latency to hot stimuli (s)	10.86±0.36	4.05±0.31***	6.41±0.98^	7.3±0.4^^	9.8±0.66^^^
Paw withdrawal latency to cold stimuli (s)	13.43±0.43	5.78±0.24***	7.9±0.61^	8.1±0.31^^	10.09±0.8^^^
Paw withdrawal latency (s)	10.89±0.40	3.85±0.8***	5.99±0.91^	6.86±0.9^	8.10±0.45^^^
Paw withdrawal threshold (g)	4.63±0.24	1.91±0.36***	2.97±0.6^	3.13±0.1	4.19±0.7^^^
Average Body weight (g)	23.18±1.11	22.65±1.23	23.85±1.99	23.99±1.20	23.21±1.32

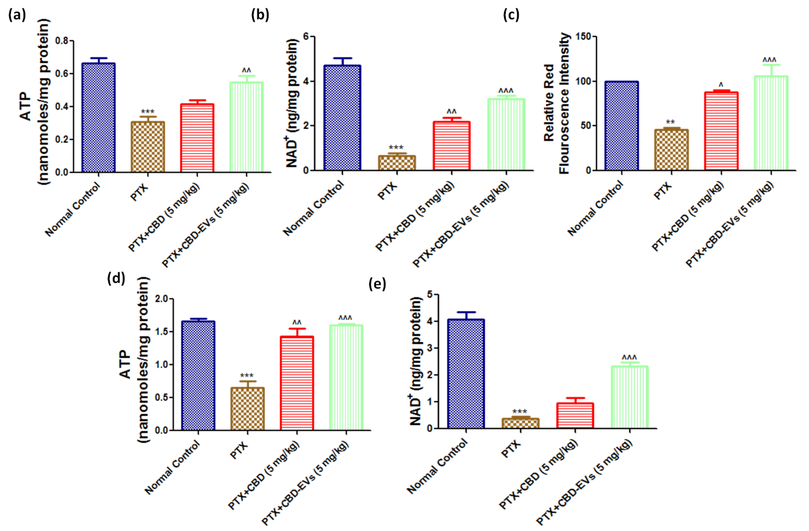
Effect of EVs, CBD and CBD-EVs on PTX induced neurobehavioral changes: *The values are expressed as mean standard error of the mean (n=6). ***p<0.001 Vs Normal control & ^p<0.05, ^^p<0.01 and ^^^p<0.001 Vs PTX.*

CBD and CBD-EVs on AMPK-SIRT1-NRF1/2 Axis



Effect of CBD and CBD-EVs on AMPK-SIRT1-NRF1/2 Axis. *(a) Western blots of DRG homogenates from PTX treated mice show treatment with CBD (5 mg/kg) and CBD-Exo (5 mg/kg) for six weeks after last dose of PTX administration. (b) Bar graphs represent the respective western blots quantification. Values are expressed as mean ± SEM (n=3). ***p<0.001 Vs Normal control, ^p<0.05, ^^p<0.01 and ^^^p<0.001 Vs PTX (8 mg/kg)*

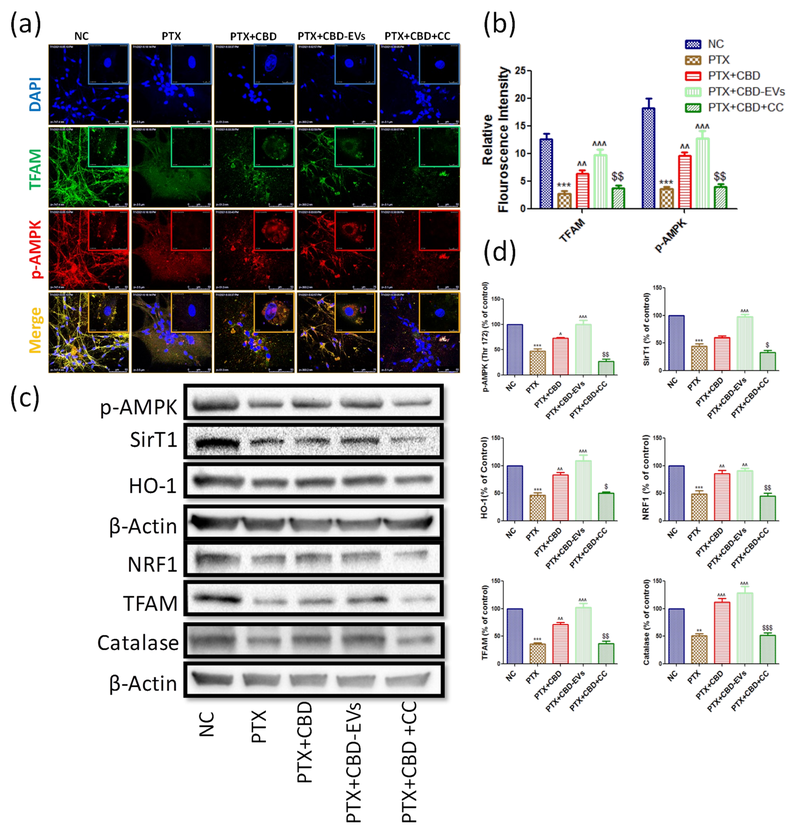
CBD and CBD-EVs on Mitochondrial Function



Effect of CBD and CBD-EVs on Mitochondrial Function and mitochondrial membrane potential (ΔΨm).

^p<0.05, ^^p<0.01 and ^^^p<0.001 Vs PTX (8 mg/kg

AMPK pathway in cultured DRG primary cells



Immunoexpressions of AMPK pathway in cultured DRG primary cells . Values are expressed as Mean ± SEM. ***p<0.001 Vs NC, ^p<0.05, ^^p<0.01 and ^^^p<0.001 Vs PTX (8 mg/kg). $P<0.05, $$P<0.01 and $$$P<0.001 Vs PTX+CBD.

Conclusion

- *In summary, the primary finding of this study is that CBD-EVs prepared from sonication method has shown potential in reducing mechanical and thermal pain sensitivities which are superior to CBD alone against PIPN in mice. CBD and CBD-EVs have a potent mitoprotective effects in neuronal cells via activating 5HT1A receptors and AMPK pathway. CBD was shown to depend on AMPK activation in improving mitochondrial function and biogenesis against PIPN in invitro and invivo.*