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Neuroprotective Propensity of 4-Allylpyrocatechol Derivatives against Oxaliplatin Induced Peripheral Neuropathy

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chemotherapy is used for the treatment of rapidly growing cell diseases in the body. It is most used for the treatment of different kinds of tumors. It can develop neuropathic pain due to damage of peripheral nerve cells and it is called Chemotherapy-Induced Peripheral Neuropathy (CIPN). In this study, we have reported the protective effects of 4-allyl pyrocatechol (4-APC) and its derivatives from biochemical and functional deficits associated with oxaliplatin (OP) induced neuropathy. The animals were submitted to mechanical and thermal hyperalgesia tests, after treatment with OP three times weekly at 0.20 mg/kg and 4-APC and derivatives (10 mg/kg & 30 mg/kg). The pain parameters were evaluated during the treatment period and at the end of treatment. 4-APC significantly prevented the mice from behavioural and biochemical alterations associated with OP-induced neuropathy. Thus, we conclude from this study, the use of 4-APC and its derivatives with OP might reduce the number of patients who develop painful peripheral neuropathy.

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Keywords: Oxaliplatin; peripheral neuropathy; nerve conduction velocity; hyperalgesia.

1. INTRODUCTION

Platinum agents are a significant antitumor medication class, broadly utilized in the treatment of advanced tumors [1]. Oxaliplatin (OP) is a third-generation platinum-based anticancer drug widely used to treat advanced colorectal malignant growth, and furthermore as an adjuvant treatment in different forms of advanced solid tumors. The incorporation of OP in the FOLFOX regimen (5-fluorouracil and leucovorin and OP), the mainline treatment for metastatic colorectal cancer has improved the survival rate in patients with this disease [2]. However, peripheral neuropathy which is seen in 10–15% of patients after total infusion of OP is the major limitation of OP [3]. A compelling body of evidence suggests neuroinflammation and oxidative stress are the major causes of OP-induced peripheral neuropathy (OIPN). To prevent OIPN, different cancer prevention agent-based treatments have been recommended alongside calming interventions. They include acetyl-L-carnitine, Vitamin-E, Vitamin-C, glutathione, and amifostine. Nonetheless, they show no adequacy in clinical investigations, along these lines, it is necessary to identify an agent to effectively prevent OIPN without affecting its anticancer efficacy [4-6].

4-Allylpyrocatechol (4-APC) is the chief constituent responsible for the anti-inflammatory, antioxidant, wound healing, and cytoprotective properties of *Piper betel* (Piperaceae). Its antioxidant and anti-inflammatory properties are attributed to its COX-2, iNOS, NF-kappaB, IL-12 p40, and JNK inhibitory effects [7-8]. We hypothesize 4-APC could inhibit calpain and result in neuroprotection from oxidative stress and inflammation in CIPN. In the present study, we report the beneficial effects of 4-APC and its derivatives against OIPN.

2. MATERIALS AND METHODS

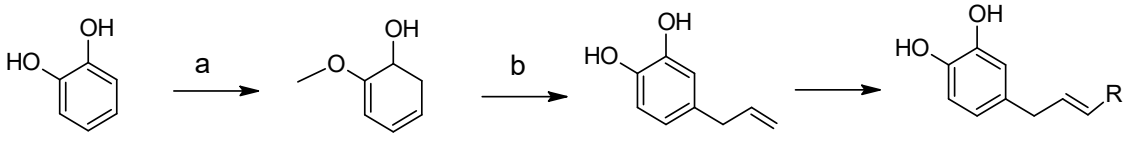
Tumour Necrosis Factor and ELISA kit were purchased from Himedia, Mumbai, Koma Biotech Inc., respectively. All other reagents and chemicals were analytical grade.

2.1 Synthesis of 4-APC Derivatives

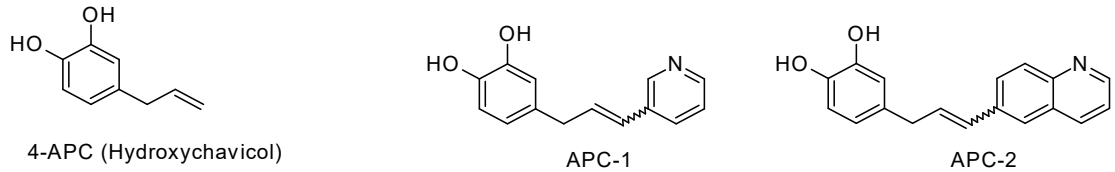
4-APC derivatives were synthesized by mono-allylation of catechol Scheme 1. The reaction of catechol with an equimolar amount of allyl bromide in presence of K_2CO_3 and acetone at 65 °C for 4 h gave the mono allyl ether **1**. The thermal rearrangement of **1** at 170°C for 1 h gave 2:1 regioisomeric mixture, *i,e,* 3-allyl catechol and 4-allylcatechol **2** respectively.

The regioisomeric mixture was separated by column chromatography to yield 4-allyl catechol **2** (31% yield). (E\Z)-4-(3-(pyridin-3-yl)allyl)benzene-1,2-diol (APC-1) and (E\Z)-4-(3-(quinolin-6-yl)allyl)benzene-1,2-diol (APC-2) were synthesised by Heck coupling of **2** with different aryl bromides.

The structural components of Allylpyocatechol derivatives that is APC-1 and APC-2 were determined by ^{1}H NMR (400 MHz; CDCl3) and ^{13}C NMR (101 MHz; CDCl3). The spectrum graphical charts (^{1}H NMR, ^{13}C NMR) for APC- 1 were shown in Fig. 1 and Fig. 2. Also, the Nuclear Magnetic Resonance spectrum for APC-2 is shown in Fig. 3 and Fig. 4.



Scheme 1. (a) Allyl bromide, K_2CO_3, acetone, reflux, 4 h; (b) 170 °C, neat, 1 h; (c) aryl bromide, $Pd_2(dba)_3$, Davephos, tetrabutyl ammonium acetate, 1,4-dioxane, 85 °C, 3 h



Scheme 2. 4- allyl catechol and its derivatives

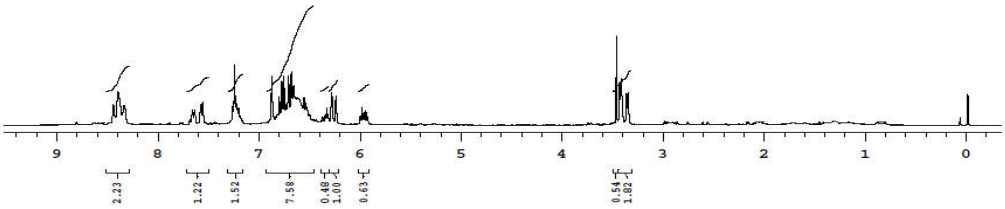


Fig. 1. ^{1}H NMR (400 MHz; CDCl3) of APC-1

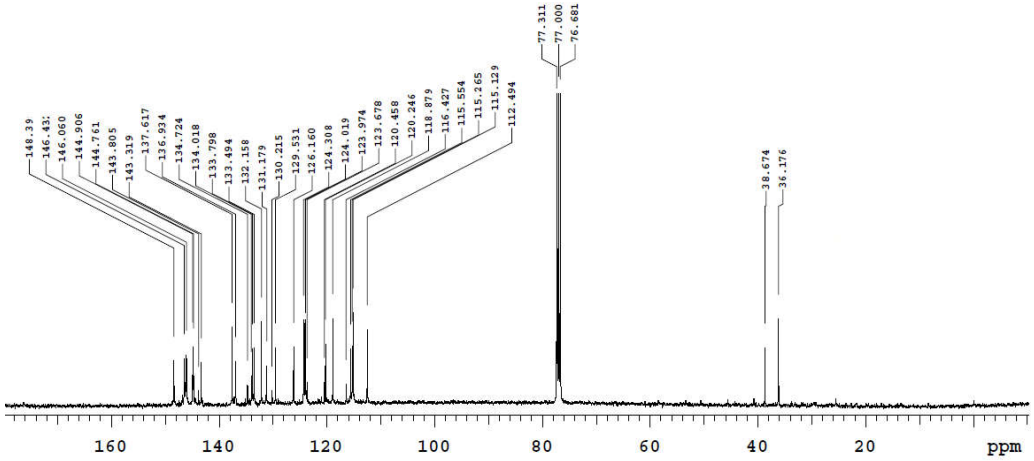


Fig. 2. ^{13}C NMR (101 MHz; CDCl3) of APC-1

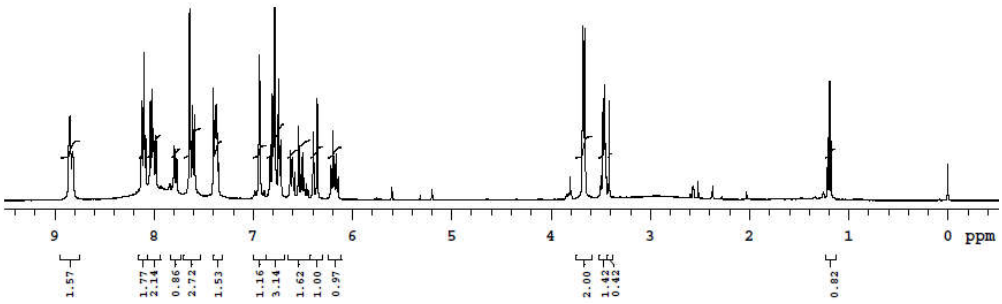


Fig. 3. ^{1}H NMR (400 MHz; CDCl3) of APC-2

2.1.1 APC-1 ((E\Z)-4-(3-(pyridin-3-yl)allyl)benzene-1,2-diol)

Appearance : Pale brown gummy; Yield: 55%; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8 51 - 8 30 (m, 2H) 7 71 - 7 51 (m, 1H) 7 33 - 7 13 (m, 1H) 6 90 - 6 49 (m, 5H) 6 38 - 6 19 (m, 1H) 6 02 - 5 89 (m, 1H) 3 44 - 3 33 (m, 2H); ^{13}CNMR (100 MHz, CDCl3) δ ppm 148 3, 146 4, 146 0, 144 8 (2 C) 143 3 137 6 (2 C), 136 9, 134 7, 133 7, 133 4, 132 1, 131 1, 129 5 , 126 1, 124 3, 124 0, 120 2, 118 8, 115 5 (2 C) 115 2, 115 1, 112 4, 38 6 36

1; FT-IR (KBr): 3448, 2923, 1595, 1515, 1495, 1276, 764, 750 cm-1 LCMS (ES): m/z 228 08 (M+H)+; HRMS (ESI): m/z calcd for $C_{14}H_{14}NO_2$ ($[M+H]^+$): 228 1025 found: 228 1010.

2.1.2 APC-2 ((E\Z)-4-(3-(quinolin-6-yl)allyl)benzene-1,2-diol)

Appearance: off white solid; Yield: 63%; MR: 84 - 86 °C; ^{1}HNMR (400 MHz, $CDCl_3$) (Mixture of E\Z isomers) δ ppm 8 95 – 8 73 (m, 1H), 8 22 - 8 06 (m, 1H), 8 06 – 7 97 (m, 1H), 7 86 – 7 53 (m, 2H) 7 49 – 7 31 (m, 1H), 7 53 – 7 27 (m, 2H), 7 03 – 6 66 (m, 2H), 6 67 – 6 44 (m, 1H), 6 37 (br d, J = 15 78 6 9 Hz, 1H),6 26 - 6 04 (m, 1H) 3 74 - 3 43 (m, 2H); ^{13}CNMR (100 MHz, $CDCl_3$) δ ppm 149 2, 149 1, 147 1, 146 5, 144 5, 144 47, 144 1, 142 8, 138 2, 135 6, 135 5, 135 4, 131 5, 131 2, 131 1, 130 8, 129 3, 129 2, 128 7, 128 6, 128 1, 128 0, 127 0, 126 0, 125 0, 124 6, 121 0, 120 7, 119 6, 118 1, 115 4, 115 0 (2C), 112 5, 38 6, 38 3; FT-IR (KBr): 3414, 3022, 2924, 2559, 1595, 1503, 798, 766 cm^{-1} LCMS (ES): m/z 278 18 $(M - H)^+$; HRMS (ESI): m/z calcd for $C_{18}H_{16}NO_2$ ($[M+H]^+$): 278 1103 found: 278 116.

2.2 Cells and Culture Conditions

SH-SY5Y cells were maintained at 37°C with 5% CO_2 in DMEM medium supplemented with 10% of FBS and 1% penicillin-streptomycin.

2.3 Cell Viability Assay

SH-SY5Y cells (1.5 x 10^4 $well^{-1}$) were seeded in a 96 well plate and incubated at 37°C. The cells were exposed to varying concentrations of OP/4-APC/APC-1/APC-2 in DMSO. After incubation for 24h, the cell viability was assessed using MTT assay [9].

2.4 In vivo Neuroprotective Study in Mice

24 Swiss Albino mice were divided into 4 groups of 6 each. The mice in Group 1 (normal) & Group 2 (control) have received 5% w/v Glucose solution, 10 ml/kg, i.p.. Groups 3 and 4 received a daily dose of 4-APC, 10 and 30 mg/kg, i.p., respectively. All the mice except Group 1 received OP (1mg/kg. i.p., twice a week). The study was conducted for 6 weeks. The body weights, mechanical and thermal nociceptive thresholds were evaluated during the study period. Mice were sacrificed at the end of the study under deep ether anesthesia, to collect sciatic nerves and dorsal root ganglion (DRG) for estimation of biochemical parameters [10].

2.5 Hyperalgesia (Thermal)

The thermal hyperalgesia to both hot and cold, studied using tail immersion in hot (45°C) and cold water (10°C). In the tail immersion test, the tail-flick latency is the endpoint. The cutoff time is 15 s. Three consecutive readings were taken at 30 min intervals [11].

2.6 Hyperalgesia (Mechanical)

Mechanical hyperalgesia was studied by the pressure stimulation method as described by Randall and Selitto [12].

2.7 Biochemical Analysis

Biochemical analysis was done in sciatic nerve tissue homogenate.

2.7.1 Preparation of tissue homogenate

The sciatic nerve and DRG homogenate (0.5 g) was prepared by homogenizing the tissue in ice-cold PBS (5.0 ml, 0.1 M, pH 7.4) to obtain 10% w/v homogenate. Then centrifuged at 10,000 rpm (20 min, 4 °C).

2.7.2 Estimation of oxidative stress parameters

Malondialdehyde (MDA) and superoxide dismutase (SOD), reduced glutathione (GSH), nitrate were measured as described previously [13].

2.7.3 Estimation of neuroinflammatory parameters

2.7.3.1 Myeloperoxidase (MPO) assay

The tissue supernatant (10µl) was mixed with potassium phosphate buffer (290 µl, 50 mM, pH 6.0) containing o-dianisidine dihydrochloride (0.167 mg/ml) and hydrogen peroxide (0.0005% v/v). Then change in absorbance was measured at 460 nm for 2 min [14].

2.7.3.2 Estimation of Tumor necrosis factor-alpha (TNF α)

Commercially available ELISA kits from Biovision(CA, USA) for assaying TNF-α and IL-6

proteins were used and levels expressed as pg/mg of protein in sciatic nerve homogenate [15].

2.7.4 Statistical analysis

Data are expressed as means ± SEM. Statistical significance was done using t-test and ANOVA. If $p < 0.05$ it is considered as significant. GraphPad Prism 6 software was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Cell Viability

From Table 1, the protective effect of APC and its derivatives against the OP-induced neuropathic pain was observed. The APC-2 Provide increased cell viability to 93.8±9.6.

Table 1. CTC 50 values of different compounds In SH-SY5Y Cells

Groups	CTC 50(µM)
4-APC	68.6±17.8
4-APC-1	79.9±14.5
4-APC-2	93.8±9.6
OP	28.9±13.5

Values are mean±S.D., n=3

3.2 Effect of 4-APC and Derivatives on Pain Behaviour

OP treatment is associated with a significant decline in paw withdrawal pressure as compared to the normal group. OXA significantly prevented the lowering of paw withdrawal thresholds compared to the normal group on days 16, 20, and 28 ($p<0.05$). OP treatment at 10 and 30 mg/kg showed dose-dependent protection against OP-induced altered mechanical nociceptive threshold ($p<0.05$), Table 2.

3.3 Effect of 4-APC and Derivatives on Oxidative Stress Parameters

OP caused a significant change in oxidative stress markers such as lipid peroxidation Fig. 5A, superoxide dismutase (Fig. 5B), catalase Fig. 5C, glutathione Fig. 5D. Pre-treatment with APC-1 and APC-2 showed a significant protective effect from OP-induced alterations in oxidative stress markers ($P<0.05$). The significant change was shown in Fig. 5.

3.4 Effect of 4-APC and Derivatives on Inflammatory Markers

OP resulted in a significant change in the inflammatory markers such as nitrate Fig. 6A,

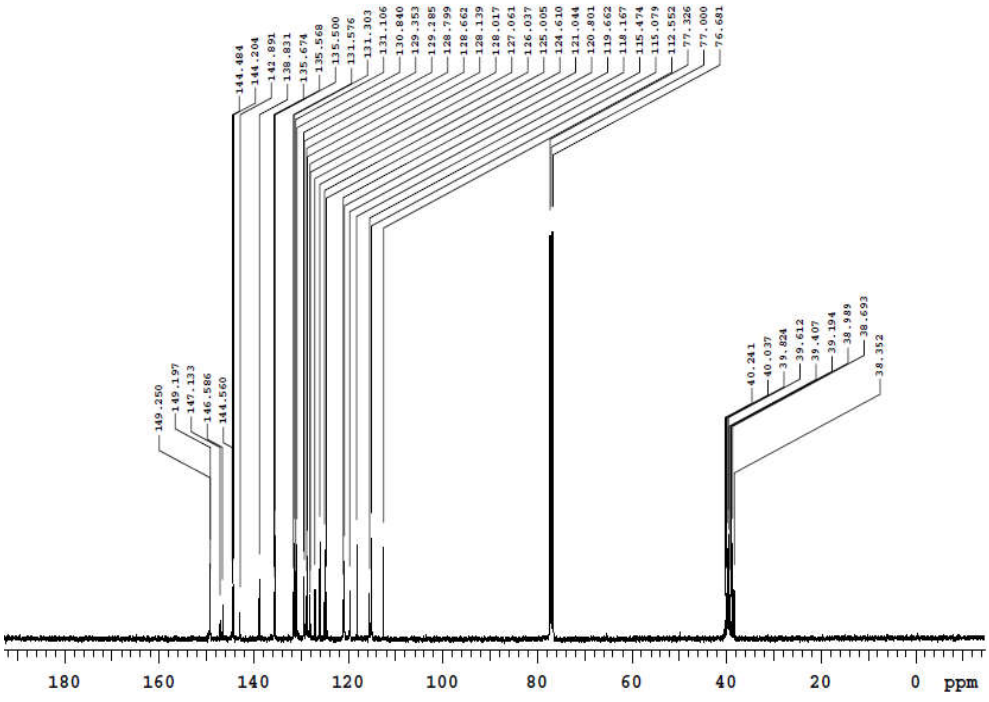


Fig. 4. ^{13}C NMR (101 MHz; CDCl3) of APC-2

myeloperoxidase Fig. 6B, and TNF alpha Fig. 6C. 4-APC, APC-1, and APC-2 significantly protected from OP-induced inflammatory effects Fig. 6.

Table 2. Effect of 4-APC and derivatives on pain behaviour

	Normal	OP Control	4-APC	APC-1 (10mg/Kg)	APC-1 (30 mg/kg)	APC-2 (10 mg/kg)	APC-2 (30 mg/kg)
Hot plate test	17.1±2.7	5.3±2.1#	6.4±1.3*	9.6±1.7*	12±2.5*	15.6±1.4*	16±1.5*
Randallsellitto test (gm)	95.1±4.3	25.6±5.2#	28.6±1.2	38.6±4.4	58.6±5.2*	57.5±3.2*	79.6±4.1*

*All values are mean±SD; # p<0.05 vs Normal, *p<0.05 vs OP*

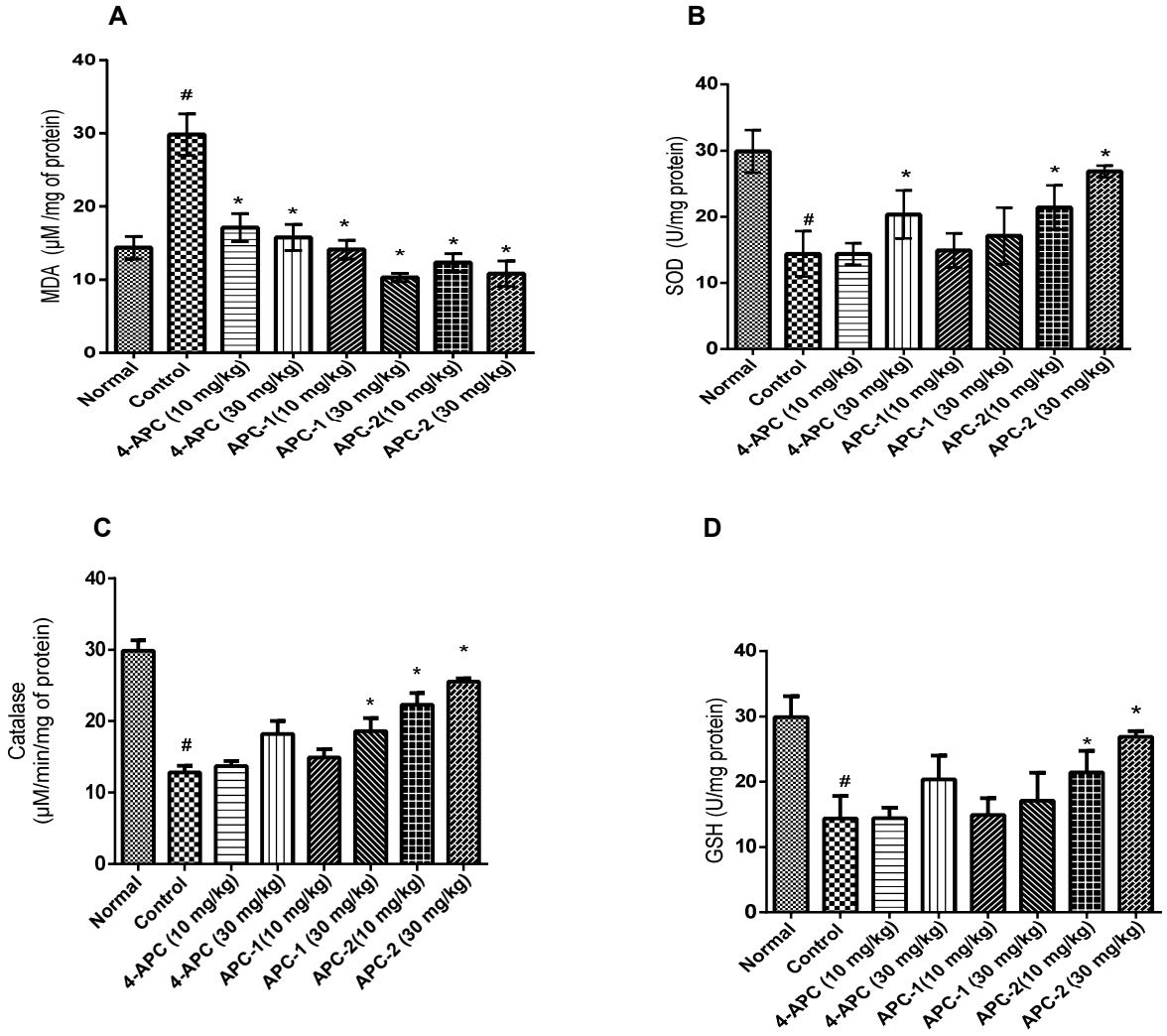


Fig. 5. Effect of 4-APC, APC-1, and APC-2 on antioxidant parameters in OPIN (A) Lipid peroxidation (B) Superoxide dismutase (C) Catalase (D) Reduced Glutathione

*Values are mean ± SEM, n=6, #P<0.05 when compared to normal; *P<0.05 when compared to control*

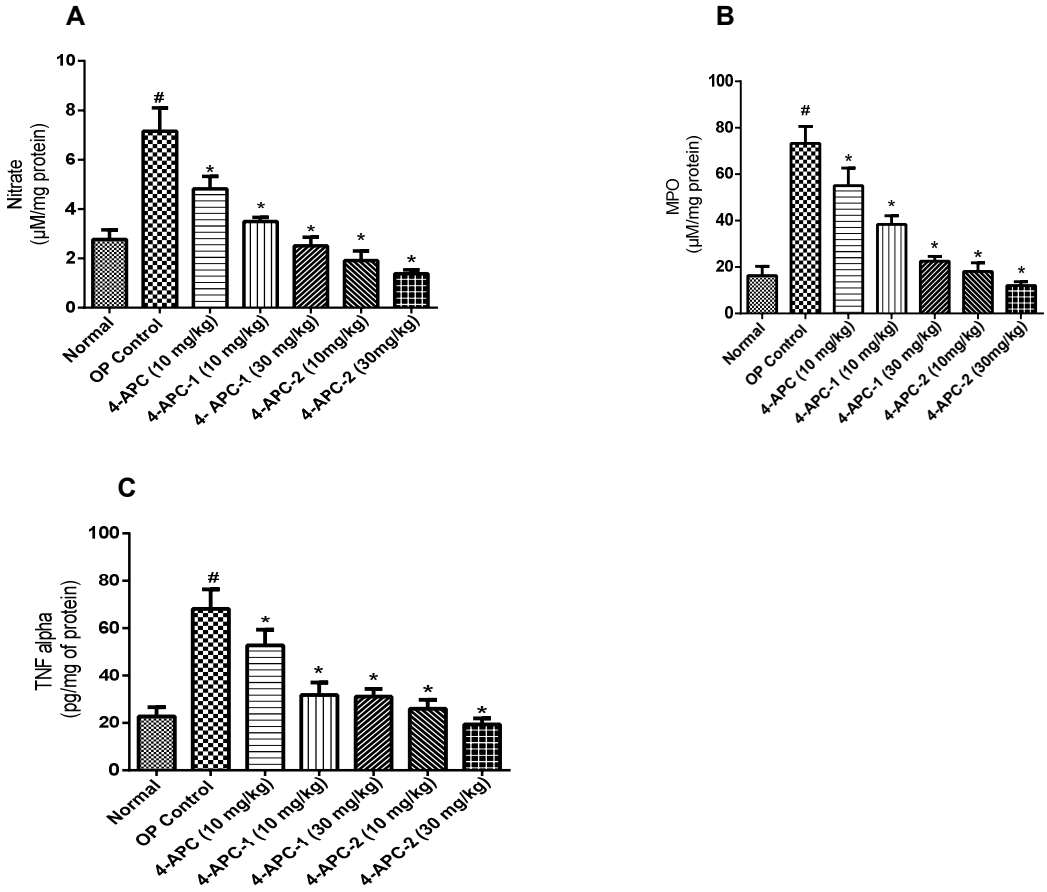


Fig. 6. Effect of 4-APC, APC-1, and APC-2 on anti-inflammatory parameters (A) Nitrate (B) Myeloperoxidase (C) Tumor necrosis factor alpha

*Values are mean ± SEM, n=6, #P<0.05 when compared to normal; *P<0.05 when compared to control*

4. CONCLUSION

A compelling body of evidence suggests the key role of neuroinflammation and oxidative stress in OP-induced CIPN. It was reported that calpain activation results in oxidative stress and neuroinflammation in CIPN. 4-APC is a polyphenol with antioxidant and anti-inflammatory propensity [5,16-17]. In the present study, 4-APC administration prevented OP-induced alterations in MDA level which means that its administration decreased the level of lipid peroxidation. OP administration significantly decreased the levels of SOD in sciatic nerves of mice. 4-APC treatment improved the SOD levels in the sciatic nerve. Hence, we can conclude that 4-APC can effectively combat chemotherapy-induced oxidative stress in rodents. The molecular mechanism of CIPN has also been reported to modulate TNF-alpha expression and myeloperoxidase (MPO) activation which plays a Crucial role in axonal degeneration, suggesting that oxidative stress and neuroinflammation play an important role in CIPN [18]. In the present study OP administration significantly increased the levels of TNF-α and MPO. The administration of 4-APC significantly reduced the levels of these pro-inflammatory mediators. Hence the 4-APC mediated neuroprotection may be partly attributed to its inhibitory activity against cytokines. In conclusion, the results of the present study demonstrated the prophylactic effect of 4-APC against OP-associated CIPN.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is approved by IAEC having the reference number of 1176/PO/Re/S/08/CPCSEA.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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