World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Effects of βeta-caryophyllene extracted from *Piper guineense* fruit (Uziza pepper) on motor deficit and muscle function (gastrocnemius) in oxaliplatin-induced peripheral neuropathy of Wistar Rat

kelechi Collins Onwuka [1,*], Daniel Nwachukwu [2], Akunneh-wariso Chris [1], Chinedu Barry Iheukwumere [1] and Clement Dimgba Ubani [1]

[1] *Department of Physiology, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria.*
[2] *Department of Human Physiology, Faculty of Basic Medical Sciences, University of Nigeria, Enugu, Enugu State, Nigeria.*

World Journal of Advanced Research and Reviews, 2025, 25(03), 1961-1979

Publication history: Received on 14February 2025; revised on 23 March 2025; accepted on 26 March 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.25.3.0912

Abstract

Introduction: Apart from its financial burden, Chemotherapy presents a induced Peripheral Neuropathy with motor deficits and muscle cachexia. This has lead to discouragement and stoppage of cancer treatment regimen, and poor quality of life with heavy financial Implications. This study was aimed to determine the effects of β-caryophyllene (BCP) on muscle function of gastrocnemius in oxaliplatin induced peripheral neuropathy of male wistar rat.

Method: The rats used in this study were grouped into 5 groups (n=9).Except for normal control , all animals were treated with Oxaliplatin (4 mg kg-1) to induce Peripheral neuropathy. Apart from the Negative control, others were treated daily with Doluxetine at 10 mg kg-1 (control 3), BCPat 25mg kg-1 (Group 4) and BCP at 50 mg kg-1(group 5). For each week of the 21 days, the rats were assessed for motor coordination deficit and muscle strength while their samples were assessed for muscle injury and muscle weight of the gastrocnemius weekly. Means less than 0.05 (<0.05) were considered statistically significant.

Result: BCP caused an improvement in motor co-ordination with decreases in muscle inflammation, creatine kinase and myoglobin levels with a weekly decrease in muscle inflammation. Muscle endurance also improved in BCP-treatment with increased relative gastrocnemius muscle weight.

Discussion: This study emphasized the importance of muscle function as needed diagnostic tool in assessment of recovery of cancer patients while demonstrating the positive impact of BCP on pain sensitivity, motor deficit and muscle function during peripheral neuropathy.

Keywords: Oxaliplatin; Peripheral neuropathy; Motor-co-ordination; Gastrocnemius; Injury

1. Introduction

Induced Peripheral Neuropathy has been one of the life challenging situations of patients worldwide. The major causes include chemotherapy besides Diabetics and Alcoholism [1]. About 1.5 million cases have been reported In Nigeria [2]. The worldwide prevalence of peripheral neuropathy in diabetes, especially for the aged, is estimated to be within 47-50% [3,4] with highest prevalence of 84.4% in Nigeria [5]. In chemotherapy, it is generally estimated to be 68,60 and 30% within first, three and six months after chemotherapy respectively [6]. Characterized with increased uncontrolled nociception, muscle numbness and weakness, it is believed to decreased patient's daily activities and quality of life [7,8].

*Corresponding author: kelechi Collins Onwukaa

The prevalence of Chemotherapy Induced peripheral Neuropathy (CIPN) is about 70-100% when platinum base drugs are used for therapy, with acute to chronic neuropathy in Oxaliplatin [9].

The oxidative stress initially caused by antineoplastic drugs is useful in apoptosis of cancer cells **[**10, 11]. However, they sponsor systemic oxidative stress and inflammation through various mechanisms[12,13, 9, 8, 14]**.**The Inflammation from OIPN targets the skeletal muscles and their nerves resulting to altered muscle excitability, muscle fiber atrophy, skeletal muscle loss (cachexia), stem cell (satellite cells) dysregulation, apoptosis, and notably pain with heightened sciatica [13,15, 9,16, 17].This may contribute to poor survival, muscle weakness and even slow death. This cachexia is very evident in oxaliplatin therapy [18]. Additionally, Oxaliplatin-treated mice displayed reduced weight gain and reduced hindlimbs muscle mass particularly, the gastrocnemius, with behavioral deficits [19].The major neurotoxic manifestations of Oxaliplatin induced Peripheral neuropathy (OIPN) are cold and mechanical allodynia**.** The sensation of pain may manifest in days and even continue for months and more into years after cessation of oxaliplatin chemotherapy, that is, after patients is cancer free [9]

The high cost of chemotherapy (presenting a heavy financial burden on patients costing about N600,000 to N1.5 million per session depending on the type of cancer [21] together with the resultant peripheral neuropathy and muscle cachexia may cause patients to discontinue treatment regimen. These factors can even stigmatize the patients and worsen their situations while frustrating the efforts of their caregivers. To highlight the obvious, Most researchers emphasize on the chemotherapy-induced neuropathic pain of oxaliplatin as regards to neuroinflammation. Little research emphasis has been made on the muscles of cancer and post-cancer patients which may have undergone atrophy, loss and evidently weakness affecting their quality of life and physical activity for life. This calls for the need of a compound from a cheap and available natural source that can protect and preserve the muscles from chemotherapy while expressing anticancer and antinoceptive properties in cancer patients. Beta-caryophyllene (β-caryophyllene, trans-caryophyllene, BCP) is one of such compounds that have recently observed due to its efficacies in other researches. Being one of the most abundant component in *Piper guineense* [17,26]. This terpene has shown promising ameliorating effects in other models of inflammation and oxidative stress such as colitis [29], hypoxia [20, 27]and arthritic rat models of neurotoxicity [69] or brain injury [65] with evidence of anti-nociceptive properties [66].It has even shown ameliorating effect in **paclitaxel** treatment [32]. Furthermore, it is approved by the United states food and drug administration, and European Agencies as a culinary agent [33]. This may be due to the fact that no toxic effects have been noted following oral administration of β-caryophyllene in mice up to 2000 mg/ [25].

Piper Guineese fruits (African black pepper), are very common in the Nigerian market and used in Nigerian dishes [31]. It is used in culinary, as a preservative, in perfumery and also as a medicinal agent [30], possessing pharmacological properties such as antioxidant, anti-inflammatory, anti-tumour, anti-allergic and antiplatelet properties, anti-malarial, antihypertensive, antiarrhythmic and anti-cancer. The aim of this study was to investigate the effect of β-caryophyllene on motor deficit and muscle functionof the gastrocnemius muscle in oxaliplatin induced peripheral neuropathy of male wistar rat.

2. Material and methods

2.1. Purchase of Rats and *Piper Guineense* Fruits

Forty-five Wistar rats (200 ± 10g) and 30 wistar rats (150 ± 20g) was purchased and used for the experimental and acute toxicity studies respectively. Their acclimatization period was for 14 days before the commencement of the study. They were also exposed to the beam walk balance test to acclimatized them to the instrument. *Piper guineense* fruits were bought from a dealer in Ogbete market, Enugu. Nigeria. It was authenticated at the Department of Plant science and biotechnology, University of Nigeria. A sample of it was kept in the herbarium for reference purposes. The Herbarium Voucher number is UNH813b

2.2. Extraction of the Essential Oil from Piper Guineense Fruits

The method according to Liu et al (2018) [20] was used. They were dried to a moisture content of < 17%. They were grounded with high speed grinder and slightly crushed. They were slight crushed to reduced temperature increases then, sieved at particle size of425 µm o obtained fine powder. The fine powder was used immediately for the extraction of essential oil.

The extraction of the oil was done using the standard of Official Methods Of Analysis Of Aoac International [21]. About 50g of the fine powder of *piper guineense* fruits was weighed using a chemical balance. It was properly wrapped in a filter paper. The wrapped sample was then put in an extraction thumble that was washed, and dried using a drying oven,

then allowed to cool down in dessicators prior weighing. It was placed in a soxhlet Extraction chamber. Before then, about 500ml of 99% n-Hexane solvent was put into the flask and the oil was extracted within an average period of4 hours. After extraction, the solvent was recovered with rotary evaporator and crude extract recollected. The flask and its contents were allowed to cool down in a desiccators and further weighed.

The percentage of the oil gotten was calculated as follows:

(Weight of the extracted oil / weight of the sample) x 100=

(Beaker with extract- empty beaker)/ weight of sample x 100 =

(122.18g— 121.97)/5x 100= 4.2%

A sample of the essential oil was used to test for the presence of Terpenes where the method according to Godswill *et al* (2014) [22]was used. 0.5ml of acetic anhydride was mixed with 1ml of sample extract and a few drops of conc. H2SO4. A blush green precipate indicated the presence of terpenes.

2.3. Isolation Of Trans-Caryophyllene (Beta-Caryophyllene, B.C.P) From *Piper guineense* Fruits

This was done according to the method of Dante, A (2022)[23]. The oil content was further measured into a flask and 2.5% NaOH was added to it at a volume ratio (oil to NaOH) of 1:5. The mixture was allowed to settle and thereafter stirred using a magnetic stirrer for about 3 hours. After wards, it was left in the separating funnel until two layers were formed. The top layer in the funnel contained beta-caryophyllene. The content in the top layer was isolated and further subjected to fractional distillation which was under reduced pressure of cmHg. Afterwards, the content was subjected to re-distillation to isolate beta-caryophyllene at temperatures above 170^0 C. The isolated beta-caryophyllene was analyzed using GC-MS Analysis.

BCP yield was calculated as:

Weight of beaker before Extraction – weight of beaker after extraction=1.6 g

BCP after fractional distillation = 1.02g

Gas-Chromatographic-Mass Spectrum Analysis And Separation

For analysis, 2ul of the sample that had been extracted in the manner previously mentioned was put into the Gas Chromatography column. The capillary column for the GC (Agilent 6890N) and MS (5975B MSD) is a DB-5ms model (30 m×0.25 mm; film thickness 0.25 μm). A starting point of 40°C was established. This was raised by 10°C every minute to 150°C. By 5°C every minute, the temperature was raised once again to 240°C. At a pace of 20°C/ min, the procedure was repeated until the temperature reached 280°C. It was decided to maintain this ultimate temperature for eight minutes. At that time, the detector temperature was 250°C, while the injector port temperature stayed constant at 280°C. The carrier medium gas was helium, flowing at a rate of 1 ml/min. The ionization voltage and split ratio were 70 eV and 110:1, respectively. The National Institute of Science and Technology 2014 database was utilized to compare each extract's unique mass spectral peak value with potential undiscovered phytochemical components. Next, by comparing the unknown peak value and chromatogram from GC-MS with the known chromatogram and peak value from the NIST Library database, the active chemicals were found. Following that, information was also acquired on their molecular formula, molecular weight, retention period, and percentage content.

2.4. Purchase and concentration of Oxaliplatin and Standard ; Duloxetine

Oxaliplatin was purchased in powder form from a pharmaceutical store in Enugu. For storage purposes for 30 days, as tock solution of 1mg/ml in 5% dextrose solution will be prepared for administration by injection as against unstable saline solutions (Mehta et al., 2015) [28]. A dose of 4mgkg-1 will be used for induction of peripheral neuropathy.

Duloxetine was purchased from a pharmaceutical store in Enugu. It was prepared according to the method of Meng *et al* (2019)[24].Duloxetine HCL was mixed in 0.9% sterile saline and used at a dose of 10 mg/kg (1 hour prior to treatment with Oxaliplatin) as the standard drug of this experiment. Precautions were taken to minimize animal suffering.

Duloxetine is effective in combating the effects of CIPN by oxaliplatin, Paclitaxel, Vincristine or Bortezomib in patients [25]. Over the years, the drug has shown the most promise for the treatment of CIPN compared to other drugs. [27,29,

30,31, 17]. Duloxetine was recommended by American Society of Clinical Oncology and European Society for clinical situation such as in Medical Oncology. It is one of the drugs used to combat peripheral neuropathy [32,33]

2.5. Induction of Oxaliplatin-Induced Peripheral Neuropathy

The modified method of Jonathan, *et al* (2021) [35] was adopted due to its close resemblance to clinical dosage regimen of Oxaliplatin (OXA) and acute neuropathy. From the first day of treatments onwards , the treatments with OXA (4 mg/kg) was injected intraperitoneally every 48 hours for 20 days, totaling 11 doses of OXA (days 0, 2, 4, 6, 8, 10, 12, 14,16,18,20).Administration was done in the morning. Beta-caryophyllene Treatment was given daily by oral gavage for low and high dose starting from day 1 for 21 days. Administration was done in the late hours of the afternoon.

2.6. Experimental Design of the study

The experimental animals were divided into five (5) group of Nine (9) male wistar group. Groups were treated with normal saline, Oxaliplatin, doluxetine and beta-caryophyllene accordingly as tabulated below.

Table 1 Groups and the dose of agents used for treatment

Group	Group Name	Doses of Agents administered to the group
Group 1	Normal Control	Normal Saline
Group 2	Negative Control	Oxaliplatin(4mg/kg).
Group 3	Positive Control	Oxaliplatin (4mg/kg) and Doluxetine (10mg/kg)
Group 4	Low Terpernoid Group	Oxaliplatin (4mg/kg) and beta-caryophyllene (25mg/kg)
Group 5	High Terpernoid Group	Oxaliplatin (4mg/kg) and beta-caryophyllene (50 mg/kg)

Mg/kg refers to the amount of substrate given to body weight of animals. 3 rats for each group were chosen randomly from each group and assessed for each week. Date of Outcome measures was collected weekly for the three (3) of experiment.

2.7. Tests for Pain Threshold

At random, the experimental animals were selected for pain threshold test

2.7.1. Test for Cold allodynia (Acetone Drop Test)

This test for Cold allodynia was evaluated using method of Wan *et al* (2020)and Wang *et al* (2020) where acetone drop were used [36,37]. The experimental rats were separately placed in plastic chambers which was put on a wire mesh shelf. Then a syringe with flat blunt needle was used to collect a quantity of some acetone compound. This was then used to aspirate one drop calmly on the top of the rat's hind paw. The time of withdrawal or licking reaction was recorded within a given time of 40 sec. Acetone was aspirated within every 10 minutes and the mean of the responses was calculated in each measurement.

2.7.2. Test for Acetic Pain Threshold

This was done using the method of Mamun-Or-Rashid *et al* (2017)[40]. Acetic acid was administered to the animals intraperitoneally to induce pain (usually used for chemical pain sensation). Due to the sensation of pain, the animals squirms their body from time to time at regular interval which is recorded as “writhing”. Each writhing is counted within a given period of time and recorded as an indication of pain sensation. Any compound that possesses analgesic/antinociceptive property is expected to reduce the number of recorded writhings within a given time.

2.7.3. Test for Tail Flick Pain Threshold

This was done according to the method of Zhou *et* al (2014) [41]. In tail flick test, the distal half of tail is immersed in hot water and temperatures of 45^0C maintained. When the animal flicked its tail, the timer was stop and the recorded time (latency) will be a value of Pain threshold. Tail Flick test is used to ascertain or measure the sensitivity of the experimental animal to heat effects of drugs or certain modification made to the experimental animal.

2.7.4. Beam Walk Balance Test

The beam walk test is used to assess motor-coordination especially in the hindlimb . It was done using the method ofFeng *et al* (2014) [42]. The experimental rats were exposed for 2 days prior the experimental use of the beam walk. Before the administration of treatment agents, animals from each group were selected for beam walk balance to determine if there is a significance in motor co-ordination before the administration of treatment agents

In beam walk test, experimental rats were placed at corner of a narrow beam with ledges and watched as they walk across the beam to the other end with some level of elevation to attract rat to the finish point. It was done 3 times. The following were recorded:

- Number of turns
- Number of foot slips/foot drift
- Number of lines crossed
- Number of fall from the beam

2.8. Assessment of Four Limbs Muscle Strength

This was done according to the method of Assessment of muscle/ Physical strength. The hanging net was used according to Castro and Kuang (2017) [43] and Bonetto *et al* (2015) [44].The animal is gently placed with its four limb under on a metal net elevated a short distance above the ground. The animal is gently released and the timer was started. The timer is stopped at when the animal loses its grip and falls. The time is recorded to used to assess Holding endurance.

2.9. Preparation of Gastrocnemius Muscle Homogenates

This was prepared according to the method of Schneider *et al* (2012)[45] and Yin *et al* (2009) [46]. The gastrocnemius muscle was carefully dissected from the experimental animal post-euthanasia, with extraneous fat and connective tissues removed to isolate the muscle tissue. The dissected muscle weighed approximately 100 mg and was processed immediately with phosphate-buffered saline (PBS) solution, protease and phosphatase inhibitors. It was subjected to Homogenization was conducted using an electric tissue homogenizer. The supernatant was separated and used for biochemical essays of creatine kinase and Myoglobin.

2.10. Determination of Creatine Kinase Muscle-Muscle isoensyme (Ck-MM) and Myoglobin

Creatine kinase Muscle-Muscle isoensyme bound was measured using the methods of Steen *et al.,* (2010) [47], as contained in Abnova assay kit while Diagnostic Automation, Inc. offered an enzyme immunoassay kit for quantifying the myoglobin concentration known as the Myoglobin ELISA test kit. ELIZA laboratory procedures as listed were conducted for the determination of creatine kinase and Myoglobin.

2.11. Relative muscle weight and Histological Studies of the gastrocnemius muscle

The gastrocnemius muscles were isolated at the head and caudal ends, cleaned and absolute weight determined using a weighing balance. The relative muscle weight was calculated as:

$$\text{Relative muscle weight } = \frac{\text{Absolute muscle weight (g)}}{\text{body weight of rat on the day of sacrifice}} \text{ X } 100$$

Histological studies was done according to Peter *et al* (2016) [48] at the end of the 21 days where the gastrocnemius (GSN) was isolated and prepared for histological findings. Morphological parameters such as Fiber Cross-sectional area, organelles integrity and microscopic injury will be observed using staining techniques and microscopy. The histopathology was conducted by qualified veterinary pathologists according to procedures recommended for nonclinical safety biomarker qualification studies in Burkhardt *et al* (2011) [49].

2.12. Statistical Analysis and Data Analysis

Every parameter was analyzed statistically using One-Way Analysis of Variance (ANOVA) and the student Newman-Keul Post-hoc test using IBM SPSS version 20. The data were displayed as mean ± standard error of mean (SEM) in a tabular form. he parameters of motor coordination and muscle function were also examined using Graph Pad Prism version 22, and the results were statistically assessed using Two-Way Analysis of Variance (ANOVA) to compare group differences during the course of the three-week therapy, and then the Tukey post-hoc test. Bar charts were used to

display the results of the analysis with significance displayed as subscripts. For the mean of the values, a value of< 0.05 was considered statistically significant.

3. Results of the Study

3.1. Effect of Beta-caryophyllene Administration on Pain Threshold Tests in experimental rats

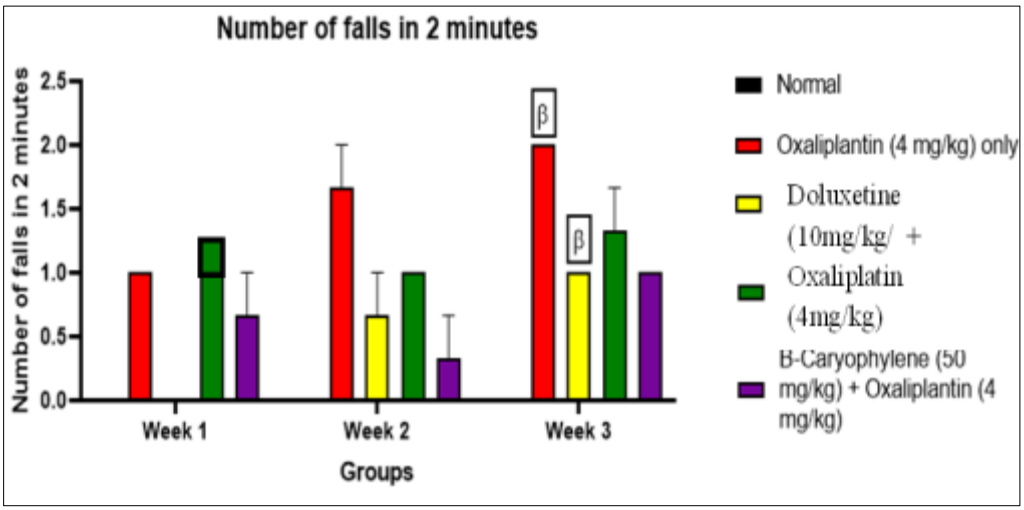
Table 2 Effect of Beta-caryophyllene administration on Pain Thresholds

Groups	acetone cold pain test (Cold Allodynia)		Acetic acid-induced pain test (chemical Hyperalgesia)		tail flick pain model (Heat Hyperalgesia)	
	Number of licking responses in 30 minutes	Percentage inhibition of pain	Number of writhes in 30 minutes	Percentage inhibition of pain	Reaction time in minutes	Percentage inhibition of pain
1	21.00±2.08^{a}	73.51±2.63^{c}	33.33±2.40^{b}	57.59±3.06^{c}	92.00±1.15^{c}	116.96±1.91^{d}
2	79.33±2.40^{c}	0.00±0.00^{a}	78.67±2.40^{d}	0.00±0.00^{a}	42.67±3.71^{a}	0.00±0.00^{a}
3	48.67±2.40^{b}	38.62±3.03^{b}	25.00±1.73^{a}	68.19±2.21^{d}	73.33±2.40^{b}	74.60±5.72^{bc}
4	54.00±2.31^{b}	31.90±2.91^{b}	44.00±2.31^{c}	44.02±2.94^{b}	67.33±4.06^{b}	60.32±9.65^{b}
5	46.67±4.06^{b}	41.15±5.12^{b}	36.00±2.00^{b}	54.18±2.55^{c}	75.33±2.03^{b}	79.35±4.83^{c}

In comparison to paired values across the column, the means with separate letter superscripts differ significantly (P < 0.05). The data is displayed as mean ± standard deviation (n = 3). 1 –Control, 2 –Negative control (Oxaliplantin, 4 mg/kg), 3 – Positive control; Doluxetine (10 mg/kg) + Oxaliplantin (4 mg/kg), 4 – B-Caryophyllene (25 mg/kg + Oxaliplantin (4 mg/kg) 5 – B-Caryophyllene (50 mg/kg + Oxaliplantin (4 mg/kg)

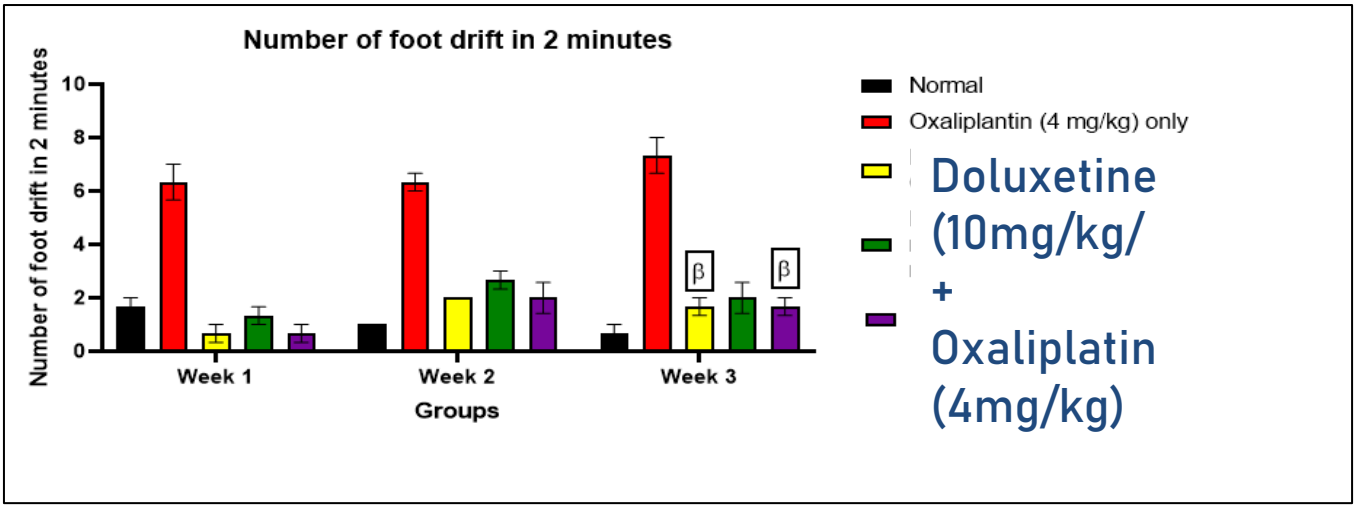
3.2. Effect of Beta-caryophyllene Administration on Beam walk balance Motor Co-ordination Test in experimental rats

For each of the weeks, doluxetine and beta-caryophyllene significantly improved the motor coordination by increasing the number of turns and line crossed while decreasing the number of falls and foot drifts when compared to the negative control. However Post hoc test for number of falls across the weeks shows a statistically significant difference at p <0.05 for group 2 and 3 at week 1 compared to week 3 (fig 1)and a statistically significant difference at p <0.05for group 3 and 5 at week 1 compared to week 3 (Fig 2) for number of foot drift. Post hoc test for differences across the weeks also showed a statistically significant difference at p <0.05for group 4 at week 1 compared to week 2 and group 1 at week 1 compared to week 3 for number of turns (fig 3)



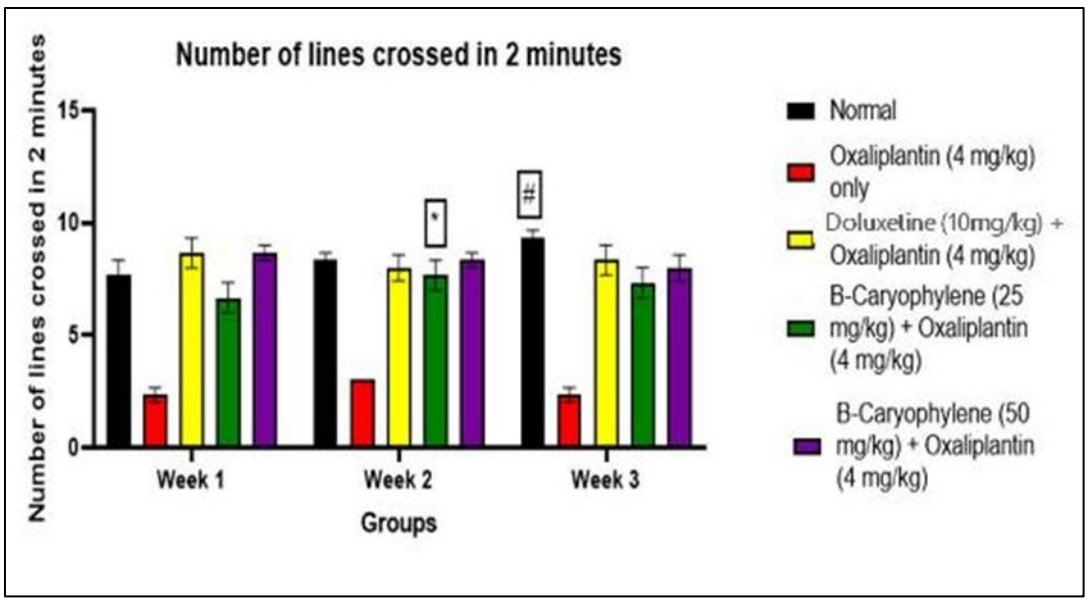
Key:β represents significant difference for Week 1 vs Week 3 (p<0.05)

Figure 1 Effect of Beta-caryophyllene administration on number of falls in 2 minutes in oxaliplatin therapy in wistar rats



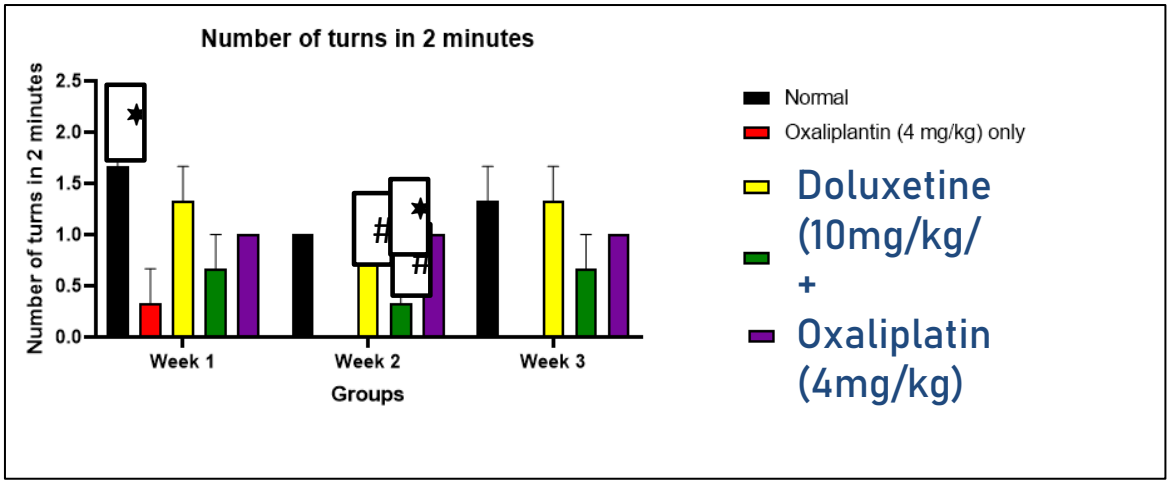
Key: β represents significant difference for Week 1 vs Week 3 ($p<0.05$)

Figure 2 Effect of Beta-caryophyllene administration on number of foot drift in 2 minutes in oxaliplatin-induced peripheral neuropathic rats



Key: * representssignificant difference for Week 1 vs Week 2 ($p<0.05$). # represents significant difference for Week 2 vs Week 3 ($p<0.05$).

Figure 3 Effect of Beta-caryophyllene administration on number of lines crossed in 2 minutes in oxaliplatin-induced peripheral neuropathic rats



Key: * represents significant difference for Week 1 vs Week 2 ($p<0.05$). # represents significant difference for Week 2 vs Week 3 ($p<0.05$).

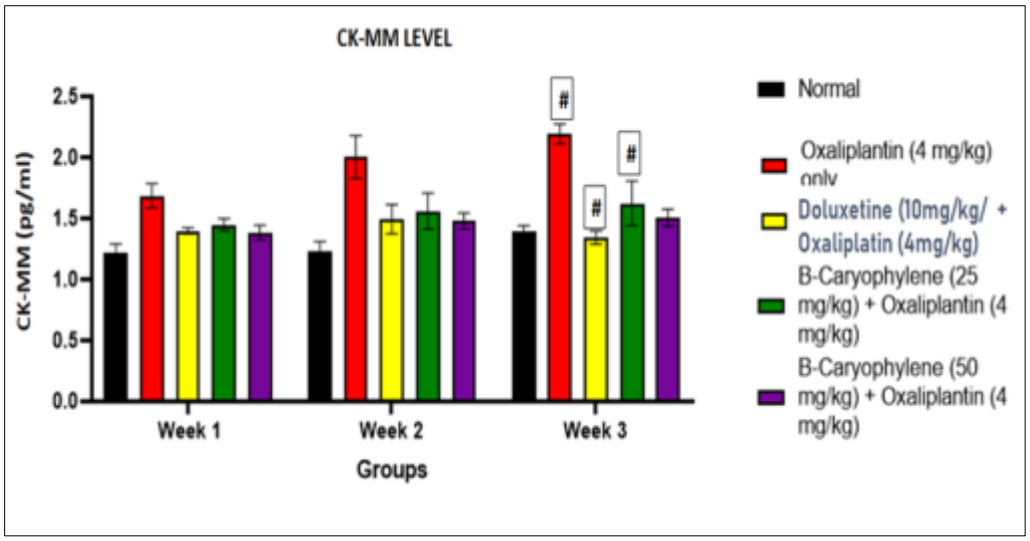
Figure 4 Effect of Beta-caryophyllene administration on number of lines crossed in 2 minutes in oxaliplatin-induced peripheral neuropathic rats

3.3. Effect of Beta-caryophyllene Administration On muscle creatine kinase (CK-MM) and myoglobin levels in experimental rats

FOR THE FIRST WEEK of study The levels of Muscle kinase kinase (CK-MM) and myoglobin levels were increased in the groups treated with beta carophyllene and doluxetine when compared to the negative control (1.69 ± 0.06^{c} pg/ml and 47.17 ± 0.33^{d} pg/ml).There was no significant difference between the effect of beta carophyllene and doluxetine on muscle creatine kinase and myoglobin levels in the first week of experiment. However in the second week, there was a statistically significant difference effect of beta carophyllene ($29.56\pm0.44^{4)}$ on the levels of myoglobin when compared to administration of oxaliplatin only (55.28 ± 2.69) and doluxetine (50.49 ± 0.37).At the third week, the standard drugshowedmore significant effect on the creatine kinase levels than that of myoglobin.

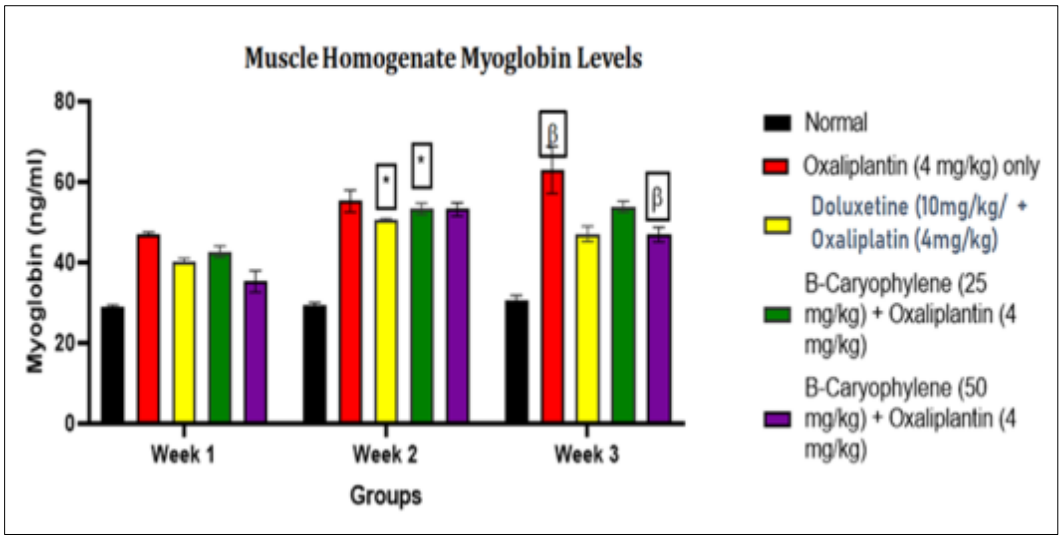
Though beta carophyllene significantly decreased the the creatine kinase and myoglobin levels when compared to the negative control ($.19\pm0.05$, 2.97 ± 5.86).

Post hoc test across the weeks showed a statistically significant difference at $p<0.05$, for CK-MM levels for groups 2,3 and 4 at week 3 when compared to week 1 (fig 5) and at groups 3 and 4 for week 1 compared to week 2 and for group 5forweek 1 compared to week 3for the Myoglobin levels (Fig 6)



represent significance when compared to group 1. # represents significance between week 3 and week 1

Figure 5 Effect of Beta-caryophyllene administration on CK-MM levels in oxaliplatin-induced peripheral neuropathic rats



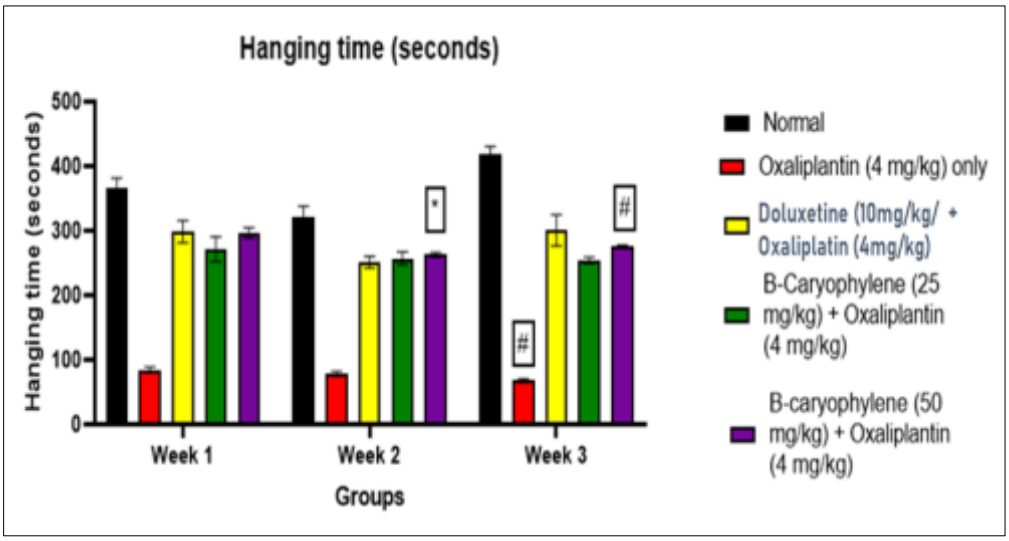
Key: * representssignificant difference for Week 1 vs Week 2 ($p<0.05$). β represents significant difference for Week 1 vs Week 3 ($p<0.05$).

Figure 6 Effect of Beta-caryophyllene administration on Skeletal Muscle Homogenates myoglobulin levels in oxaliplatin-induced peripheral neuropathic rats

3.4. Effect of Beta-caryophyllene administration on muscle strength (hanging time) and muscle mass in oxaliplatin Therapy of Wistar rats

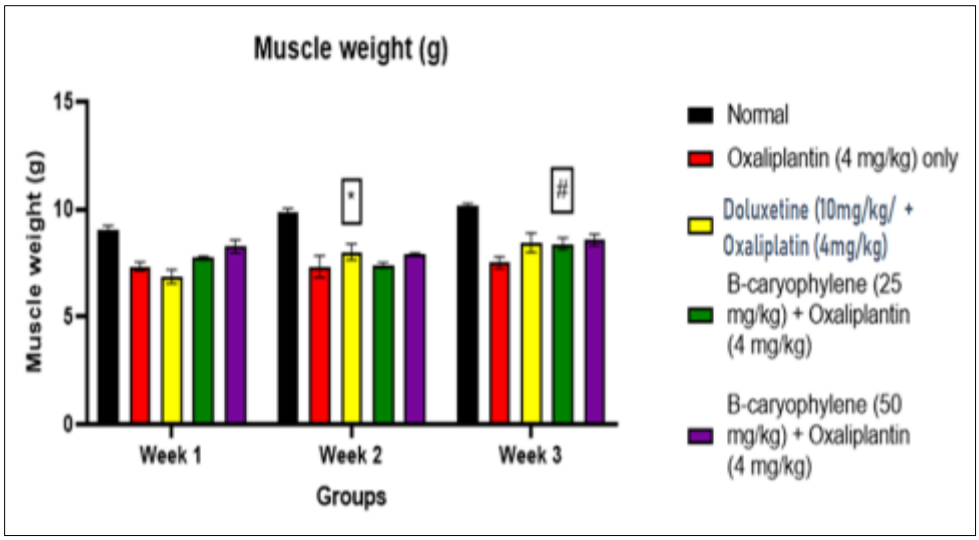
At the first week, Oxaliplatin significantly reduced the hanging time , gastrocnemius muscle weight and body weight of the experimental animals when compared to other experimental groups. However beta carophyllene caused increased muscle weight of gastrocnemius muscle when compared to both the standard drug (6.86±0.32g) and negative control. However in the second week, Beta carophyllene significantly improve the hanging time (indicating improved muscle strength and endurance) and the Gastrocnemius muscle weight when compared to the groups treated with Oxaliplatin only (negative control). At the third week, the Beta-caryophyllene and the standard drug significantly improved the hanging time of the experimental animals

In addition, Post hoc test for hanging time showed a statistically significant difference at $p <0.05$forgroup 5 for week 1 compared to week 2 and for groups 2 and 5 forweek 1 compared to week 3 (Fig 7) while indicating significant difference at $p <0.05$forgroup 5 for week 1 compared to week 2 and for groups 2 and 5forweek 1 compared to week 3 (Fig 8) for gastrocnemius muscle weight.



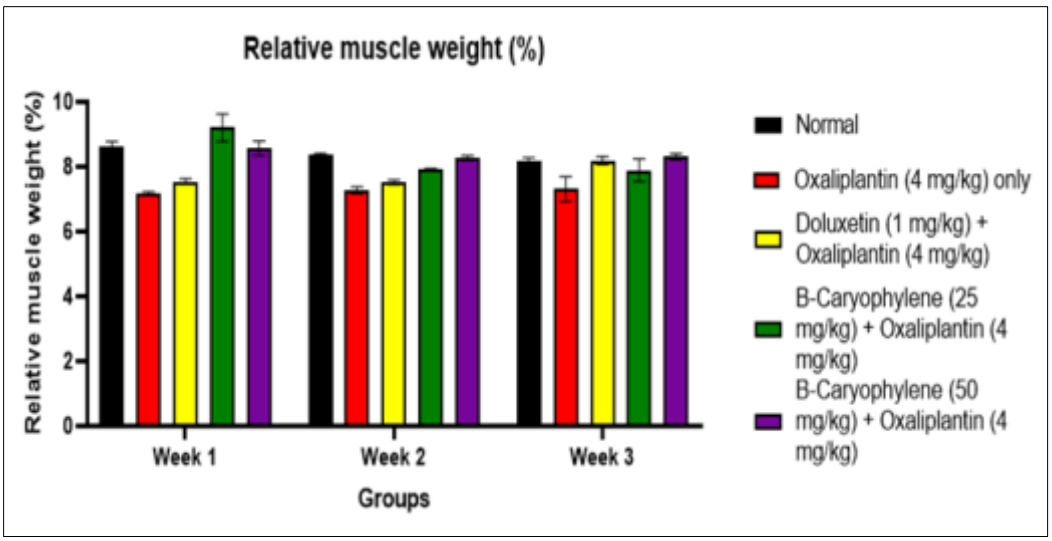
Key: * represents significant difference for Week 1 vs Week 2 ($p<0.05$). # represents significant difference for Week 2 vs Week 3 ($p<0.05$).

Figure 7 Effect of Beta-caryophyllene administration on hanging time in oxaliplatin-induced peripheral neuropathic rats



Key: * representssignificant difference for Week 1 vs Week 2 ($p<0.05$). # represents significant difference for Week 2 vs Week 3 ($p<0.05$).

Figure 8 Effect of Beta-caryophyllene administration on muscle weight in oxaliplatin-induced peripheral neuropathic rats

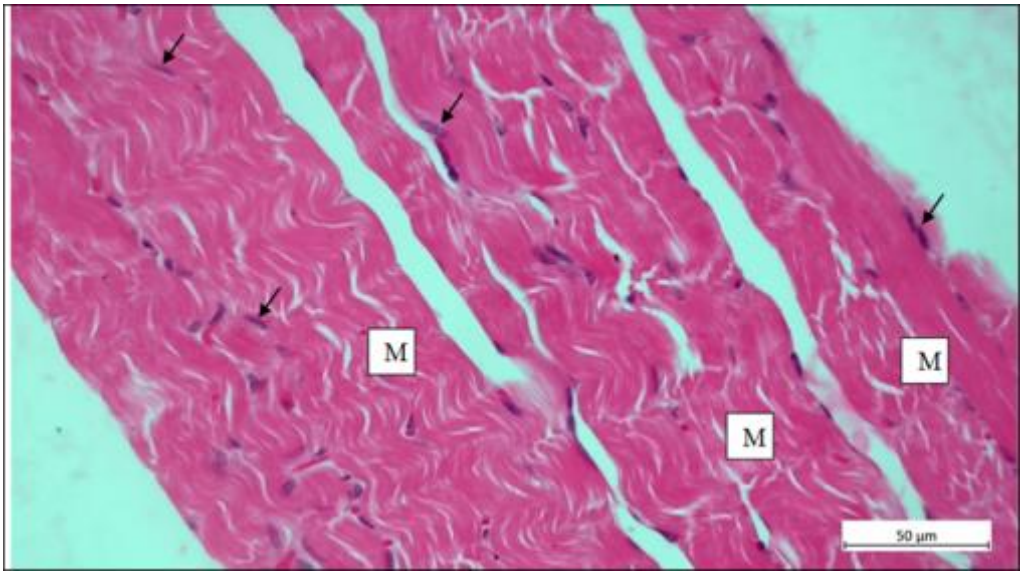


No Significance was noted across the weeks.

Figure 9 Effect of Beta-caryophyllene administration on relative muscle weight in oxaliplatin-induced peripheral neuropathic rats

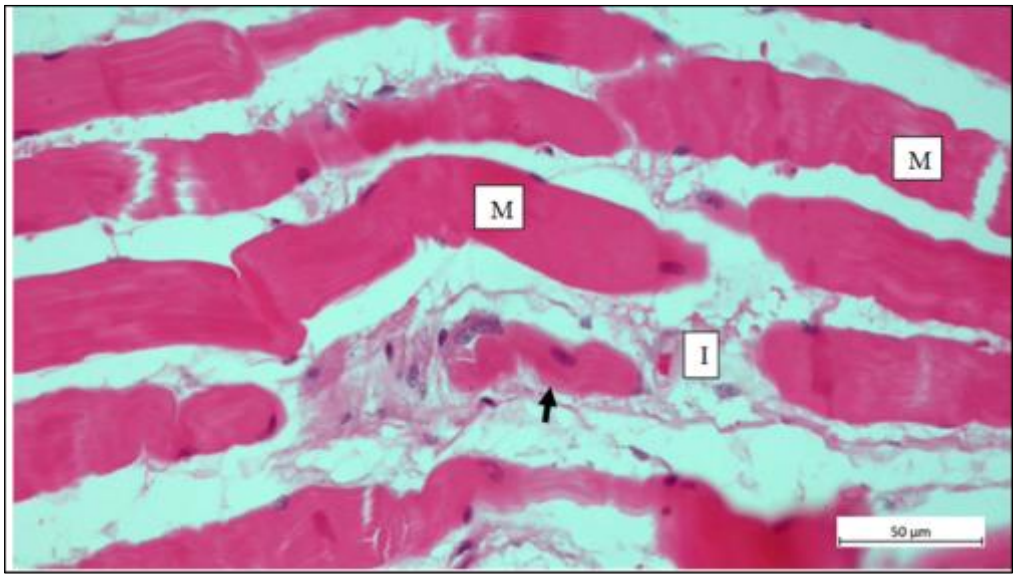
3.5. Histological Findings of the Gastrocnemius muscle in the Study

The Histological Findings, taken at the end of the three weeks clearly shows the denegerative effect of the Oxaliplatin on the gastrocnemius muscle of the rats which was ameliorated by the treatment of Doluxetine and Beta-caryophyllene. However there are mild differences between the effect of doluxetine and the phytochemical , within the cross sectional areas of the muscle bundles.



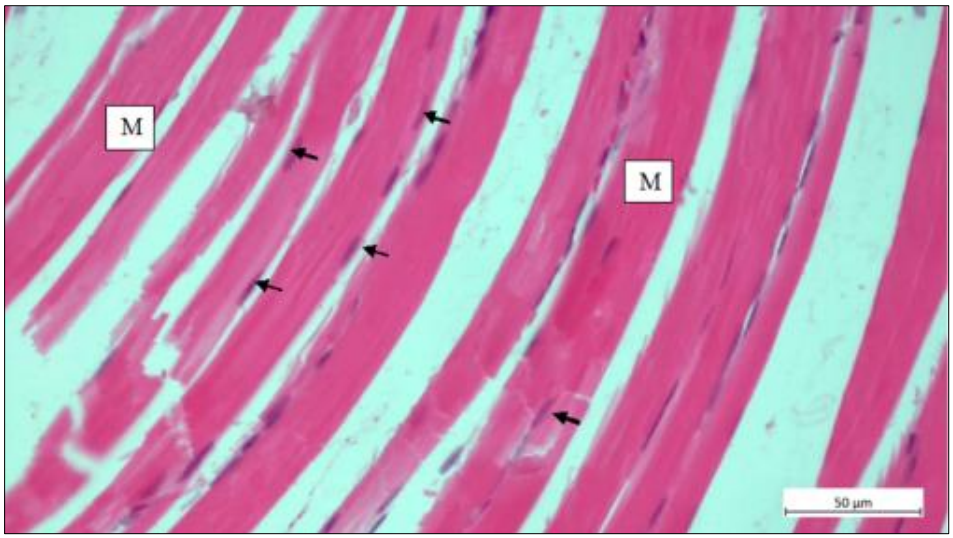
Sections of the gastrocnemius muscle showing normal histomorphology. Normal muscle bundles (M) with peripheral round-to-elongated nuclei (arrow) were observed. H&E x400

Figure 10 Histological Findings For Normal Control (Group 1)



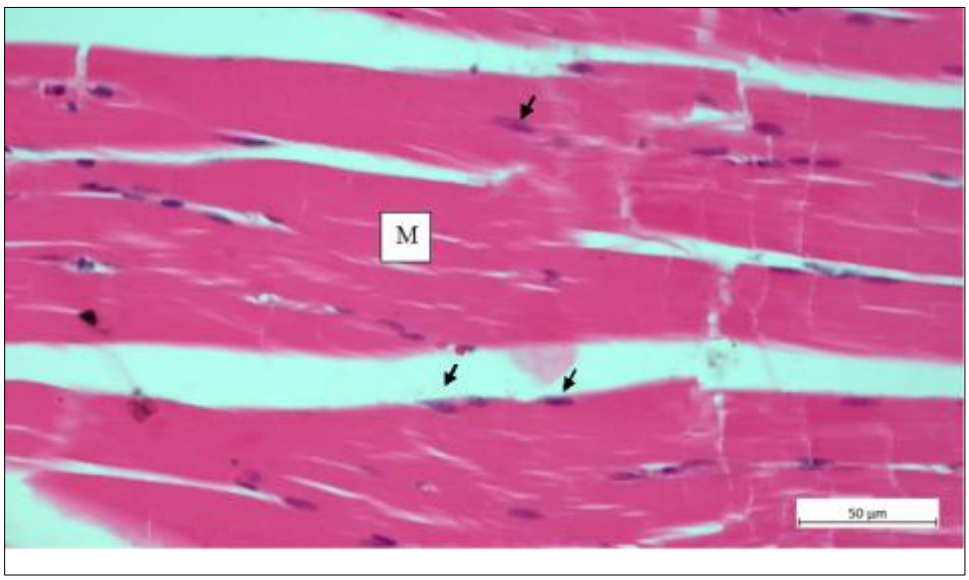
Sections of the gastrocnemius muscle presented in this group showed loss of cross striations as well as fragmentation of the muscle fibers. Muscle fiber (M); Interstitium (I). Fragmented muscle fiber (black arrow). HE x400

Figure 11 Histological Findings ForNegative Control (Group 2)



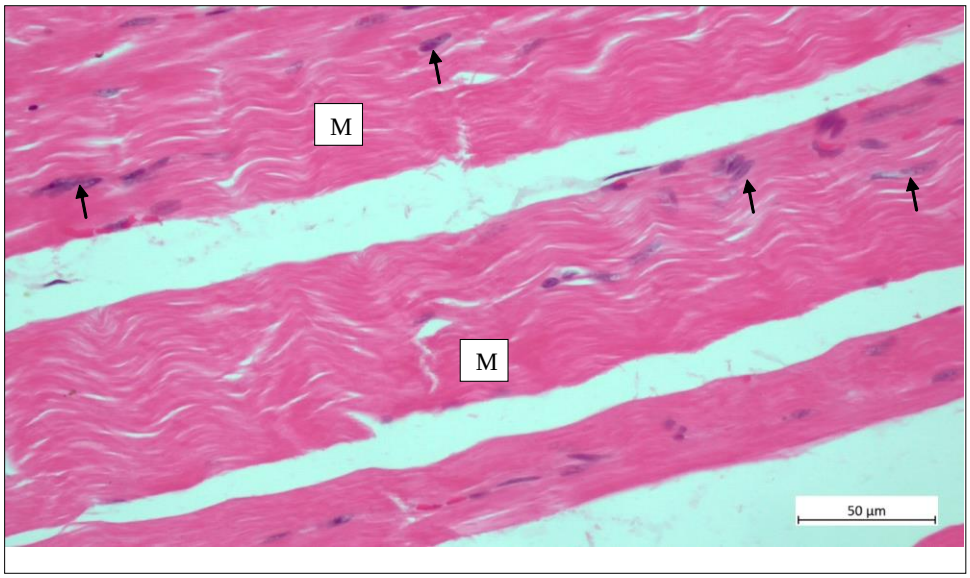
Sections of the gastrocnemius muscle showing normal histomorphology. Normal muscle bundles (M) with peripheral round-to-elongated nuclei (arrow) were observed. H&E x400

Figure 12 Histological Findings For Positive Control (Group 3)



Sections of the gastrocnemius muscle showing normal histomorphology. Normal muscle bundles (M) with peripheral round-to-elongated nuclei (arrow) were observed. H&E x400.

Figure 13 Histological Findings For The Low Terpernoid Group (GROUP 4)



Sections of the gastrocnemius muscle showing normal histomorphology. Normal muscle bundles (M) with peripheral round-to-elongated nuclei (arrow) were observed. H&E x400.

Figure 14 Histological Findings for High Terpernoid Group (GROUP 5)

4. Discussion

4.1. Lethal Dose Evaluation

The lethal dose evaluation confirmed that beta-caryophyllene oral consumption is safe for up to 2000mg/kg as reported by Da Silva Olivenia et al (2018) [50]. However, safety data sheet (2019) [51]of Hazard Communication Standard of Occupational Safety And Health Administration (OSHA's HCS) indicated that it has an oral toxicity of 4,716 mg/kg [51].

4.2. Effect Of Beta-Caryophyllene To Doluxetine And Oxaliplatin Therapy In The Study

The functions of beta-caryophyllene have been primarily linked to its activation of CB (cannabinoid) 2 receptors of the endocannobinoid system [52,53,54]. However some other mechanisms will be included.

4.3. Effects on Motor Coordination in the Study

Clinically, Oxaliplatin therapy presents an induced acute peripheral neuropathy during or immediately after infusion, that may be accompanied with motor changes with vary intensity and duration. This poses a major problem to both cancer patients, survivors and their health care providers [9].

Oxaliplatin therapy presents muscle weakness, particularly in cold environment as it causes cold allodynia, coupled with its ability to damage motor neurons that results to altered nerve conductions [62] especially as disturbances in the sodium Voltage-gated (NaV) channels of muscle cells [9] resulting to muscle cramps and spasms [63]. This may be the reason for the motor deficit observed in oxaliplatin therapy in this study. However, Beta-caryophyllene improved the motor responses in this study as it may have caused protection against neuroinflammation. [52] as it has demonstrated ability to suppress motor paralysis like in models of Alzeheimer's disease [64], induced brain Injury [65], multiple sclerosis [66] and Parkinson's disease [56]. The difference of this effect between beta-caryophyllene and doluxetine was not significant in this study and it may due to dose regimen of beta-caryophyllene (25mg/kg and 50 mg/kg of body weight).

In this study, the Increase in number of falls across the weeks in the groups treated with oxaliplatin only may be due to accumulation of oxaliplatin at the Dorsal Root Ganglion (DRG) resulting to neuroinflammation that led to dysfunction induced on the sensory-motor pathway or the loss of purkinje cells (the principal cells of the cerebellar cortex)as stated by Paz et al (2017)[67].

In this study however, the number of falls were increased across the weeks in the experimental design of the groups treated with oxaliplatin. This may be due to accumulation of oxaliplatin at the dorsal root ganglia resulting to dysfunction induced on the sensory-motor pathway or the cerebellum as it is seen to cause loss of purkinje cells, (the

principal cells of the cerebellar cortex), and activation of microglial (the brain's resident immune cells) with alterations in cerebellar structure [67,68]. This will definitely result to disruptions in learning in motor co-ordination. Beta-caryophyllene can ameliorate this effect by offering neuroinflammation to these delicate brain cells as shown in other works [69,70], thus improving the coordination, control, and learning of movements (functions of the purkinje cells).

According to Jin et al (2020), It may be also due to excess activation of microglial cells causing alterations in cerebellar structure that manifest as disruptions of learning in motor co-ordination [68]. Beta-caryophyllene may haveameliorated this effect by offering neuroinflammation to these delicate brain cells as stated in other works [69,70], thus improving the coordination, control, and learning of movements (functions of the purkinje cells). This study has shown that beta-caryophyllene may provide protection against loss of motor memory as it has shown to improve working memory according to Lindsey et al (2019) [71].

Oxaliplatin therapy is believed to cause dysfunction of Astrocytes which plays a role in maintenance of the brain homeostasis (disturbances in cognitive function, a condition often referred to as "chemobrain" resulting in diminished number of line crossed indicative of reduced motor memory. Beta-caryophyllene and doluxetine may have prevented this loss of motor memory by offering protection against loss of motor memory[66].

4.4. Effects on Muscle Function and Mass, Injury in the Study

Oxaliplatin therapy presented a weekly increase in muscle weakness in this study attributed to the reduced hanging time. Other studies reported that this weakness is usually accompanied by cold allodynia and damage to motor neurons [62] as a result of disturbances in the sodium voltage gated (NaV) channels of muscle cells[9] resulting to muscle cramps and spasms [63]. Beta-caryophyllene, in this study, demonstrated protection over the muscle strength showing that it may stabilize the disturbances in NaV channels of muscles improving endurance and strength of skeletal muscles.

In this study, Oxaliplatin was seen to cause reductions in gastrocnemius muscle weight which was reported by Feather et al (2018) who implied that it causes loss of hindlimb muscle mass and function, particularly the gastrocnemius muscle [72]. The loss may negatively impacts the quality of life of cancer patients. This study suggests that the muscle loss and atrophy, especially at the hindlimbs like the gastrocnemius, should be monitored and used as a diagnostic tool in recovery and post-recovery of cancer patients and survivors respectively.

However, beta-caryophyllene treatment, in this study, increased relative muscle weight showing it could indirectly boost muscle synthesis. A report by Geddo et al (2021) indicated that it also enhances skeletal muscle glucose metabolism which is needful for maintenance of skeletal muscle mass, a useful property in the face of aging and other myopathies [73. The direct anti-inflammatory and antioxidant effect of beta on skeletal muscle could protect the muscle cell integrity reducing leak of creakine kinase and myoglobin. This will maintain the muscles cells' intrinsic functions and prevent further damage of muscle cells.

4.5. Contribution ToExisting Knowledge

This research provided strong evidence that muscle recovery should be included as a diagnostic tool in assessment of cancer patients and survivors respectively. It presented the activities of beta-caryophyllene regarding Protection of the skeletal muscles from the adverse effect of Oxaliplatin therapy. These include protection of muscle cells from injury incurred from oxidation and inflammation, preservation of muscle mass which is a needed advantage for patients with degenerative skeletal muscle loss, as seen in cancer patients. It showed that piper guineense could be beneficial as part of diet recommendations for cancer patients, especially since it has shown to contain beta-caryophyllene in good quantities especially with the knowledge that beta-caryophyllene consumption is safe up to 2000mg/kg

This study further added to the growing knowledge on amelioration of the various pain sensitivities in Oxaliplatin therapy showcasing beta-caryophyllene as a very potential antinociceptive agent in oxaliplatin therapy. It also showed that beta-caryophyllene could ameliorate the sensorimotor deficit in Oxaliplatin-induced peripheral neuropathy adding to the existing knowledge in other models of dysfunctional motor coordination.

5. Conclusion

This study suggested that beta-caryophyllene extracted from *Piper guineense* exhibits anti-nociceptive properties and can improve motor deficit and muscle cachexia. This should effectively reduce the cost of chemotherapy and also the cost of managing its associated neuropathy. Further and clinical studies should be designed for management of muscle cachexia induced by oxaliplatin, and other antineoplastic drugs in cancer patients. Further works should be carried on other classes to exhaust its clinical potentials in alternate Chemotherapy. There should also be model where beta-

caryophyllene is directed tested on heavy and light chains of skeletal muscle fibers to further elucidate their mechanism of action beyond skeletal muscle glucose metabolism and protection against lipid peroxidation in muscle cells.

Compliance with ethical standards

Acknowledgments

This is to acknowledge and appreciate all the authors who participated with their knowledge, editorial skills, technical and financial aids to the success of this research. We will continue to aim for excellence in academic research to further improve the quality of knowledge available for clinical usage. We also want to state that there was no conflict of interest among authors in respect to this publication.

Disclosure of conflict of interest

There is no conflict of interest that he has with research towards its publication, or product mentioned in this study.

Statement of ethical approval

Ethical Approval was obtained from the College of Medicine and Health Sciences, University of Nigeria, Enugu Campus, Enugu State, Nigeria.

References

[1] Kanzawa-Lee GA, Larson JL, Resnicow K, Smith EM. Exercise effects on chemotherapy-induced peripheral neuropathy: a comprehensive integrative review. *Cancer Nursing*.2000; 43:E172–85.

[2] Augustine B, Abdulrahman FM, Yahaya G, Adebayo A, Obiako, R.O., Muktar, H.M. Vinca Alkaloid-induced peripheral Neuropathy in Zaria,North Western Nigeria: a case Report. Journal *of Medicine in the Tropic.*2018; 20 :135-139

[3] Hicks CW, and Selvin, E. (2019). Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. *Current diabetes reports.*2019;19(10):86.

[4] Medscape. Diabetic Neuropathy. Endocrinology: Drugs and Diseases. Last updated August 24, 2021.https://www.mayoclinic.org/diseases-conditions/diabetic-neuropathy/diagnosis-treatment/drc-20371587

[5] Shiferaw WS, Akalu TY, WorkY, and Aynalem YA. Prevalence of Diabetic Peripheral neuropathy in Africa: a systematic review and meta-analysis. *BMC endocrine disorders,*2020; 20(1): 49.

[6] Kim E. Chemotherapy-induced peripheral neuropathy: bench to Clinical Practice. *The Korean Journal ofPain.* 2020; 33(4), 291-293.

[7] Park SB, Goldstein D, Krishnan AV, Lin CS-Y, Friedlander ML, Cassidy J, et al. Chemotherapy-induced peripheral neurotoxicity: a critical analysis. *CA Cancer Journal ofClinics,* 2013; 63:419–37.

[8] Fumagalli G, Monza L, Cavaletti G, Rigolio R, and Meregalli C. (2021). Neuroinflammatory Process Involved in Different Preclinical Models ofChemotherapy- Induced Peripheral Neuropathy. *Frontiers in* Immunologyl; 2021;11:626687.

[9] Zajaczkowska R, Kocot-kepska M, Leppert W, Wrzosek A, Mika J, Wordliczek J Mechanisms of Chemotherapy induced peripheral neuropathy. *International Journal of Molecular Science,* 2019.20:1451

[10] Trachootham D, Zhou Y, Zhan H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus R B, Liu J, & Huang P . Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer cell*. *2006;10*(3), 241–252.

[11] Branca JJV, Morucci G, Malentacchi F, Gelmini S, Ruggiero M, and Pacini S. Effects of oxaliplatin and oleic acid Gc-protein-derived macrophageactivating factor on murine and human microglia: effects of oxaliplatin and GcMAF on microglia. *Journal ofNeuroscience. Research*. 2015; 93, 1364–1377.

[12] Brandolin L, d'Angelo M, Antono Sante A, Allegrelli M, Cimini A. Chemotherapy signalig in chemotherapy-induced neuropathic pain. *International journal of molecular Sciences.* 2019; 20 (12), 2904.

[13] Hu S, Huang KM, Adams EJ, Loprinzi CL, & Lustberg MB. Recent Developments of Novel Pharmacologic Therapeutics for Prevention of Chemotherapy-Induced Peripheral Neuropathy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2019; 25(21), 6295–6301

[14] Flynn KE, Atallah E, Lin L, Shah NP, Silver RT, Larson RA, Panilla- Ibarz J, Thompson JE, Oehler VG, Radich JP, Kota V, Mauro MJ, Schiffer CA, Cortes J, and Weinfurt KP.Patient- and physician- reported pain after tyrosine kinase inhibitor discontinuation among patient with chronic myeloid leukemia. *Haematologica.*2022;107(11), 2641-2649.

[15] Dario, C. Chemotherapy-induced muscle wasting: an update.*European Journal ofTranslational Myology.*2018;28 (2): 153-157, 2018.

[16] Nicolas K, David T, Côme L, Denis P, Karine Le M, et al.Efficacy of donepezil for the treatment of oxaliplatin-induced peripheral neuropathy: DONEPEZOX, a protocol of a proof of concept, randomised, triple-blinded and multicentre trial. *BMC Cancer*. 2022;22 (1),1-11.

[17] Wikipedia . Chemotherapy-induced peripheral Neuropathy. Last edited on 14 February 2024. Last visited 20 August 2024.https://en.wikipedia.org/wiki/Chemotherapy-induced_peripheral_neuropathy.

[18] Lees JG, Abdulla M, Barkl-Luke ME, Livni L, Keating BA, Hayes J, Fiore NT, Park SB, Moalem-Taylor G, & Goldstein D. Effect of exercise on neuromuscular toxicity in oxaliplatin-treated mice. *Muscle & nerve*.2021; *64*(2), 225–234.

[19] Claire EF, Justin GL, Preet GS M, David G, Fracp John, B K, Gila MT And Patsie P. Oxaliplatin Induces Muscle Loss And Muscle-Specific Molecular Changes In Mice. *Muscle Nerve.*2018; 57: 650–658, 2018.

[20] Liu H, Zheng J, Liu P, Zeng F. Pulverizing processes affect the chemical quality and thermal property of black, white, and green pepper (*Piper nigrum* L.). *Journal of Food Science and Technology*. 2018;55: 2130-2142.

[21] Agilent (2019). Safety Data Sheet according to OSHA HCS (Occupational Safety and Health): Beta-Carophyllene Standard (IXL ml). Last Prepared on 30/03/2019.

[22] Godswill NA, Onajobi F, Osilesi O, Adebawo O, Efere MO. Chemical constituents in *n*-butanol fractions of *Costus afer* kerGawl leaf and stem. *Journal of Intercultural Ethnopharmacology*. 2014; 3(2)

[23] Dante, A. Isolation and Antifungal Activity of CaryoPhyllene from Clove Leaf Oil (*Syzygium aromaticum L)* on Mahogany Leaf Composites. *Science and Community Pharmacy Journa. 2022; 1*(1),1-6

[24] Meng J, Zhang Q, Yang C, Xiao L, Xue Z. and Zhu J. Duloxetine, a Balanced Serotonin-Norepinephrine Reuptake Inhibitor, Improves Painful Chemotherapy-Induced Peripheral Neuropathy by Inhibiting Activation of p38 MAPK and NF-B. *Frontier for Pharmacology.* 2019; *10:365*.

[25] da Silva Oliveira GL, Machado K.C, Machado KC, Feitosa CM, de Castro Almeida FR. Non-clinical toxicity of β-caryophyllene, a dietary cannabinoid: Absence of adverse effects in female Swiss mice. *Regulatory toxicology and pharmacology.* 2018; 92, 338–346.

[26] JirovetzL, Buchbauer G, Ngassoum MB, Geissler M. Aroma compound analysis of Piper nigrum and Piper guineense essential oils from Cameroon using solid-phase microextraction–gas chromatography, solid-phase microextraction–gas chromatography– mass spectrometry and olfactometry. *Journal ofChromatography*.2002; 976:265-275.

[27] Taejoon K., Bokyeong S, Kyoung SC, and Im-Soon L. Therapeutic Potential of Volatile Terpenes and Terpenoids from Forests forinflammatory Diseases. *Internal Journal ofMolecular. Science.*2020; 21, 2187.

[28] Mehta AM, Van Den Hoven JM, Rosing H, Hillebrand M J, Nuijen B, Huitema AD, Beijnen JH, and Verwaal VJ. Stability of Oxaliplatin in Chloride- containing carrier solutions used in hyperthermic intraperitoneal chemotherapy. *International Journal of Pharmaceutic.*2015; 479(1), 23-27.

[29] Zhang Z, Shen P, Lu X, Li Y, Liu J, Liu B, Fu Y, Cao Y, Zhang N.In Vivo and In Vitro Study on the efficacy of terpinen-4-ol in dextran sulfate sodium-induced mice experimental colitis. *Frontiers in Immunology*. 2017;8 (558).

[30] Iwu, M. M. (2014). Handbook of African medicinal plants (2nd ed.). CRC Press. (Chapter on Piper guineense discusses its traditional uses and pharmacological activities). ttps://doi.org/10.1201/b16292

[31] Ogunwande IA, Avoseh NO, Flamini G, Hassan A S, Ogunmoye AO, Ogunsanwo AS, Ogunmoye AO, Ogunsanwo AO, Yusuf KO, Kelechi AO, Tiamiyu ZA, and Tabowei GO.Essential oils from the leaves of six Medicinal Plants of Nigeria. *Natural product Communications*. 2013;8(2): 243-248.

[32] Fidyt K, Fiedorowics A, Strazadala L, and Szummy A.β-Caryophyllene and β Caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Medicine.*2016; 5(10): 3007-3017.

[33] Sharma C, Al Kaaabi JM, Nurulain SM, Goyal SN, Kamal MA and Ojha S. Polypharmacological Properties and Therapuetic potential of β- caryophyllene: a Dietary Phytocannabinoid of Pharmaceutical Promise. *Current Pharmaceutival* Design. 2016;22(21):3237-3264.

[34] Orieke D, Ohaeri OC, Irene II, & Ijioma SN . Semen quality, hormone profile and histological changes in male albino rats treated with *Corchorus olitorius* leaf extract. *Avicenna journal of phytomedicine*. 2019; *9*(6): 551–562.

[35] Jonathan PA, Vitoria W. dos Santos, Raquel, N. das N, et al. Antioxidants Improve Oxaliplatin-Induced Peripheral Neuropathy in Tumor-Bearing Mice Model: Role of Spinal Cord Oxidative Stress and Inflammation. *The Journal of Pain.* 2021;22(8) : 996–1013

[36] Wan N, Xiaolan Z, Ling H, Chao K, and Qingbang X. Preventing Oxaliplatin-induced Neuropathic Pain: Using Berberine to Innhibit the activation of NF-kB and release of pro-inflammatory cytokines in dorsal root ganglion. *Experimental and Therapeutic Medicine.* 2020; 21:135.

[37] Wang G, Yang Y, Wang C, Huang J, Wang X, Liu Y, & Wang H.Exploring the role and mechanisms of diallyl trisulfide and diallyl disulfide in chronic constriction-induced neuropathic pain in rats. *The Korean journal of pain*. 2020; *33*(3): 216–225.

[38] Ruan Y, Gu L, Yan J, Guo J, Geng X, Shi H, Yu G, Zhu C, Yang Y, Zhou Y, Wang C, & Tang Z. An effective and concise device for detecting cold allodynia in mice. Scientific reports. 2018; 8(1): 14002.

[39] Scheich B, Vincze P, Szőke É, Borbély É, Hunyady Á, Szolcsányi J, Dénes Á, Környei Z, Gaszner B, & Helyes Z.Chronic stress-induced mechanical hyperalgesia is controlled by capsaicin-sensitive neurones in the mouse. *European journal of pain.* 2017; *21*(8): 1417–1431.

[40] Mamun-Or-Rashid, AshiqulI.,Shah A, and Aslam H. Evaluation of Analgesic Activity by Acetic Acid Induced Writhing Method of Crude Extracts of Acacia nilotica. *Scholars Academic Journal of Pharmacy.* 2017; 6(4): 126-138.

[41] Zhou Q, Bao Y, Zhang X, Zeng L, Wang L, Wang J, & Jiang W. (2014). Optimal interval for hot water immersion tail-flick test in rats. Acta neuropsychiatrica. 2014;26(4): 218–222.

[42] Feng Q, Ma Y, Mu S, Wu J, Chen S, Ouyang L, & Lei W.(2014). Specific Reactions of Different Striatal Neuron Types in Morphology Induced by Quinolinic Acid in Rats. PloS one. 2014; 9. e91512.

[43] Castro B, & Kuang S. (2017). Evaluation of Muscle Performance in Mice by Treadmill Exhaustion Test and Whole-limb Grip Strength Assay. *Bio-protocol*. 2017; *7*(8): e2237.

[44] Bonetto A, Andersson DC, & Waning DL. (2015). Assessment of muscle mass and strength in mice. *BoneKEy reports*. 2015; *4*, 732.

[45] Schneider CA, Rasband WS, & Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods.*2012; *9*(7), 671-675.

[46] Yin W, Li Z, & Zhou Q. Preparation of tissue homogenates. In T. Zhao (Ed.), *Basic methods for cell culture* (pp. 79-84). Humana Press. 2009

[47] Steen C, Wilczak N, Hoogduin JM, Koch M, & De Keyser J. (2010). Reduced creatine kinase B activity in multiple sclerosis normal appearing white matter. *PloS one*. 2010; *5*(5): e10811.

[48] Peter NH, Mattheus U, Hirt B, Just L, & Mack AF. Large-scale tissue clearing (PACT): Technical evaluation and new perspectives in immunofluorescence, histology, and ultrastructure. *Scientific reports*. 2016; *6*, 34331.

[49] Burkhardt JE, Pandher K, Solter PF, Troth SP, Boyce RW, Zabka TS, & Ennulat D. (2011). Recommendations for the evaluation of pathology data in nonclinical safety biomarker qualification studies. *Toxicologic pathology*. 2011; *39*(7): 1129–1137.

[50] da Silva Oliveira GL, Machado KC, Machado KC, Feitosa CM, de Castro Almeida FR.Non-clinical toxicity of β-caryophyllene, a dietary cannabinoid: Absence of adverse effects in female Swiss mice. *Regulatory toxicology and pharmacology. 2018;* 92, 338–346.

[51] Agilent (2019). Safety Data Sheet according to OSHA HCS (Occupational Safety and Health): Beta-Carophyllene Standard (IXL ml). Last Prepared on 30/03/2019

[52] Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie X Q, Altmann KH, Karsak M, & Zimmer A.Beta-caryophyllene is a dietary cannabinoid. *Proceedings of the National Academy of Sciences*. 2008; 105(26):9099-9104.

[53] Russo E B.Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacolog.* 2011; 163(7): 1344-1364.

[54] Klauke A.-L, Racz I, Pradier B, Markert A, Zimmer A, Gertsch J, & Zimmer A. The cannabinoid CB2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. *European Neuropsychopharmacology.* 2014; 24(4): 608-620.

[55] Alberto H, María EG, Fernando N, Gimena P, Fausto R, and María IA. Role ofBeta-caryophyllene in the Antinociceptive and Anti-Inflammatory Effects of Tagetes lucida Cav. Essential Oil. *Molecules*. 2020; 25 (675).

[56] Vijayakumar K, Ajith TA and Pillai S. Protective effect of β-carophyllene against MPTP-induced striatal neuronal injury in mice. *Neuroscience Letters*. 2020;735,1350833.

[57] AgnesJP, dos Santos B, das Neves RN, Luciano VMM, Benvenutti L, Goldoni FC, Schran RG, Santin JR, Quintão NLM, Zanotto-Filho A. β-Caryophyllene Inhibits Oxaliplatin-Induced Peripheral Neuropathy in Mice: Role of Cannabinoid Type 2 Receptors, Oxidative Stress and Neuroinflammation. *Antioxidants*1893*,* 12.

[58] Fotio Y, Aboufares el Alaoui, A, Borruto AM, Acciarini S, Giordano A, and Ciccioppo R. Efficacy of a combination of N-Palmitoylethanolamide, Beta- carophyllene, Carnosic Acid and Myrrh Extract on Chronic Neuopathic Pain: a Preclinical Study. *Frontiers of Pharmacology*. 2019; 10 (711).

[59] Yakimov ID, KolmogorovIM, Le-Deygen IM. Beta-Caryophyllene Induces Significant Changes in the Lipid Bilayer at Room and Physiological Temperatures: ATR-FTIR Spectroscopy Studies. *Biophysica*.2023; ,2:501-512.

[60] Gromley J, and Sweeney JA. Doluxetine in the treatment of depression and anxiety. *Current Medical Research and Opinion.2012;* 28(11): 1879-1889.

[61] Goh MJ, and Jang JH. (2015). Doluxetine and its effects on cognitive function in patients depression and anxiety: A meta-analysis. *Neuropsychology Revie.*2015;25(4): 341-355.

[62] Park SB, Lin CS, and Krishnan AV. Oxaliplatin-induced Neurotoxicity: Changes in excitability precede development of neuropathy. *Brain*. 2013; 136(12): 3666-3675.

[63] Staff NP, Grishold A, Grishold W, and Winderbank AJ. (2017) Chemotherapy induced peripheral Neuropathy: A current review.*Annals of Neurology.* 2017; 81(6): 772-781

[64] ScuderiC, FilippisDD, Luvone T, Blasio A, Steardo A, and Esposito G.Cannabidiol in Medicine: A review of its therapeutic Potential in CNS disorders, with a focus on preclinical models. *Frontiers in Bioscience (Landmark Edition.*2014;19:317-335.

[65] Gabon EA, Butawan M, Khan MA, Williams AL, Kelly LS, McGriffCP, Alwhaibi A, Fagan JM, and Fuqua MB. Beta-caryophyllene improves motor deficit and reduces neuroinflammationin a mouse model of traumatic brain injury. *Journal of Neurotrauma.*2016; 33(13): 1141-1153.

[66] Alberti TB, Barbosa WLR, Vieira JLF, Raposo NRB, Dutra RC. β-caryophyllene, a CB2 receptor-selectivephytocannabinoid, suppresses motor paralysis and neuroinflammation in a murine model of multiple sclerosis. *InternationalJournal of Molecular Scences*. 2017; 8, 691.

[67] Paz AC, Costa DM, Lima RR, Silva AC, Carvalho JF, & Pires MM. Oxaliplatin induces cerebellar damage and motor deficits in rats. *Neurotoxicology.*2017; 60:118-127.

[68] Jin, H., Lu, W., Zhang, X., & He, X. Oxaliplatin-induced oxidative stress and neurotoxicity in cerebellar neurons. *Frontiers in Neuroscience.*2020; 14, 533821.

[69] Salles J P, da SilvaFS, Vanzela EC, & Salgado JV. Beta-caryophyllene protects against neuroinflammation and cognitive deficits induced by neurotoxic injury in rats. *Frontiers in Pharmacology.2020; 11*, 580536.

[70] Zhang L, Cheng X, Wang C, Li X, Liu Y, & Zhang J. Beta-caryophyllene attenuates neuroinflammation and improves motor function in a mouse model of Parkinson's disease. *Journal of Neuroinflammation. 2021; 8*(1):155.

[71] Lindsey LP, Daphney CM, Oppong-Damoah A, Uchakin PN, Abney SE, Ucheakina ON, Khusial RD, Akil A, and Munane KS.The Cannabinoid receptor 2 agonist, β-Carophyllene, improves working memory and reduces circulating levels of specific pro-inflammatory cytokines in aged mice. *Behavioural brain Research.*2019;372, 112012.

[72] Feather CE, Lees JG, Makker PG, Goldstein D, Kwok, JB, Moalem-Taylor G, and Polly P. Oxaliplatin induces muscle loss and muscle-specific molecular changes in Mice. *Muscles & nerve.*2018; 57(4):650-658.

[73] Geddo F, Antoniotti S, Querio G, Salaroglio IC, Costamagna C, Riganti C, & Gallo M P. Plant-Derived Trans-β-Caryophyllene Boosts Glucose Metabolism and ATP Synthesis in Skeletal Muscle Cells through Cannabinoid Type 2 Receptor Stimulation.*Nutrients.*2021;13(3).