

Immunomodulatory, anti-inflammatory and antioxidant activities of aqueous and ethanolic extracts of *Cissus quadrangularis* Linn. (Vitaceae) in chronic pain

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Abstract

Cissus quadrangularis Linn. (Vitaceae) is a plant used to treat injured tendons, broken bones, asthma, stomach ache, scurvy and digestive disorders. The present study purposed to evaluate the antihyperalgesic effects (*in vivo*) and the immunomodulatory, antioxidant and anti-inflammatory properties (*in vitro*) of aqueous and ethanolic extracts of *Cissus quadrangularis* (*C. quadrangularis*). Immunomodulatory (chemiluminescence, cytokines and cell proliferation), anti-inflammatory (protein denaturation, 5-lipoxygenase, cyclooxygenase 1 and 2) and antioxidant (DPPH, ABTS and NO) tests were performed *in vitro*, while the anti-hyperalgesic (vincristine) investigations were conducted *in vivo* on Wistar rats. The results revealed that extracts developed immunomodulatory activity by inhibiting the production of ROS (intracellular/extracellular), of TNF α , IL-1 β , IL-6 as well as inhibiting cell proliferation, and by stimulating the production of IL-10. The anti-inflammatory activity of the extracts was demonstrated by an inhibition of 5-LOX, protein denaturation and cyclooxygenases 1 and 2. In addition, extracts showed interesting scavenging effects, attesting their antioxidant potential. The extracts administered to the animals (180 and 360 mg/kg) inhibited ($p < 0.001$) hyperalgesia and allodynia in animals. These extracts also led to the reduction in serum and sciatic nerve levels of TNF α , IL-1 β and IL-6, as well as to an increase in cell growth factors (NGF and IGF) production of treated animals. These results suggest that extracts of *C. quadrangularis* use immunomodulatory, anti-inflammatory and antioxidant capacity to prevent and/cure painful neuropathy after vincristine administration. *C. quadrangularis* is therefore a promising natural substance for the management of neuropathic pain.

Introduction

Pain, known as the most common manifestation of anyone undergoing primary care, also known as one of the worlds' most debilitating problems causes a feeling that ranges from mild discomfort to extreme pain, and can be in a well-localized wound and/or be diffuse (Nunes et al. 2020; Karrat et al. 2022). Known as a sensory experience associated with an unpleasant emotion, pain is subdivided into nociceptive pain and neuropathic pain. When nociceptive or acute pain is poorly controlled, many peripheral and central pathophysiological changes can transform it into persistent pain or a state of chronic pain (Glare et al. 2019). Neuropathic pain has an extremely complicated mechanism that can be caused by ischemia, immune disorders, metabolic disorders, infections, toxins, hereditary and traumatic disorders (Woolf and Mannion 1999; Nishikawa and Nomoto 2017). Chronic pain in general affects nearly 10% of the world's population (Bergman et al. 2001; Mourao et al. 2010); while chronic neuropathic pain affects 19% of the population in European countries (Royds and McCrory 2018). In addition, in 61% of adults affected by this pain, there is a cessation of activities, absenteeism, early retirement, a drop in productivity, an increase in health care accompanied by very negative social and economic impacts (Breivik et al. 2006). Although acute pain plays a protective role, chronic pain is of no physiological interest since its chronicity causes depression, sleep disturbances, lethargy and hyperalgesia (Konsman et al. 2002). Many neuropeptides and inflammatory cells (macrophages, astrocytes, microglia, T cells)

provide bidirectional communication between the inflammatory system and the nervous system in the modulation of pain (Grace et al. 2014).

One of the most common neuropathic pains is peripheral neuropathy, which is usually caused by taking anticancer drugs (cisplatin, paclitaxel, vincristine) (Hershman et al. 2014; Cavaletti et al. 2000). Vincristine is a widely used cancer treatment to relieve people with cancer of the cervix, breasts, head, uterus and neck (Rowinsky et al. 1988; Scripture et al. 2006). However, prolonged use of this drug causes neuropathy with numbness, tingling and burning pain (Lipton et al. 1989; Wasserheit et al. 1996). During the development of neuropathic pain, nerve damage causes the release of neurotransmitters (glutamate and substance P) and adenosine triphosphate, which activates microglia and other immune cells resulting in the production of numerous cytokines and the derived neurotrophic factor of the brain (Royds and McCrory 2018). Moreover, many other proteins such as nerve growth factor (NGF), fatty acid binding protein and creatine kinase are also associated with nociceptor sensitivity and also in inflammatory response (Mendell et al. 1999; Seidel et al. 2010; Olausson et al. 2012). After painful trauma, phospholipase A2 transforms membrane phospholipids into arachidonic acid, which in turn is transformed into inflammatory prostaglandins by the enzyme COX (Pertusi 2004) and into leukotriene by the enzyme 5-lipoxygenase (5-LOX) (Oliviera et al. 2016). Furthermore, the production of cytokines in the inflammatory site or in the wound can cause the activation of COX-2 and increase the production of prostaglandins (Samad et al. 2001). Furthermore, it is known that leukotrienes and prostaglandins have a synergistic effect on the development and maintenance of painful symptoms; in addition, treatment with inhibitors of the 5-LOX enzyme and the COX enzyme is very beneficial for patients suffering from neuropathic pain (Cortes-Burgos et al. 2009).

Generally, the treatment of neuropathic pain is based on a combination of analgesics and many classes of other drugs, which unfortunately cause kidney failure, gastric ulceration, coronary heart disease, gastrointestinal bleeding and thrombotic cardiovascular events (Vonkeman and van de Laar 2010; Rezabakhsh et al. 2021). Scientific progress has allowed the understanding of the characteristics of chronic neuropathic pain, as well as the knowledge and understanding of their molecular and cellular mechanisms; which has allowed the development of new therapies for the management of this type of pain (Edinoff et al. 2021). Thus, the use of plants has become an essential alternative medicine for diseases resistant to modern drugs. Moreover, many plants and isolated compounds tested against vincristine-induced neuropathic pain have been shown to be useful (Mbiantcha et al. 2017; 2018).

Cissus quadrangularis Linn., a creeping shrub with a quadrangular thick stem, fleshy, fibrous and smooth, with four winged internodes (Brahmkshatriya et al. 2015), belonging to the Vitaceae family, is an edible plant found in the hottest regions of India, Sri Lanka, Asia and Africa (Thakur et al. 2009). In traditional medicine, this plant is used to heal injured tendons, broken bones and injured ligaments (Suzu et al. 2013). The stem and roots of this plant are known for their antimicrobial and antioxidant activities. They are also used to treat asthma, insect stings and dog bites, stomach ache, scurvy and digestive disorders (Mukhopadhyay et al. 2003; Mohammed and AlBayati 2009; Daniet et al. 2011; Nan et al. 2004). The stem of this plant is also used as an anthelmintic, digestive, analgesic for ear and eye aches, or against

spinal and back complaints (Eswaran et al. 2012). In Africa, this plant is used against gout, venereal diseases, leucorrhoea, syphilis, haemorrhoids, diarrhoea, dysentery and as an aphrodisiac (Shirwaikar et al., 2003). Work carried out on *C. quadrangularis* has shown its analgesic (Dora et al. 2017), anti-inflammatory (Banu et al. 2012; Nie et al. 2015), anti-osteoporotic (Potu et al. 2009) and antioxidant (Lekshmi et al. 2015) properties, its effects on the skeletal system (Bhagat et al. 2009), antibacterial, antifungal, deworming, antihemorrhoidal and analgesic (Mishra et al. 2010) effects. In addition, extracts of this plant have demonstrated antiviral properties and free radical scavengers (Chidambara et al. 2003; Panthong et al. 2007; Balasubramanian et al. 2010). On the other hand, ethyl ethanoate extracts from *C. quadrangularis* suppress NF-Kb activation and heme oxygenase-1 stimulation (Srisook et al. 2011). This plant is rich in triterpenoids, carotenoids, ascorbic acid, β -sitosterols (Prabhavathi et al. 2016), steroids, flavonoids and iridoids (Bhutani et al. 1984; Singh et al. 2007). Moreover, many biologically active compounds have already been isolated from *C. quadrangularis*, these include hexadecanoic acid, stilbene glucoside trans-resveratrol-3-O-glucoside (Thakur et al. 2009), β -sitosterol, δ -amyrone, δ -amyrin (Prabhavathi et al. 2016), 3 resveratrol, β -sitosterol (Shirley et al. 1996; Jainu et al. 2004). In addition, the aerial parts of this plant contain 7-OxoOnocer-8-ene-3 β 21 α diol (Pluemjai et al. 1986; Gupta et al. 1991), 4-hydroxy 2 methyltricos-2 ene- 22-one, 9-methyloctadec-9-ene, heptadecyl-octadeca-noate, icosanilico-sanoate, 31-methyl tritriacontan-1-ol, 7-hydroxy-20-oxodocosanyl cyclohexane, 31-methyl tritriacontanoic acid, taraxerol, isopentacosanoic acid, friedelan-3-one and 3,3',4,4'- tetrahydroxybiphenyl (Dora et al. 2017). By the fact that *C. quadrangularis* is traditionally used to relieve many painful disorders of the body, and starting from the fact that *in vitro* studies of the extracts of this carried out in this study have shown that they have anti-inflammatory properties (inhibition of denaturation of proteins, activities of COX-1, COX-2 and 5-LOX), antioxidant properties (DPPH, ABTS and NO), and immunomodulatory (inhibition of the production of proinflammatory cytokines (TNF α , IL-1 β , IL6), the production of ROS, cell proliferation, and stimulation of the production of anti-inflammatory cytokines (IL-10), and moreover, these extracts are rich in phenolic compounds, flavonoids and tannins, we can conclude that this plant could have antihyperalgesic properties. The objective of this work was to evaluate the ability of extracts of *C. quadrangularis* Linn. (Vitaceae) to reduce chronic pain by derived from its immunomodulatory, antioxidant and anti-inflammatory properties.

Material And Methods

Plant material and extraction

The plant material consisted of the aerial part of *C. quadrangularis* which was harvested in June in the Cameroon coastal region, more precisely in the Department of Mougo (Nkongsamba). The botanical authentication of this specimen was carried out at the national herbarium of Yaoundé in Cameroon by comparison with a sample kept under number 36966 HNC/Cam. After the harvest, the plant was dried in the shade, then it was crushed with a grinder to was obtained by decoction of 300 g of *C. quadrangularis* powder in 3.25 l of distilled water for 20 min then filtered using filter give a fine powder which was used to prepare the various extracts. The aqueous extract paper. The filtrate obtained was evaporated in an oven at a temperature of 40 °C and the powder obtained (17 % yield) representing the aqueous extract was

transferred to a dry bottle, then kept for the various pharmacological tests. The ethanolic extract was obtained by maceration of 300 g of *C. quadrangularis* powder in 3.25 l of 99% ethanol for 48 h and then filtered using filter paper. The filtrate was concentrated using a rotary evaporator at 70 °C. The extract which was obtained (3 % yield) was placed in the open air for complete evaporation of the ethanol, then placed in an oven for evaporation of the water. The powder obtained was transferred to a dry vial and then stored for the pharmacological tests.

Quantitative phytochemistry

Dosage of total phenols

The oxidation of phenols, the mixture of phosphomolybdic acid (H₃PMo₁₂O₄₀) and phosphotungstic acid (H₃PW₁₂O₄₀) is reduced to a solution consisting of molybdenum and tungsten blues therefore the absorption varies according to the quantitative/qualitative composition of the pH and phenolic mixtures obtained by adding sodium carbonate (Luis et al. 2009). For this test, a solution of extract (20 µL, 2 mg/ml) or distilled water (white tube), Folin-Ciocalteu reagent (100 µL, diluted 10 times), sodium carbonate (80 µL, 20%) was stirred, incubated (water bath, 20°C, 30 minutes), and the absorbance was read (spectrophotometer, 765 nm). Gallic acid (0.015 to 2 mg/ml) was used to draw the standard curve which made it possible to obtain the results in milligram equivalent of gallic acid per gram of powder (Ramde-Tiendrebeogo et al. 2012).

Assay of total flavonoids

A solution of extract (100 µL, 2 mg/ml), aluminum chloride (50 µL, 1.2%) and potassium acetate (50 µL, 120 mM) was incubated (30 minutes, temperature ambient) and the optical density was read (415 nm). Quercetin (0.015 to 2 mg/ml) was used to draw the standard curve which made it possible to obtain the results in milligram equivalent of quercetin per gram of powder (Chang et al. 2002).

Quantification of total tannins

A solution of extract (100 µL, 2 mg/ml) or distilled water (white tube), Folin-Ciocalteu reagent (500 µL, diluted 10 times), sodium carbonate (1000 µL, 35%) and distilled water (8.4 mL) was stirred, incubated (30 minutes, room temperature), then the optical density read at 700 nm. Tannic acid (100, 200, 300, 400 and 500 µg/mL) was used to draw the standard curve which made it possible to obtain the results in milligram equivalent of tannic acid per gram of powder (Govindappa et al. 2011).

In vitro assays

Immunomodulatory activity

The production of ROS (intracellular/extracellular) through the chemiluminescence test, the production of cytokines through the ELISA test and cell proliferation were used to determine in vitro the

immunomodulatory properties of the extracts of *C. quadrangularis* according to the protocols described by Mbiantcha et al. (2017) and Matah Marte et al. (2020).

Isolation of human polymorpho neutrophils (PMNs)

The method described by Zimmerman (1983) was used to isolate neutrophils. In a 30- year-old voluntary donor (having given his consent for the purposes of this study), 10 ml of blood was taken from the bend of the elbow and introduced into a tube (with heparin). At equal volume, this blood was combined with Hanks Balance Salts Solution (HBSS) (MP Biomedicals Inc., Sigma and Research Organics) and lymphocyte separation medium (LSM) (MP Biomedicals Inc., Sigma and Research Organics) and left to stand (30 minutes). The supernatant was removed, mixed with LSM (5 ml) and centrifuged (400 g, 20 minutes, room temperature). The pellet collected was mixed with distilled water (1 ml, 1 minute, lysis of the red blood cells), then 1 ml of HBSS⁻ (2x) (stopping of the lysis). HBSS was added again and the tube centrifuged (10 minutes, 4 °C, 300g) and the supernatant discarded. Then 1 ml of HBSS⁻ was then introduced into the tube and kept cold. Trypan blue exclusion (viability test), hemocytometer (cell count) were used and for each test, 1 x 10⁶ cells/ml was the cell concentration used.

Peritoneal macrophages isolation from mice

Mice (Roswell Park Memorial Institute medium (NMRI)) weighing 22 to 24 g, having received Fetal bovine serum (FBS) (1 ml, i.p.), after 72 hours and sacrifice of the animals, the RPMI (Roswell Park Memorial Institute medium, MP Biomedicals Inc., Sigma) 10% (10 ml), was introduced into the peritoneal cavity which was massaged for 2 minutes. Using a syringe, the macrophage-rich RPMI was extracted from the peritoneum and centrifuged at 400g at 4 °C for 20 minutes. In the pellet, the incomplete RPMI was introduced and centrifuged again at 4 °C (10 minutes, 300 g), then addition of incomplete RPMI/HBSS (1 ml). The same methods of viability, counting and the same number of cells per ml for each test were used (Mesaik et al. 2006; Mahomoodally et al. 2007).

Chemiluminescence assay

The chemiluminescence method in 96-well plates described by Mesaik et al. (2006) and Mahomoodally et al. (2007) was used. *C. quadrangularis* extracts (25 µl, 3.125 to 100 µl/ml), ibuprofen (25 µl, 3.125 to 100 µl/ml), whole blood (25 µl) or neutrophils (25 µl, 1 x 10⁶ cells/ml) or macrophages (25 µl, 1 x 10⁶ cells/ml) were mixed in the test wells. The control wells did not receive the plant extracts. Plates were introduced into a thermostatically controlled chamber of the luminometer (20 minutes, 37 °C), then the Zymosan/Phorbol 12-myristate 13- acetate (PMA) (Fluka, European Cell Culture Collection) mixture (25 µl) and/or the luminol/lucigenine mixture (25 µl, 7 × 10⁵ M) were added to each well and the plate was read in RLU (relative light units) and the percent inhibition was calculated:

$$\text{Inhibition (\%)} = \frac{(\text{RLU}_{\text{control}} - \text{RLU}_{\text{sample}})}{\text{RLU}_{\text{control}}} \times 100$$

Cytokines, T-Cell proliferation and MTT assay

Cell Culture

The human monocytic cell line THP-1 (ATCC® TIB-202) (American Type Culture Collection (Manassas, VA, USA)), grown in the Roswell Park Memorial Institute (RPMI-1640) containing glucose (4.5 g/l), inactivated fetal calf serum (10%), L-glutamine (1%), nonessential amino acids (1%), streptomycin (10 µg/ml) and 100 U/ml penicillin.

Immunoassay for Cytokines

THP-1 cells (2.5×10^5 cells/ml) (American Type Culture Collection (Manassas, VA, USA)) were mixed with vitamin D3 (0.1 µM) and PMA (100 ng/ml) to be differentiated into macrophages for 24 hours. Each well containing the cells received the extracts (2, 10 and 50 µg/ml) and Lipopolysaccharide (LPS, 2 µg/ml). TNF-α, IL-6, IL-1β and IL-10 were quantified using commercial kits (R&D Systems, Minneapolis, USA) to the ELISA test (Enzyme-linked immunosorbent assay). After 4 hours, optical density (450 nm) was read by a microplate reader (Model 680; BioRad Laboratories, Mississauga, ON, Canada), then TNF-α, IL-6, IL-1β and IL-10 quantity was calculated using a standard curve constructed (serial dilutions of cytokine standards) supplied with the kit (Mahajna et al. 2015).

T-Cell proliferation assay

The cell proliferation test in 96-well white plates (Rita et al., 2014). *C. quadrangularis* extracts (50 µl; 1, 10 and 100 µg/ml), prednisolone (50 µl; 1, 10 and 100 µg/ml), T lymphocytes (50 µl, 2×10^6 cells/ml) and phytohaemagglutinin-L (PHAL) (50 µl, 7.5 µg/ml) were mixed. Negative (T lymphocytes and RPMI (150 µl)) and positive (T lymphocytes, PHAL and RPMI (100 µl)) controls were also prepared. The plate was incubated (5% CO₂, 37 °C, 72 hours), then addition of 0.5 µCi/well (methyl 7 3H) of thymidine (25 µl), further incubation for 18 hours and cell harvesting (filter in fiberglass). The counts per minute (CPM) were used to determine the percent inhibition (%):

$$\text{Inhibition (\%)} = \frac{(\text{CPM}_{\text{control}} - \text{CPM}_{\text{sample}})}{\text{CPM}_{\text{control}}} \times 100$$

MTT Assay

For this test carried out in 96-well plates, seeded THP-1 (20,000 cells), differentiated into macrophages (0.1 µM of vitamin D3 and 100 ng/ml of PMA) for 24 hours, then addition of extracts of *C. quadrangularis* (1 – 100 µg/ml) and incubated (24 hours, 37 °C). The culture medium was then removed, the cells washed (PBS), addition of 100 µl (500 µg/ml) of 3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and RPMI, incubated (4 hours, 37°C), removal of the medium, addition of isopropanol (100 µl, 0.08N) and further incubation (15 minutes, 37 °C). An ELISA plate reader set to 570 nanometers was used to read the absorbance (Mahajna et al. 2015).

Anti-inflammatory activity

The *in vitro* evaluation of the anti-inflammatory properties of the aqueous and ethanolic extracts of *C. quadrangularis* in this study was made on the enzymes cyclooxygenase (COX1 and COX2), 5-lipoxygenase (5-LOX) and on the denaturation of proteins according to the protocols described by Mbiantcha et al. (2020; 2022) were used.

Lymphocyte isolation

A mixture of 3 ml of Ficoll solution and 3 ml of human blood was centrifuged (400 g, 30 minutes) at room temperature, then the lymphocytes were removed by aspiration and the trypan blue method made it possible to check cell viability, then by microscopic observation the lymphocytes were confirmed by their reduced cytoplasm and large nucleus. A suspension of 1×10^6 cells/ml was cultured in tubes containing filled with 1 ml of fetal bovine serum, 1 ml of RPMI medium, 1 ml (100 µg/ml) of antibiotic (streptomycin and penicillin), 1 ml phytohemagglutinin, filtered (cellulose acetate filter, 0.2 µm) and incubated (37 °C, 72 hours); then addition of lipopolysaccharides (1 µl, 1 µg/ml) to each new incubation tube (24 h, 37 °C) (Viji and Helen, 2008). The cyclooxygenase inhibition test was performed following the protocol described by Viji and Helen (2008).

The tubes containing 1 ml of the different extracts (62.5, 125, 250 and 500 µg/ml) or ibuprofen (62.5, 125, 250 and 500 µg/ml) or 0.9% NaCl were incubated (24 hours at 37 °C), centrifuged (400 g, 10 minutes), then cell lysis buffer (50 µL) was introduced into each tube after removal of the supernatant and further centrifugation (400 g, 10 minutes). Subsequently, Tris-HCl buffer (2.75 ml, 100 mM, pH= 7.4), human hemoglobin (5 mM), glutathione (5 mM), cyclooxygenase (10 µl) and arachidonic acid (200 µM) were added, the tubes were incubated (37 °C, 20 minutes), 0.2 ml of thiobarbituric acid (TBA) and 0.2 ml of trichloroacetic acid (TCA, 10% in HCl) were also added, then the tubes were heated (boiling water bath, 70°C, 20 minutes), cooled and centrifuged (1000 rpm, 3 minutes). The optical density of the supernatant (variation of arachidonic acid) was read at 632 nm.

As for the 5-lipoxygenase activity inhibition test, the tubes were again prepared as before (cyclooxygenase test), then a solution (2 ml) containing phosphate buffer (2.75 ml; pH 7.4 ; 50 mM), 5-lipoxygenase (50 µl) and sodium linoleate (0.2 ml) were added and incubated (25 °C, 30 minutes). The increase in absorbance (234 nm) characteristic of the formation of hydroperoxy-linoleic acid materialized the activity of 5-lipoxygenase (Viji and Helen 2008).

$$\text{Pourcentage d'inhibition (\%)} = \frac{\text{Absorbance}_{(\text{témoin})} - \text{Absorbance}_{(\text{Test})}}{\text{Absorbance}_{(\text{témoin})}} \times 100$$

For protein denaturation, 1 ml of distilled water or Diclofenac (62.5, 125, 250 and 500 µg/ml) or different extracts (62.5, 125, 250 and 500 µg/ml) was added 1 ml of bovine serum (5 %), incubated (15 minutes,

27 °C), placed for 10 minutes at 70 °C; then the optical density (OD) at 660 nm was read and the percentage inhibition was determined (Padmanabhan and Jangle 2012).

$$\% \text{ Inhibition} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{test}}}{\text{OD}_{\text{control}}} \times 100$$

Antioxidant activity

The in vitro evaluation of the antioxidant properties of extracts of *C. quadrangularis* in this study was made on the DPPH radical scavenging tests, the ABTS^{•+} discoloration test and the nitric oxide scavenging test according to the protocols described by Mbiantcha et al. (2020) were used.

DPPH radical scavenging activity: Three (3) ml of a working solution was obtained by diluting DPPH (24 mg/100 ml methanol) (MP Biomedicals Inc., Sigma and Research Organics) with methanol for an absorbance of 0.98 ± 0.02 at 517 nm, was mixed with each extract (100 µl, 1 mg/ml) or standard or methanol (100 µl). Absorbance (OD) (517 nm) was measured for 30 min (Brand Williams et al. 1995). The following formula was used to calculate antioxidant activity:

$$\% \text{ Antioxidant activity} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{test}}}{\text{OD}_{\text{control}}} \times 100$$

ABTS^{•+} Discoloration Test: Ten (10) ml of a solution of ABTS (7 mmol/l, 9.5 ml), potassium persulfate (100 mmol/l, 245 µl) and distilled water was diluted with 0.1 mol/l potassium phosphate buffer (pH 7.4) to an absorbance of 0.70 ± 0.02 (734 nm, dark, room temperature, 18 hours). The absorbance of the mixture of 10 µL of each extract (62.5, 125, 250 and 500 µg/ml) and 2.99 ml of the ABTS solution was read at 734 nm (Re et al. 1999) and allowed the percentage of antioxidant activity to be calculated using the formula previously described.

Nitric oxide (NO) scavenging test: a mixture of 0.5 ml of sodium nitroprusside (pH7.4, 5 mmol/l) and each extract (62.5, 125, 250 and 500 µg/ml) or ascorbic acid or methanol was incubated (25 °C, 180 minutes), then 1.5 mL of Griess' reagent (phosphoric acid (2%), sulfanilamide (1 %), N-(1 %) dihydrochloride-naphthyl) ethylenediamine (0.1 %)) was added, then a new incubation (30 minutes) was made. The percentage of activity was determined from the absorbance (Abs) read at 546 nm (Benzie and Strain 1999).

$$\% \text{ NO scavenging} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100$$

In vivo assays

Neuropathic Pain, Animal Treatment, Induction and Behavioral Assessment

Fifty-four (54) Wistar rats (male and female, 160 - 200 g, 3-4 months) and reared under 19-23 °C, 12 hours light at the Research Unit of Animal Physiology and Phytopharmacology (Department of Animal Biology, Faculty of Science, University of Dschang). These animals were treated as follows : group 1 (n=6, normal control) received orally distilled water, group 2 (n = 6, negative control) received orally distilled water , group 3 (n = 6, positive control) received orally pregabalin (50 mg/kg), groups 4, 5 and 6 (n = 6, tests) treated orally with the aqueous extract of *C. quadrangularis* at respective doses of 90, 180 and 360 mg/kg, groups 7, 8 and 9 (n = 6, tests) treated orally with the ethanolic extract of *C. quadrangularis* at respective doses of 90, 180 and 360 mg/kg. All treatments were administered on the first day before the start of induction. After treatments, an intraperitoneal injection of vincristine sulphate (100 µg/kg) was administered to the rats (except the animals in group 1) for two series of five days (1st, 2nd, 3rd, 4th, 5th day, then 8th, 9th, 10th, 11th, 12th day) of successive work with 2 days off. After the start of induction, the different treatments were administered daily until the 15th day (Chiba et al. 2017; Mbiantcha et al. 2018).

For behavioral tests, mechanical hyperalgesia was induced by placing the left hind paw of each rat on an analgesimeter (UGO BASILE) on days 0, 1, 3, 5, 7, 9, 11, 13 and 15. The pain was caused by increasing the pressure (cut-of 250 g) until the paw was withdrawn. The value of the nociceptive threshold was considered as the force (g) obtained (Mbiantcha et al. 2018). While mechanical allodynia was performed using Von Frey filaments. The animals were first placed individually in cages for acclimatization over a period of 30 minutes before taking the values. Subsequently, these animals gradually received the Von Frey filaments in order of masses perpendicular to their right plantar arches. Pressure was exerted for 5 seconds until the filament bent. One test consisted of applying the same filament three times in a row for a period of 1 minute. The animals' response was considered a painful reaction if it lifted its paw or leg and/or moved its body; licked the stimulated paw or spread the toes. The mass of the filament to which the animal responded was considered as the threshold intensity.

Sample collections and assessment of proinflammatory cytokines and Growth Factors levels

On day 15, the animals are anesthetized (thiopental, 50 mg/kg, *i.p.*), rib cage opened, then the blood collected (catheterization of the abdominal artery) is introduced into a dry tube and centrifuged (3000 rpm, 15 minutes), the serum collected was used to determine the amounts of IL-1β, TNFα, IL-6 and IL10. Then, the sciatic nerve of the animals is also removed, crushed, centrifuged (room temperature, 3000 rpm, 15 min, 4°C, Eppendorf 5804 R, Hamburg), the supernatant allowed the analysis of IL-1β, TNFα, IL-6, NGF and IGF) (Fatani et al. 2015).

Statistical analysis.

The individual values of each test were used to calculate the Mean and it was used to present the results of this study. Some results are expressed with standard deviation (SD) or standard error of the mean

(SEM). One-way analysis of variance (Tukey's test, biochemical assays and in vitro tests) and two-way analysis of variance (Bonferroni's test, behavioral tests) were used to compare group tests with controls (normal and/or negative). At $p < 0.05$, the differences were considered significant.

Results

Quantitative analysis of extracts

The quantitative phytochemistry carried out on the aqueous and ethanolic extracts of *C. quadrangularis* shows that the two extracts contain flavonoids, phenols and tannins. As for the aqueous extract, the phenolic compounds, the flavonoids and the tannins represent respectively 15.57 mg equivalent gallic acid/g, 2.07 mg equivalent quercetin/g and 1.48 mg equivalent tannic acid /g. In addition, 15.53 mg equivalent gallic acid/g of phenolic compounds, 2.55 mg equivalent of quercetin/g of flavonoids and 8.02 mg equivalent of tannic acid/g of tanning.

In vitro test results

Immunomodulatory effects extracts of *C. quadrangularis*

Extracts of *C. quadrangularis* inhibited their production of intracellular ROS, in whole blood (19.00 and 12.31 $\mu\text{g/ml}$ for aqueous and ethanolic extracts, respectively), polynuclear neutrophils (12.20 and 7.22 $\mu\text{g/ml}$ (IC_{50}) for aqueous and ethanolic extracts, respectively) and macrophages (15.05 and 6.66 $\mu\text{g/ml}$ for aqueous and ethanolic extracts, respectively). For extracellular ROS, extracts reduced their production with 22.34, 16.23 and 13.45 $\mu\text{g/ml}$ (IC_{50} , aqueous extract) in mouse whole blood, polymorphonuclear neutrophils and peritoneal macrophages respectively; while the ethanolic extract with 16.45; 11.03 and 7.77 $\mu\text{g/ml}$ (IC_{50}) respectively in whole blood, polymorphonuclear neutrophils and mouse peritoneal macrophages (Table 2). Ibuprofen inhibits intracellular ROS with 13.09 $\mu\text{g/ml}$ (IC_{50}) in whole blood and extracellular ROS with 15.39 $\mu\text{g/ml}$ (IC_{50}) in polymorphonuclear neutrophils.

The effect of the extracts on the release of cytokines by THP-1 cells is shown in Figures 1 and 2. It emerges from these figures that the concentrations of IL-1 β , TNF- α , IL-6 increase, while the concentration of IL-10 decreases in the tubes having received only LPS (2 $\mu\text{g/ml}$). In the tubes containing the extracts of *C. quadrangularis*, at all the concentrations tested, a significant decrease ($p < 0.001$) in the production of IL-1 β , TNF- α , IL-6 (Figure 1) and a significant ($p < 0.001$) increase IL-10 (Figure 2).

Regarding the proliferation of T lymphocytes, the ethanolic extract (4.27 $\mu\text{g/ml}$), aqueous extract (22.39 $\mu\text{g/ml}$) and prednisolone (2.39 $\mu\text{g/ml}$) showed antiproliferative activity (Table 3). In addition, the aqueous (IC_{50} = 89.98 $\mu\text{g/ml}$) and ethanolic (IC_{50} = 19.33 $\mu\text{g/ml}$) extracts of *C. quadrangularis* showed no toxic effect on the cells compared to cyclohexamid (IC_{50} = 0.1 $\mu\text{g/ml}$) (Table 3).

Anti-inflammatory effects of extracts of *C. quadrangularis*

Table 4 shows the effect of extracts of *C. quadrangularis* on protein denaturation, cyclooxygenase and 5-lipoxygenase inhibition. Diclofenac, extracts (aqueous and ethanolic) at 500 µg/mL had maximum inhibition of 76.03%, 50.11% and 63.22%, respectively. In addition, the aqueous (54.11 and 46.33%) and ethanolic (68.55 and 80.33%) extracts as well as ibuprofen (70.11 and 79.02%) at 500 µg/mL respectively exhibited maximum percent inhibition on COX1 and COX2 activities. Regarding 5-lipoxygenase, aqueous and ethanolic extracts of *C. quadrangularis* and ibuprofen inhibited with 65.67%, 72.33% and 78.11%, respectively.

Antioxidant effects of extracts of *C. quadrangularis*

Table 5 shows that the extracts of *C. quadrangularis* have remarkable antioxidant properties. The result of the DPPH test revealed that the aqueous and ethanolic extracts (500 µg/ml) had a percentages activity of 67.41% and 77.41%, respectively. Regarding the ABTS decolorization test, the aqueous extract at 500 µg/mL had a percentage activity of 62.43%, while the ethanol extract showed a maximum percentage inhibition of 69.58%. For the NO inhibition test, the maximum inhibition percentages of 58.97% (aqueous extract) and 72.81% (ethanolic extract) were obtained at a concentration of 500 µg/ml.

***In vivo* test results**

Effects of extracts on some behavioral parameters of rat submitted to Vincristine-induced neuropathic pain

Figures 3 and 4 show the effects of *C. quadrangularis* extracts on mechanical hyperalgesia and mechanical allodynia induced by the analgesimeter and the Von Frey filament, respectively. Compared to the neutral control group, the animals having received vincristine, developed a hypersensitivity marked by a decrease in the latency of sensitivity to pain ($P < 0.001$) from the 5th day for hyperalgesia and from the 4th day for allodynia in animals of the negative control group. Oral administration of aqueous and ethanolic extracts of *C. quadrangularis*, combined with pregabalin, protected the animals against hypersensitivity and allodynia by increasing the latent time. For all the behavioral parameters studied, the effect of the aqueous and ethanolic extracts of *C. quadrangularis* (180 and 360 mg/kg) was significant ($p < 0.01$; $p < 0.001$) from day 6 to day 15, compared to the negative control group.-

Effects of extracts on production of cytokines of rat submitted to Vincristine-induced neuropathic pain

In figures 5 and 6, we observe that the injection of vincristine in rats led to a significant increase ($p < 0.001$) of pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6) in the sciatic nerve and in serum in non treated group compared to animals in the normal control group. Administration of *C. quadrangularis* (180 and 360 mg/kg) and pregabalin (50 mg/kg) significantly reduced ($p < 0.001$) pro-inflammatory cytokines compared to the negative control group in both serum (Figure 5) and sciatic nerve (Figure 6).

Effects of the extracts of *C. quadrangularis* on the Growth Factors Levels

Figure 7 shows the effects of *C. quadrangularis* on growth factor (NGF, IGF) levels in the sciatic nerve. It was observed that the level of growth factors (sciatic nerve) was significantly increased ($p < 0.001$) in the animals of the non treated group compared to the animals of the normal control group. The treatment of the animals with the aqueous extract of *C. quadrangularis* (360 mg/kg) significantly reduced ($p < 0.05$) the level of NGF compared to the negative control ; while the treatment with the ethanolic extract (360 mg/kg) significantly inhibits ($p < 0.001$) the levels of NGF and IGF.

Discussion

This study was carried out with the aim of evaluating *in vitro* the immunomodulatory properties (chemoluminescence tests, pro- and anti-inflammatory cytokines, cell proliferation and cytotoxicity), anti-inflammatory properties (protein denaturation tests, cyclooxygenase inhibition (COX 1 and COX 2) and 5-lipoxygenase), antioxidants (DPPH, ABTS and NO tests), then *in vivo* the antihypernociceptive properties (hyperalgesia and allodynia) of aqueous and ethanolic extracts of *C. quadrangularis*.

Neuropathic pain, which is a consequence of damage to the nervous system that can induce hypersensitivity (hyperalgesia and/or allodynia), remains a very difficult pathology to treat today (Dowdall et al. 2005; Colloca et al. 2017); thus, a simple sensation of touching or accidentally rubbing door frames or furniture (allodynia) or a pat on the back (hyperalgesia) becomes very painful (Jeffrey 2009). After nerve damage, there is activation and proliferation of microglia in the spinal cord, there is release of pro-inflammatory factors (IL-1 β , TNF α , IL-6) with development of peripheral nerve inflammation ; subsequently, there is a decrease in the activity of intermediate neurons (which are inhibitory) and an increase in the activity of excitatory neurons leading to central hypersensitization with the consequence of the development of allodynia and mechanical hyperalgesia (Fu et al. 1999; Jin et al. 2003; Zhao et al. 2017). During the process of development of neuropathic pain, an accumulation of ROS is observed in the nervous system, these ROS will lead to the phosphorylation of sensory receptors (peripheral and central), leading to a structural disturbance of the nerve and hypersensitivity (Zhang and al. 2014; Duggett et al. 2016). It is also known that ROS cause hyperalgesia by activating transient vanilloid receptors with release of many chemical pain mediators (Hassler et al. 2014; Goel and Tyagi 2016). In addition, nerve damage accompanied by the activation of microglial cells and infiltrating macrophages will also lead to the expression of cyclooxygenase 1 and 2 in the spinal cord resulting in the release of prostaglandins which will stimulate sleeping nociceptors thus causing hyperalgesia and allodynia (Tegeder et al. 2001; Kang et al. 2004; Zanjani et al. 2014).

The results of this study showed that the aqueous and ethanolic extracts of *C. quadrangularis* inhibited the production of intracellular and extracellular ROS stimulated by zymosan and PMA respectively. Indeed the aqueous and ethanolic extracts produced maximum IC₅₀ of 12.20 μ g/ml (PMNs) and 6.66 μ g/ml (macrophages) for intracellular ROS, then IC₅₀ of 13.45 μ g/ml (macrophages) and 7.77 μ g/ml (macrophages) for extracellular ROS. Moreover, the two extracts also inhibited the production of proinflammatory cytokines (TNF α , IL-6 and IL-1 β) and stimulated the production of IL-10 (anti-inflammatory cytokine). The immunomodulatory activity of the extracts was also confirmed by the T cell

proliferation test where the two extracts showed interesting activities very close to that of prednisolone used as reference substance. The anti-inflammatory properties of *C. quadrangularis* extracts have been verified on protein denaturation, 5-lipoxygenase and cyclooxygenase inhibition tests. The aqueous extract of *C. quadrangularis* inhibited protein denaturation, COX1, COX2 and 5-LOX activities by 50.11%, 54.11%, 46.33% and 65.67% respectively, while the ethanolic extract inhibits them by 63.22%, 68.55%, 80.33% and 72.33% respectively. The antioxidant properties evaluated showed activities of 67.41% (DPPH), 62.43% (ABTS) and 58.97% (NO) for the aqueous extract, then 77.41% (DPPH), 69.58% (ABTS) and 72.81% (NO) for the ethanolic extract. These results show that *C. quadrangularis* is rich in compounds capable of modulating the immune system, regulating the inflammatory process and boosting the antioxidant activity of the organism. Indeed, certain phenolic, flavonoid and tannin compounds whose presence has been demonstrated in these extracts have already shown their immunomodulatory, antiinflammatory and antioxidant capacity (Carla de Agostino et al. 2008; Musfizur et al. 2012; Liu et al. 2014; Devasena et al. 2014; Abood et al. 2014; Adedapo et al. 2015; Hosseinzade et al. 2019; Dejeni et al. 2021).

In this study, Wistar rats given an intraperitoneal injection of vincristine developed mechanical hyperalgesia and allodynia that are familiar symptoms of neuropathic pain (Moini Zanjani et al. 2014), and analgesimeter use (hyperalgesia) and Von Frey filaments (allodynia) for the evaluation of antinociceptive substances is widely adopted (Ishola et al. 2014; Owoyele and Bakare 2018). In 38–100% of people with cancer receiving chemotherapy treatment with herbal cancer drugs (paclitaxel, vincristine, docetaxel), there is development of peripheral neuropathy with allodynia and hyperalgesia as the main symptoms (Han and Smith 2013; Kerckhove et al. 2017; Carrasco et al. 2018); which in most cases resist analgesics that are effective on other pain models (Griffiths and Flatters 2015) which affects the survival and quality of life of cancer patients (Kerckhove et al. 2017). This study showed that extracts of *C. quadrangularis* are effective in reversing hyperalgesic and allodynic symptoms induced by vincristine injection. Interestingly, pretreatment with the *C. quadrangularis* extracts prevented Wistar rats from developing hyperalgesia and allodynia, indicating that pretreatment with the extracts attenuates neuronal plasticity that could have resulted in hyperalgesia and allodynia. The results of this study suggest that the extracts possess an antihyperalgesic effect similar to that of pregabalin, an anticonvulsant drug. These anticancer drugs can cause nerve damage by damaging nuclear and/or mitochondrial DNA, altering axonal transport, disrupting ion channels, causing loss of antioxidant enzymes, altering the inflammatory process, increasing oxidative stress (Han and Smith 2013; Massicot et al. 2013; Kerckhove et al. 2017). The overproduction of ROS during neuropathic pain, materialized by an increase in oxidative stress markers (DNA oxidation and lipid peroxidation) justifies the decrease in antioxidant defense (Han and Smith 2013); this incipient oxidative stress causes on the one hand the damage of non-neuronal and neuronal cells and on the other hand the activation of macrophages with overproduction of TNF α and IL-1 β (Kerckhove et al. 2017). At the level of the immune system, the treatment of neurons by anticancer drugs is characterized by a significant production of TNF α , IL-1 β , IL-6 and prostaglandin E2 which are capable of generating and rendering chronic the painful process. (Carrasco et al., 2018). The antioxidant (DPPH, ABTS and NO), anti-inflammatory (protein denaturation, COX1, COX2 and 5-LOX) and immunomodulatory (ROS, pro- and anti-inflammatory cytokines, cell proliferation) effects demonstrated

in vitro in this study are evidence that shows the neuroprotective effect of extracts of *C. quadrangularis*. Furthermore, *in vivo* studies, extracts of *C. C. quadrangularis* also significantly inhibited the production of pro-inflammatory cytokines (TNF α , IL-1 β , IL-6) in the serum and in the sciatic nerve of treated animals compared to untreated animals. In addition, the levels of growth factors (IGF, NGF), which play an important role in the growth of nerve cells (Fatani et al. 2015), were significantly increased in animals treated with the different extracts. In this study, the extracts significantly inhibited the activities of cyclooxygenases (COX1 and COX2) as well as that of 5-LOX. The immunomodulatory and anti-inflammatory activities of the extracts of *C. quadrangularis* are mainly due to the inhibition of the metabolism of cyclooxygenase, arachidonic acid, lipooxygenase, cytokines (TNF α , IL-1 β , IL-6), protein denaturation and cell proliferation most likely through downregulation of NF-kB activation, which is involved in the regulation of cyclooxygenase 2 expression (Shishodia et al. 2005 ; Kohli et al. 2005; Orlando et al. 2010). These results suggest that treatment with extracts of this plant may play an important role in the regulation of pain at central and peripheral levels.

The immunomodulatory, anti-inflammatory and antioxidant properties *in vitro*, followed by the *in vivo* antihyperalgesic properties of aqueous and ethanolic extracts of *C. quadrangularis*, were demonstrated in this study. Extracts of *C. quadrangularis* also induced a significant reduction of hyperalgesia and allodynia observed after Vincristine injection. The results of this study suggest that extracts of *C. quadrangularis* use immunomodulatory, anti-inflammatory and antioxidant capacity to prevent the development of painful neuropathy after vincristine administration. This shows that *C. quadrangularis* is a promising of new molecule for the management of anti-cancer drugs induced peripheral neuropathic pain.

Abbreviations

DPPH
1,1-Diphenyl-2-picrylhydrazyl
EC50
median effective concentration
NO
Nitric oxide
TNF α
Tumor necrosis factor alpha
IL
Interleukin
5-LOX
5-Lipoxygenase
COX
Cyclooxygenase
H3PMo12O4O
phosphomolybdic acid

H3PW12O40
phosphotungstic acid
ELISA
Enzyme-Linked Immuno Assay
HBSS
Hanks Balance Salts Solution
LSM
lymphocyte separation medium
PMNs
polymorpho neutrophils
FBS
Fetal bovine serum
RPMI (Roswell Park Memorial Institute medium
PMA
Phorbol 12-myristate 13- acetate
PHAL
phytohaemagglutinin-L
MTT
3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
TBA
thiobarbituric acid
SD
standard deviation
SEM
standard error of the mean
EDTA
Ethylene Diamine Tetra-acetique acid
ROS
reactive oxygen species
NF- κ B
Nuclear factor- κ B
NGF
nerve growth factor.

Declarations

Authors' contributions

FYMO, MM and AG designed the work. FYMO, AG, MM, TEG, DNSF, TNW, NMC, and AMLG conducted the work and collected and analysed the data. FYMO, AG and MM drafted the manuscript and revised it critically. All authors agree to be accountable for all aspects of the work.

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None

Competing interests

The authors declare that they have no competing interests.

Data availability

All data supporting our findings are adequately contained within the manuscript.

Consent for publication

Not applicable.

Ethics approval and consent to participate

For this study, the animal facility of H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan, was used. Experiments were executed under the ethical guidelines of the International Association for the Study of Pain in Conscious Animals and guidelines set by, for the scientific advisory committee, animal care, use and standards, ICCBS were followed (Protocol no. 1209004). The experimental procedures have been approved by the local ethics committee and are in accordance with the guidelines for the study of pain in awake animals, published by the NIH Publication no. 85–23, “Principles of Animal Protection,” Laboratory,” and Study of Pain, Ministry of Scientific Research and Technology, which adopted the European Union Guidelines on Animal Care and Experimentation (EWC Council 86/609).

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Disclosure

All authors agree to be accountable for all aspects of the work.

Data Availability

All data supporting our findings are adequately contained within the manuscript.

References

1. Abood WN, Fahmi I, Abdulla MA (2014) Ismail S. Immunomodulatory effect of an isolated fraction from *Tinospora crispa* on intracellular expression of INF- γ , IL-6 and IL-8. *BMC Complement Altern Med* 14:205. <http://www.biomedcentral.com/1472-6882/14/205>.
2. Adedapo AA, Falayi OO, Oyagbemi AA (2015) Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed *Moringa oleifera* in some laboratory animals. *J Basic Clin Physiol Pharmacol* 26(5): 491–499.
3. Balasubramanian P, Jayalakshmi K, Vidhya N, Prasad R, Sheriff AK, Kathiravan G, Rajagopal K, Sureban SM (2010) Antiviral activity of ancient system of Ayurvedic medicinal plant *Vitis quadrangularis* L. (Vitaceae). *J Basic Clin Pharm* 1(1):37–40.
4. Banu J, Varela E, Bahadur AN, Soomro R, Kazi N, Fernandes G (2012) Inhibition of bone loss by *Cissus quadrangularis* in mice: a preliminary report. *J Osteoporos* 2012:101206. doi: 10.1155/2012/101206.
5. Benzie IFF, Strain JJ (1999) Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol* 299: 15–27.
6. Bergman S, Herrström P, Höglström K, Petersson IF, Svensson B, Jacobsson LT (2001) Chronic musculoskeletal pain, prevalence rates, and sociodemographic associations in a Swedish population study. *J Rheumatol* 28:1369–1377.
7. Bhagat PK, Kumar MRB, Rao SM (2009) Petroleum ether extract of *Cissus quadrangularis* enhance bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. *J Clin Sao Paulo* 64(10): 10. doi: <https://doi.org/10.1590/S1807-59322009001000010>.
8. Bhutani KK, Kapoor R, Atal CK (1984) Two unsymmetric tetracyclic triterpenoids from *Cissus quadrangularis*. *Phytochemistry* 23: 407–410.
9. Bisht R, Bhattacharya S, Jaliwala YA (2014) COX and LOX inhibitory potential of *Abroma augusta* and *Desmodium gangeticum*. *J. phytopharm* 3(3): 168-175.
10. Brahmikshatriya HR, Shah KA, Ananthkumar GB, Brahmikshatriya MH (2015) Clinical evaluation of *Cissus quadrangularis* as osteogenic agent in maxillofacial fracture: A pilot study *Ayu* 36:169-73.
11. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol* 28:25–30.
12. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D (2006) Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain* 10:287e333
13. Carla de Agostino Ba, Marcos JS, Diones AD, Marcelo DB, Luciana SPC (2008) Evaluation of immunomodulatory and anti-inflammatory effects and phytochemical screening of *Alternanthera*

- tenella* Colla (Amaranthaceae) aqueous extracts. *Mem Inst Oswaldo Cruz*, Rio de Janeiro 103(6): 569-577.
14. Carrasco C, Naziroglu M, ~ Rodríguez AB and Pariente JA (2018) Neuropathic Pain: Delving into the Oxidative Origin and the Possible Implication of Transient Receptor Potential Channels. *Front Physiol* 9:95. doi: 10.3389/fphys.2018.00095
 15. Cavaletti G, Cavalletti E, Oggioni N, Sottani C, Minoia C, D'Incalci M, Zucchetti M, Marmiroli P, Tredici G (2000) Distribution of paclitaxel within the nervous system of the rat after repeated intravenous administration. *Neurotoxicology* 21:389–93.
 16. Chang C., Yang M., Wen H. et Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 10: 178-182.
 17. Chiba T, Oka Y, Sashida H, Kanbe T, Abe K, Utsunomiya I, Taguchi K (2017) Vincristine-induced peripheral neuropathic pain and expression of transient receptor potential vanilloid 1 in rat. *J Pharmacol Sci* 133: 254-260.
 18. Chidambara MKN, Vanitha A, Mahadeva SM, Ravishankar GA (2003) Antioxidant and antimicrobial activity of *Vitis quadrangularis* L. *J Med Food* 6(2):99–105.
 19. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH (2017) Neuropathic pain. *Nat Rev Dis Primers* 3 :17002.
 20. Cortes-Burgos LA, Zweifel BS, Settle SL, Pufahl RA, Anderson GD, Hardy MM, Weir DE, Hu G, Happa FA, Stewart Z, Muthian S, Graneto MJ, Masferrer JL (2009) CJ-13610, an orally active inhibitor of 5-lipoxygenase is efficacious in preclinical models of pain. *European Journal of Pharmacology* 617:59-67.
 21. Daniet A. Oyugi, Folahan O. Ayorinde, Ayele Gugssa, Adrian Allen, Ernest B (2011) Izevbigie, Broderick Eribo Biological Activity and Mass Spectrometric Analysis of *Vernonia amygdalina* Fractions. *Journal of Bioscience and Technology* 2(3): 287 – 304.
 22. Dejana NN, Elshabrawy HA, Bezerra Filho CSM, de Sousa DP (2021) Anticoronavirus and Immunomodulatory Phenolic Compounds: Opportunities and Pharmacotherapeutic Perspectives. *Biomolecules* 11 :1254. <https://doi.org/10.3390/biom11081254>.
 23. Devasena.T, Durga.M, Nathiya. S (2014) Immunomodulatory and antioxidant actions of dietary flavonoids. *Int J Pharm Pharm Sci* 6(2):50-56.
 24. Dora BB, Kumar S, Borah T (2017) Therapeutic Effect of Asthishrunkhala (*Cissus quadrangularis*) in Fracture Healing and Other Disease Conditions. *Research & Reviews: A Journal of Immunology* 7(3):11–15.
 25. Dowdall T, Robinson I, Meert T (2005) Comparison of five different rat models of peripheral nerve injury. *Pharmacol Biochem Be* 80:93-108.
 26. Duggett NA, Griffiths LA, McKenna OE, de Santis V, Yongsanguanchai N, Mokori EB, Flatters SJL (2016) Oxidative stress in the development, maintenance and resolution of paclitaxel-induced painful neuropathy. *Neuroscience* 333: 13-26.

27. Edinoff AN, Patel AS, Baker MW, Lawson J, Wolcott C, Cornett EM, Sadegi K, Kaye AM, Kaye AD (2021) Conolidine: A Novel Plant Extract for Chronic Pain. *Anesth Pain Med*. In Press(In Press):e121438. doi: 10.5812/aapm.121438.
28. Eswaran R, Anandan A, Doss A, Sangeetha G, Anand SP (2012) Analysis of chemical composition of *Cissus quadrangularis* Linn. BY GC-MS. *Asian J Pharm Clin Res* 5(2):139-140.
29. Fatani AJ, Al-rejaie SS, Halem MA, Al-assaf, Pamar MY, Ola MS, Ahmed MM(2015) Neuroprotective effects of *Gymnema sylvestre* streptozotocin-induced diabetic neuropathy in rats. *Exp Ther Med* 95(5): 1670-8. <https://doi.org/10.3892/etm.2015.2305>.
30. Fu KY, Light AR, Matsushima GK and Maixner W (1999) Microglial reactions after subcutaneous formalin injection into the rat hind paw. *Brain Res* 825:59–67.
31. Glare P, Aubrey KR, Myles PS (2019) Transition from acute to chronic pain after surgery. *Lancet* 393(10180):1537–46. doi: 10.1016/S0140-6736(19)30352-6.
32. Goel R, Tyagi N (2016) Potential contribution of antioxidant mechanism in the defensive effect of lycopene against partial sciatic nerve ligation induced behavioral, biochemical and histopathological modification in wistar rats. *Drug Res (Stuttg)* 66: 633-8.
33. Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP (2011) Antimicrobial, Antioxidant and *in vitro* Anti-inflammatory activity of Ethanol extract and Active Phytochemical screening of *Wedelia trilobata* (L.) Hitchc. *J pharmacogn phytochem* 3(3):43-51.
34. Grace PM, Hutchinson MR, Maier SF, Watkins LR (2014) Pathological pain and the neuroimmune interface. *Nat Rev Immunol* 14: 217.
35. Griffiths LA, Flatters SJ (2015) Pharmacological modulation of the mitochondrial electron transport chain in paclitaxel-induced painful peripheral neuropathy. *J. Pain* 16:981–994. doi: 10.1016/j.jpain.2015.06.008
36. Gupta MM *et al.* (1991) Lipid constituents of *Cissus quadrangularis* Linn. *Phytochemistry* 30(3): 875–78.
37. Hajimashhadi Z, Aboutaleb N, Nasirinezhad F (2017) Chronic administration of [Pyr1] apelin-13 attenuates neuropathic pain after compression spinal cord injury in rats. *Neuropeptides* 61:15–22
38. Han Y, Smith MT (2013) Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). *Front. Pharmacol* 4:156. doi: 10.3389/fphar.2013.00156
39. Hassler SN, Johnson KM, Hulsebosch CE (2014) Reactive oxygen species and lipid peroxidation inhibitors reduce mechanical sensitivity in a chronic neuropathic pain model of spinal cord injury in rats. *J Neurochem* 131: 413-7.
40. Hershman DL, Lacchetti C, Dworkin RH, Lavoie Smith EM, Bleeker J, Cavaletti G, Chauhan C, Gavin P, Lavino A, Lustberg MB, Paice J, Schneider B, Smith ML, Smith T, Terstriep S, Wagner-Johnston N, Bak K, Loprinzi CL (2014) Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 32:1941–67.

41. Hosseinzade A, Sadeghi O, Naghdipour Biregani A, Soukhtehzari S, Brandt GS, Esmailzadeh A (2019) Immunomodulatory Effects of Flavonoids: Possible Induction of T CD4+ Regulatory Cells Through Suppression of mTOR Pathway Signaling Activity. *Front Immunol* 10:51. doi: 10.3389/fimmu.2019.00051.
42. Ishola IO, Agbaje EO, Adeyemi OO, Shukla R (2014) Analgesic and anti-inflammatory effects of the methanol root extracts of some selected Nigerian medicinal plants. *Pharm Biol* 52: 1208-16.
43. Jainu M, Shyamala DCS (2004) Effect of *Cissus quadrangularis* Linn. on gastric mucosal defensive factor in experimental induce gastric ulcer-a comparative study with sucralfate. *J Med Food* 7(3): 372–76p.
44. Jeffrey SM (2009) Animal models of pain: progress and challenges. *Neuroscience* 10:283-294.
45. Jin SX, Zhuang ZY, Woolf CJ and Ji RR (2003) p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. *J Neurosci* 23:4017–4022.
46. Kang G, Kong PJ, Yuh YJ, Lim SY, Yim SV, Chun W, Kim SS (2004) Curcumin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression by inhibiting activator protein 1 and nuclear factor kappaB bindings in BV2 microglial cells. *J Pharmacol Sci* 94: 325-8.
47. Karrat L, Abajy MY, Nayal R (2022) Investigating the anti-inflammatory and analgesic properties of leaves ethanolic extracts of *Cedrus libani* and *Pinus brutia*. *Heliyon* 8:e09254, <https://doi.org/10.1016/j.heliyon.2022.e09254>.
48. Kerckhove N, Collin A, Condé S, Chaletex C, Pezet D, Balayssac D (2017) Long-term effects, pathophysiological mechanisms, and risk factors of chemotherapy-induced peripheral neuropathies: a comprehensive literature review. *Front Pharmacol* 8:86. doi: 10.3389/fphar .2017.00086
49. Kohli K, Ali J, Ansari MJ, Raheman Z (2005) Curcumin: a natural antiinflammatory agent. *Indian J Pharmacol* 37: 141-7.
50. Konsman JP, Parnet P, Dantzer R (2002) Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci* 25:154e9.
51. Kulmacz RJ, Lands WE (1983) Requirements for hydroperoxide by the cyclooxygenase and peroxidase activities of prostaglandin H synthase. *Prostaglandins* 25(4):531-540.
52. Lekshmi RK, Rajesh R, Mini S (2015) Ethyl acetate fraction of *Cissus quadrangularis* stem ameliorates hyperglycaemia-mediated oxidative stress and suppresses inflammatory response in nicotinamide/ streptozotocin induced type 2 diabetic rats. *Phytomedicine* 22(10): 952-960. doi: 10.1016/j. phymed.2015.06.014.
53. Lipton RB, Apfel SC, Dutcher JP, Rosenberg R, Kaplan J, Berger A, Einzig AI, Wiernik P, Schaumburg HH (1989) Taxol produces a predominantly sensory neuropathy. *Neurology* 39:368–73.
54. Liu JB, Ding YS, Zhang Y, Chen JB, Cui BS, Bai JY, Lin MB, Hou Q, Zhang PC, Li S (2014) Anti-inflammatory Hydrolyzable Tannins from *Myricaria bracteata*. *J Nat Prod* 78(5). DOI: 10.1021/np500953e.

55. Mahajna S, Azab M, Zaid H, Farich BA, Al Battah F, Mashner S, Saad B (2015) *In vitro* Evaluations of Cytotoxicity and Anti-inflammatory Effects of *Peganum harmala* Seed Extracts in THP-1-derived Macrophages. *European J Med Plants* DOI: 10.9734/EJMP/2015/13267.
56. Mahomoodally MF, Gurib-Fakim A, Subratty AH (2007) Effect of exogenous ATP on *Momordica charantia* Linn. (Cucurbitaceae) induced inhibition of D-glucose, L-tyrosine and fluid transport across rat everted intestinal sacs in vitro. *J Ethnopharmacol* 110(2):257–63.
57. Massicot F, Hache G, David L, Chen D, Leuxe C, Garnier-Legrand L, Rat P, Lapr v te O, Coudor  F (2013) P2X7 Cell death receptor activation and mitochondrial impairment in oxaliplatin-induced apoptosis and neuronal injury: cellular mechanisms and *in vivo* approach. *PLoS ONE* 8(6):e66830. doi: 10.1371/journal.pone.0066830.
58. Matah Marte VM, Ateufack G, Mbiantcha M, Atsamo AD, Adjouzem CF, Djuichou Nguemnang SF, Tsafack EG, Nana Yousseu W, Madjo Kouam YK, Ngoufack AE (2020) Methanolic Extract of *Distemonanthus benthamianus* (Caesalpiniaceae) Stem Bark Suppresses Ethanol/Indomethacin Induced Chronic Gastric Injury in Rats. *Gastroenterol Res Pract* 2020: Article ID 8180323, 14 pages. <https://doi.org/10.1155/2020/8180323>.
59. Mbiantcha M, Almas J, Ateufack G, Shabana US, Bomba TFD, Nida D (2018) NO-cGMP-K channel-dependent anti-nociceptive activities of methanol stem bark extract of *Piptadeniastrum africanum* (Mimosaceae) on rats. *Asian Pac J Trop Biomed* 8(3): 150-159.
60. Mbiantcha M, Almas J, Shabana SU, Nida D, Aisha F (2017) Anti-arthritic property of crude extracts of *Piptadeniastrum africanum* (Mimosaceae) in complete Freund's adjuvant-induced arthritis in rats. *BMC Complement Altern Med* 17:111. DOI 10.1186/s12906-017-1623-5.
61. Mbiantcha M, Dawe A, Atsamo AD, Ateufack G, Yousseu NW, Khalid R, Mehreen A, Fanta YS A, Njoku IS, Hamza D, Naeem UR, Izhar A (2020) In Vitro Antioxidant, Anti-inflammatory, and In Vivo Anticolitis Effects of Combretin A and Combretin B on Dextran Sodium Sulfate-Induced Ulcerative Colitis in Mice. *Gastroenterol Res Pract* 2020: Article ID 4253174, 12 pages. <https://doi.org/10.1155/2020/4253174>.
62. Mbiantcha M, Feuya Tchouya RG, Nana Yousseu W, Atsamo DA, Foundikou H, Lebibi J, Gamo Zemo F (2022) *Scyphocephalione A* isolated from the stem bark of *Scyphocephalum ochocoa* (Myristicaceae) attenuate acute and chronic pain through the anti-inflammatory activity. *Inflammopharmacology*. <https://doi.org/10.1007/s10787-022-00966-4>.
63. Mbiantcha M, Wembe NA, Dawe A, Nana YW, Ateufack G (2017) Antinociceptive Activities of the Methanolic Extract of the Stem Bark of *Boswellia dalzielii* Hutch. (Burseraceae) in Rats Are NO/cGMP/ATP-Sensitive-K⁺ Channel Activation Dependent. *Evid Based Complement Alternat Med* 2017: Article ID 6374907, 12 pages. <https://doi.org/10.1155/2017/6374907>.
64. Mendell LM, Albers KM, Davis BM (1999) Neurotrophins, nociceptors, and pain. *Microsc Res Tech* 45:252–261. doi: 10.1002/(sici)1097-0029(19990515/ 01)45:4/5<252::aid-jemt9>3.0.co;2-n (1999).
65. Mesaik MA, Ul-Haq Z, Murad S, Ismail Z, Abdullah NR, Gill HK, Yousaf M, Siddiqui RA, Ahmad A, Choudhary MI (2006) Biological and molecular docking studies on coagulin-H: Human IL-2 novel

- natural inhibitor. *Mol Immunol* 43(11):1855–163.
66. Mishra G, Srivastava S, Nagori BP (2010) Pharmacological and therapeutic activity of *Cissus quadrangularis*: An overview. *Int J Pharmtech Res* 2:1298-1310.
 67. Mohammed MJ, Al-Bayati FA (2009) Isolation, identification and purification of caffeine from *Coffea arabica* L. and *Camellia sinensis* L.: A combination antibacterial study. *Int J Green Pharm* 3:52-7
 68. Moini Zanjani T, Ameli H, Labibi F, Sedaghat K, Sabetkasaei M (2014) The attenuation of pain behavior and serum COX-2 concentration by curcumin in a rat model of neuropathic pain. *Korean J Pain* 27: 246-52.
 69. Mourao AF, Blyth FM, Branco JC (2010) Generalised musculoskeletal pain syndromes. *Best practice & research. Clin Rheumatol* 24:829–840. doi: 10.1016/j.berh.2010.10.005 (2010).
 70. Mukhopadhyay S, Mondal A, Poddar MK (2003) Chronic administration of caffeine: Effect on the activities of hepatic antioxidant enzymes of Ehrlich ascites tumor-bearing mice. *Ind J Exp Biol* 41: 283.
 71. Musfizur HMd, Shahid-Ud-Daula AFM, Jahan IA, Nimmi I, Adnan T, Abdullah-Al-Mansur, Hossain H (2012) Anti-inflammatory Activity, Total Flavonoids and Tannin Content from the Ethanolic Extract of *Ageratum conyzoides* Linn. Leaf. *Int J Pharm Phytopharmacol Res* 1(5): 234-241.
 72. Nan P, Hu Y, Zhao J, Feng Y, Zhong Y (2004) Chemical composition of the essential oils of two *Alpinia* species from Hainan island. *China Z Naturforsch* 59: 153-160.
 73. Nie TW, Shukkoor MS, Nair RS, Amiruddin FK, Ramassami S (2015) Involvement of opioidergic and serotonergic systems in the analgesic activity of *Cissus quadrangularis* L. stem extract in mice. *J Basic Clin Physiol Pharmacol* 26(1): 35-41. doi: 10.1515/jbcpp-2014-0035.
 74. Nishikawa N, Nomoto M (2017) Management of neuropathic pain. *J Gen Fam Med* 18:56-60.
 75. Nunes CR, Arantes MB, Pereira SMF, Cruz LL, Passos MS, Moraes LP, Vieira IJC, Oliveira DBarros (2020) Plants as sources of anti-inflammatory agents, *Molecules* 25:3726.
 76. Olausson P, Gerdle B, Ghafouri N, Larsson B, Ghafouri B (2012) Identification of Proteins from Interstitium of Trapezius Muscle in Women with Chronic Myalgia Using Microdialysis in Combination with Proteomics. *PLoS ONE* 7:e52560. doi: 10.1371/journal.pone.0052560 (2012).
 77. Oliviera Junior JO, Portella Junior CS, and Cohen CP (2016) Inflammatory mediators of neuropathic pain. *Rev Dor. Sao Paulo* 7(Suppl 1): S35-42.
 78. Orlando RA, Gonzales AM, Hunsaker LA, Franco CR, Royer RE, Vander Jagt DL, Vander Jagt DJ (2010) Inhibition of nuclear factor kappaB activation and cyclooxygenase-2 expression by aqueous extracts of Hispanic medicinal herbs. *J Med Food* 13: 888-95.
 79. Owoyele BV, Bakare AO (2018) Analgesic properties of aqueous bark extract of *Adansonia digitata* in Wistar rats. *Biomed Pharmacother* 97: 209-12.
 80. Padmanabhan P, Jangle SN (2012) Evaluation of in-vitro antiinflammatory activity of herbal preparation, a combination of four medicinal plants. *Int J Basic Appl Med* 2(1):109–116

81. Panthong A, Supraditaporn W, Kanjanapothi D, Taesotikul T, Reutrakul V (2007) Analgesic, anti-inflammatory and venotonic effects of *Cissus quadrangularis* Linn. J Ethnopharmacol 110(2):264–270.
82. Pertusi R.M (2004) Selective Cyclooxygenase Inhibition in Pain Management. JAOA Supplement 104(11):S19-S24.
83. Pluemjai T. (1986) Constituent of *Cissus quadrangularis* Linn. Thai J Pharm Sci 11 (4).
84. Potu BK, Bhat KM, Rao MS (2009) Petroleum ether extract of *Cissus quadrangularis* (Linn.) enhances bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. Clinics (Sao Paulo) 64(10): 993-998. doi: 10.1590/S1807-59322009001000010.
85. Prabhavathi RM, Prasad MP, Jayaramu M (2016) Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis*. Adv Appl Sci Res 7(4):11-17.
86. Ramde-Tiendrebeogo A, Tibiri A, Hilou A, Lompo M, Millogo-Kone H, Nacoulma OG, Guissou IP (2012) Antioxidative and antibacterial activities of phenolic compounds from *Ficus sue Forssk*. Int j biol chem sci 6(1): 328-336.
87. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 26: 1231–1237.
88. Rezaakhsh A, Mahmoodpoor A, Soleimanpour M, Shahsavarinia K, Soleimanpour H (2021) Clinical Applications of Aspirin as a Multi-potent Drug Beyond Cardiovascular Implications: A Proof of Concept for Anesthesiologists- A Narrative Review. Anesth Pain Med 11(5). e118909. doi: 10.5812/aapm.118909.
89. Rita C, Bruno V, Helena R, Ana NC, Nuno F, Vineet G, João EF, Luis FM (2014) Potent Anti-Inflammatory and Antiproliferative Effects of Gambogic Acid in a Rat Model of Antigen-Induced Arthritis. Mediators Inflamm 7pages.
90. Rowinsky EK, Donehower RC, Jones RJ, Tucker RW (1988) Microtubule changes and cytotoxicity in leukemic cell lines treated with taxol. Cancer Res 48:4093–100.
91. Royds J, McCrory C (2018) Neuroimmunity and chronic pain. BJA Education 18(12): 377e383. doi: 10.1016/j.bjae.2018.09.003.
92. Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, Bonventre JV, Woolf CJ (2001) Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 410:471-475.
93. Scripture CD, Figg WD, Sparreboom A (2006) Peripheral neuropathy induced by paclitaxel. recent insights and future perspectives. Cur Neuropharmacol 4:165–72.
94. Seidel MF, Herguijuela M, Forkert R, Otten U (2010) Nerve Growth Factor in Rheumatic Diseases. Semin Arthritis Rheum 40:109–126.doi: <http://dx.doi.org/10.1016/j>.
95. Shirley D (1966) High resolution Xray photoemission studies on the active constituents of *Cissus quadrangularis* Linn. Current Science 35: 317p.

96. Shirwaikar A, Khan S, Malini S (2003) Antiosteoporotic effect of ethanol extract of *Cissus quadrangularis* Linn. on ovariectomized rat. *J Ethnopharmacol* 89:245–250.
97. Shishodia S, Sethi G, Aggarwal BB (2005) Curcumin: getting back to the roots. *Ann N Y Acad Sci* 1056: 206-17.
98. Singh G, Rawat P, Maurya R (2007) Constituents of *Cissus quadrangularis*. *Nat Prod Res* 21: 522–528.
99. Singh U, James T, Senthil KV, Sridevi D, Ishwarlal J (2005) Development of an In Vitro Screening Assay to Test the Anti-inflammatory Properties of Dietary Supplements and Pharmacologic Agents. *Clin Chem* 51(12):2252–6.
100. Srisook K, Palachot M, Mongkol N, Srisook E, Sarapusit S (2011) Anti-inflammatory effect of ethyl acetate extract from *Vitis quadrangularis* Linn may be involved with induction of heme oxygenase-1 and suppression of NF- κ B activation. *J Ethnopharmacol* 133:1008–1014.
101. Suzu I, Goto H, Hiwatashi N, Hattori S, Rotjanapan K, Leeanansaksiri W, Okad S (2013) Antioxidant and Antityrosinase Activity of *Cissus quadrangularis* Extract. *Nat Prod Commun* 8(5) : 629 – 630.
102. Tegeder I, Niederberger E, Vetter G, Bräutigam L, Geisslinger G (2001) Effects of selective COX-1 and -2 inhibition on formalinevoked nociceptive behaviour and prostaglandin E(2) release in the spinal cord. *J Neurochem* 79: 777-86.
103. Thakur A, Jain V, Hingorani L, Laddha KS (2009) Phytochemical Studies on *Cissus quadrangularis* Linn. *Pharmacognosy Research* 1(4):213-215.
104. Viji V, Helen A (2008) Inhibition of lipxygenases and cyclooxygenase-2 enzymes by extracts isolated from *Bacopa monniera* (L.) Wettst. *J Ethnopharmacol* 118: 305–311.
105. Vonkeman HE, van de Laar MA (2010) Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. *Semin Arthritis Rheum* 39(4):294–312.
106. Wasserheit C, Frazein A, Oratz R, Sorich J, Downey A, Hochster H, Chachoua A, Wernz J, Zeleniuch-Jacquotte A, Blum R, Speyer J (1996) Phase II trial of paclitaxel and cisplatin in women with advanced breast cancer: an active regimen with limiting neurotoxicity. *J Clin Oncol* 14:1993–9.
107. Woolf JC, Mannion JR (1999) Neuropathic pain: etiology, symptoms, mechanisms, and management. *Lancet* 353:1959-64.
108. Zanjani TM, Ameli H, Labibi F, Sedaghat K, Sabetkasaei M (2014) The Attenuation of Pain Behavior and Serum COX-2 Concentration by Curcumin in a Rat Model of Neuropathic Pain. *Korean J Pain* 27(3): 246-252
109. Zhang EJ, Song CH, Ko YK, Lee WH (2014) Intrathecal administration of mesenchymal stem cells reduces the reactive oxygen species and pain behavior in neuropathic rats. *Korean J Pain* 27: 239-45.
110. Zhao H, Alam A, Chen Q, Eusma MA, Pal A, Eguchi S, Wu L, Ma D (2017) The role of microglia in the pathobiology of neuropathic pain development: what do we know? *Br J Anaesth* 118: 504–516.
111. Zimmerman M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16(2):109–10.

Tables

Table 1: Total phenol, flavonoid and tannins content of aqueous and methanolic extracts of *Cissus quadrangularis*.

	Total Phenols (mg Gallic acid eq/mg extract)	Total Flavonoids (mg equivalent of quercetin/mg extract)	Total Tannins (mg equivalent of tannic acide/g extract)
Aqueous extract	15.57 ± 0.16	2.07 ± 0.33	1.48 ± 0.62
Ethanollic extract	15.53 ± 0.34	2.55 ± 0.12	8.02 ± 0.13

Table 2 : IC₅₀ value of aqueous and ethanol extracts of *Cissus quadratilarus* on human whole blood evaluated by luminol and/or lucigenin amplified chemiluminescence

Treatment	Concentration ($\mu\text{g/ml}$)	Oxidative burst (IC_{50} $\mu\text{g/ml}$)					
		Luminol and Zymosan			Lucigenin and PMA		
		WB	PMNs	MQ	WB	PMNs	MQ
Aqueous extract	3.125	19.00 $\pm$ 0.22	12.20 $\pm$ 0.27	15.05 $\pm$ 1.11	22.34 $\pm$ 1.18	16.23 $\pm$ 0.09	13.45 $\pm$ 0.29
	6.25						
	12.5						
	25						
	50						
	100						
Ethanol extract	3.125	12.31 $\pm$ 0.21	7.22 $\pm$ 0.19	6.66 $\pm$ 0.22	16.45 $\pm$ 1.15	11.03 $\pm$ 0.04	7.77 $\pm$ 0.25
	6.25						
	12.5						
	25						
	50						
	100						
Ibuprofen	12.5	13.09 $\pm$ 0.26	14.53 $\pm$ 1.78	17.03 $\pm$ 1.22	16.22 $\pm$ 0.71	15.39 $\pm$ 1.14	16.21 $\pm$ 0.22
	25						
	50						
	100						

The IC_{50} values are presented as mean $\pm$ SD of triplicates. Where WB whole blood, PMNs polymorphonuclear leukocytes, MQ mice peritoneal macrophages

Table 3 : IC_{50} value of aqueous and ethanol extracts of *Cissus quadrangularis* on cell proliferation and cytotoxicity

Treatment	Concentration ($\mu\text{g/ml}$)	T-cells proliferation ($\text{IC}_{50} \mu\text{g/ml}$)	Cytotoxicity on MTT Cells ($\text{IC}_{50} \mu\text{g/ml}$)
Aqueous extract	1	22.39 ± 0.19	89.98 ± 2.45
	10		
	100		
Ethanollic extract	1	4.27 ± 0.04	19.33 ± 1.12
	10		
	100		
Prednisolone	-	2.39 ± 0.08	-
Cyclohexamide	-	-	0.13 ± 0.05

The IC_{50} values are presented as mean $\pm$ SD of triplicates.

Table 4 : *In vitro* antiinflammatory property of aqueous and ethanollic extracts of *Cissus quadrangularis*.

Treatment	Dose (µg/ml)	Inhibition (%)			
		Protein denaturation	COX1	COX2	5-lipoxygenase
Diclofenac	62.5	44.01 ± 1.09	-	-	-
	125	51.22 ± 1.11	-	-	-
	250	62.23 ± 0.99	-	-	-
	500	76.03 ± 2.01	-	-	-
Ibuprofen	62.5	-	44.16 ± 0.89	47.67 ± 1.59	49.09 ± 1.24
	125	-	52.00 ± 1.22	61.87 ± 1.29	55.89 ± 1.35
	250	-	63.03 ± 1.01	70.21 ± 1.12	63.21 ± 0.99
	500	-	70.11 ± 1.05	79.02 ± 1.54	78.11 ± 0.77
Aqueous extract	62.5	18.22 ± 1.11	21.12 ± 2.01	24.32 ± 0.93	35.04 ± 0.93
	125	29.01 ± 1.23	33.14 ± 1.32	29.44 ± 1.39	45.22 ± 1.09
	250	38.32 ± 1.21	49.23 ± 1.19	40.31 ± 1.17	52.43 ± 1.23
	500	50.11 ± 0.92	54.11 ± 1.21	46.33 ± 1.09	65.67 ± 0.87
Ethanollic extract	62.5	27.87 ± 0.88	28.04 ± 0.95	24.60 ± 1.11	41.93 ± 1.13
	125	35.07 ± 1.14	36.12 ± 0.83	33.21 ± 1.22	50.90 ± 1.06
	250	40.04 ± 0.91	53.21 ± 0.92	40.42 ± 1.33	65.45 ± 2.01
	500	63.22 ± 0.81	68.55 ± 1.16	80.33 ± 1.27	72.33 ± 1.37

The percentage values were obtained using various concentrations of test compounds and readings are presented as mean ± SD of triplicates.

Table 5 : *In vitro* antioxidant property of aqueous and methanolic extracts of *Cissus quadratarus*

Compounds	Dose (µg/ml)	Inhibition (%)		
		DPPH	ABTS	NO
Vitamine C	62.5	39.91 ± 2.44	13.75 ± 0.11	16.37 ± 2.21
	125	51.94 ± 1.24	27.72 ± 3.35	37.18 ± 4.60
	250	66.44 ± 0.61	29.81 ± 0.45	52.59 ± 2.43
	500	75.75 ± 1.18	55.12 ± 0.33	71.16 ± 0.39
Aqueous extract	62.5	16.57 ± 2.72	15.02 ± 0.59	15.68 ± 0.64
	125	36.80 ± 4.32	24.86 ± 1.39	39.59 ± 3.93
	250	57.42 ± 1.54	50.44 ± 0.69	49.20 ± 1.84
	500	67.41 ± 0.45	62.43 ± 1.15	58.97 ± 1.90
Ethanollic extract	62.5	30.37 ± 2.25	18.59 ± 0.31	25.23 ± 2.35
	125	53.94 ± 0.83	31.52 ± 0.50	46.95 ± 3.77
	250	71.23 ± 1.08	56.99 ± 1.96	63.03 ± 2.68
	500	77.41 ± 1.32	69.58 ± 0.83	72.81 ± 2.41

The percentage values were obtained using various concentrations of test compounds and readings are presented as mean ± SD of triplicates.

Figures

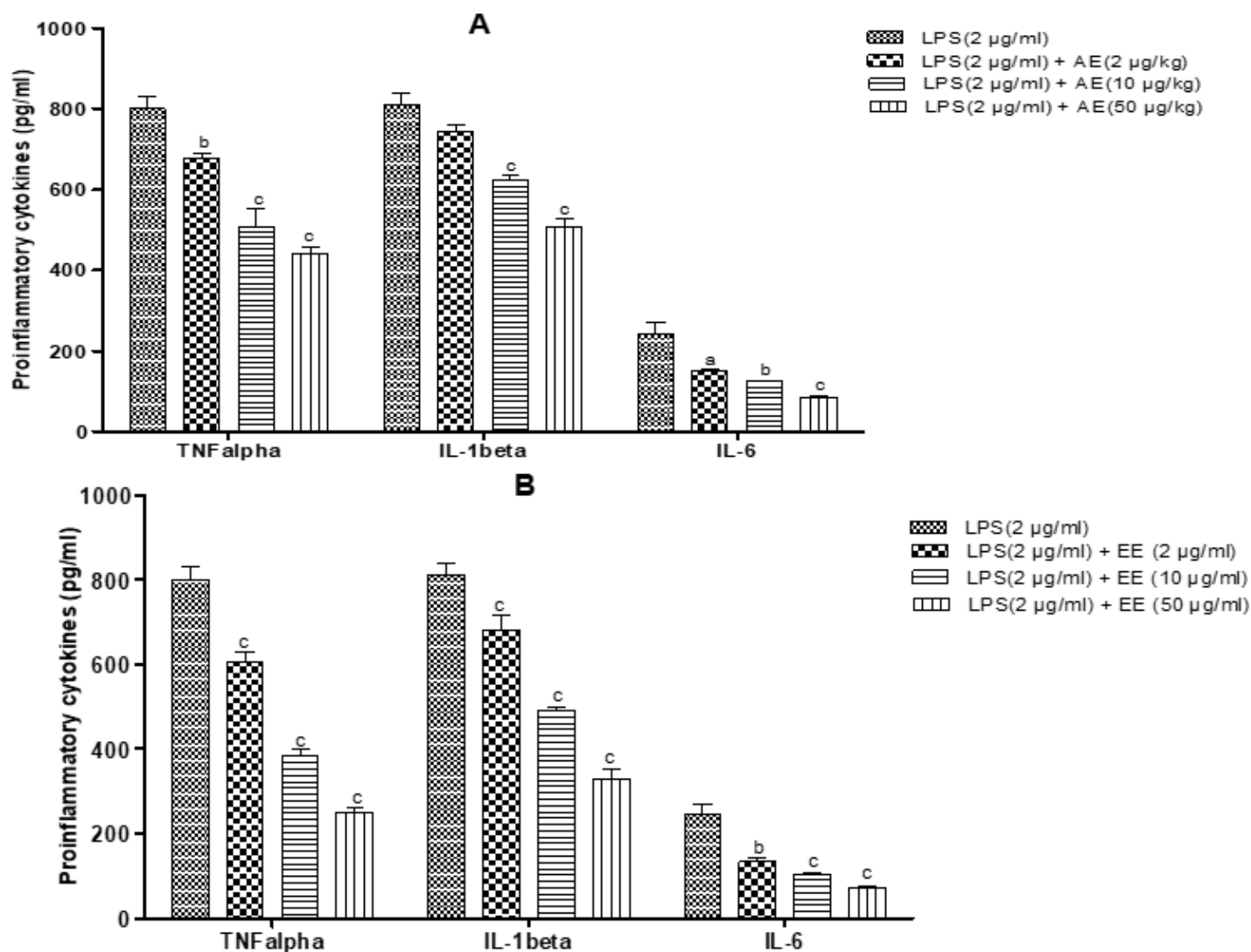


Figure 1

Effect of aqueous (A) and ethanol (B) extracts of *Cissus quadrangularis* on TNF- α , IL-1 β and IL-6 secretion levels by THP-1 activated by LPS. Data represent mean $\pm$ SD of triplicates. ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$ when compared with that of the control: PMA + LPS

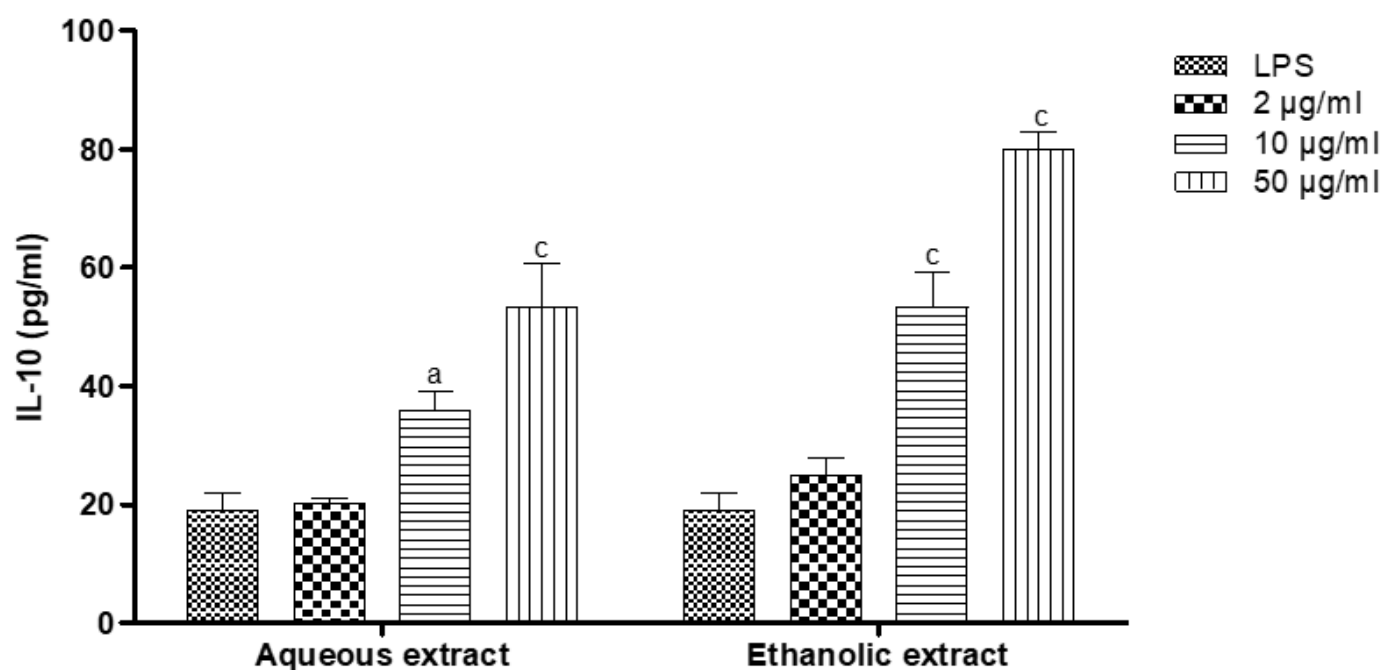


Figure 2

Effect of aqueous (A) and ethanol (B) extracts of *Cissus quadrangularis* on IL-10 secretion levels by THP-1 activated by LPS. Data represent mean $\pm$ SD of triplicates. ^ap < 0.05 and ^cp < 0.001 when compared with that of the control: PMA + LPS.

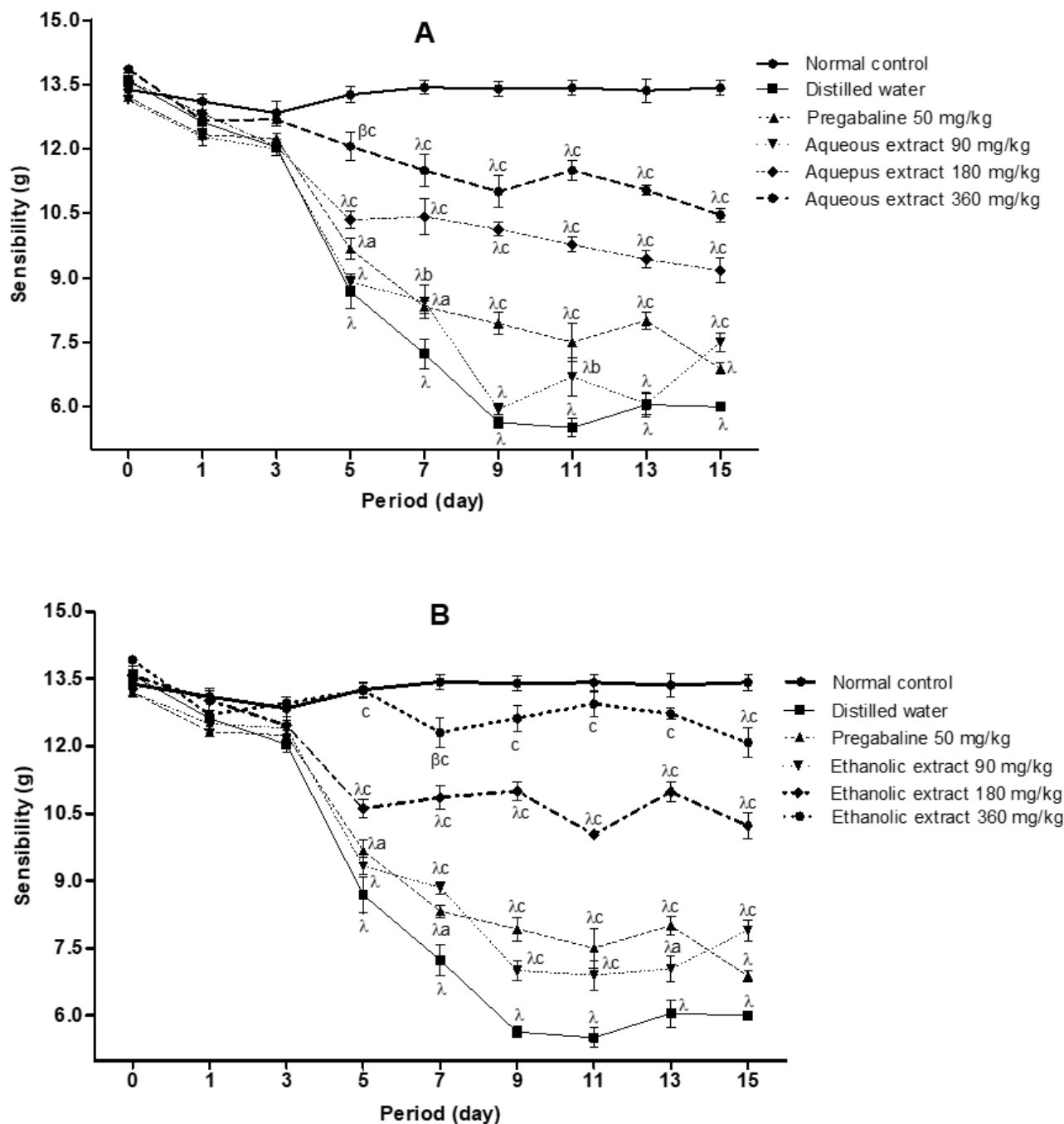


Figure 3

Effect of aqueous (A) and ethanolic (B) extract of *Cissus quadrangularis* on paw hyperalgesia in vincristine-induced neuropathic pain. N = 6 rats per group. Data are expressed as the mean $\pm$ SEM of six animals per experimental group, compared by two-way analysis of variance followed by Bonferroni's test; $^{\beta}P < 0.01$; $^{\lambda}P < 0.001$ significantly different compared to normal control; $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.001$ significantly different compared to distilled water.

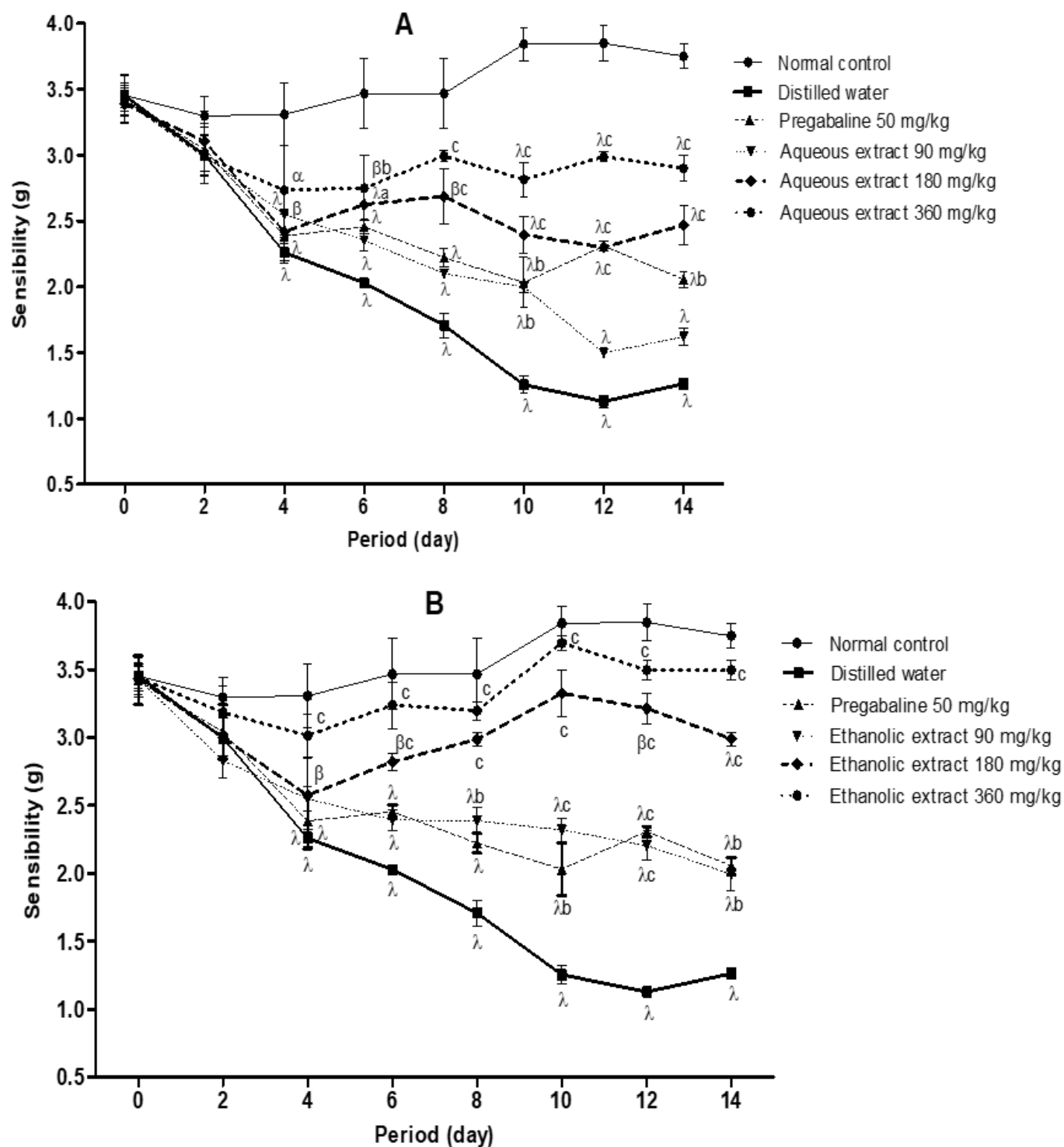


Figure 4

Effect of aqueous (A) and ethanolic (B) extract of *Cissus quadrangularis* on paw allodynia in vincristine-induced neuropathic pain. N = 6 rats per group. Data are expressed as the mean $\pm$ SEM of six animals per experimental group, compared by two-way analysis of variance followed by Bonferroni's test; $^{\alpha}P < 0.05$; $^{\beta}P < 0.01$; $^{\lambda}P < 0.001$ significantly different compared to normal control; $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.001$ significantly different compared to distilled water.

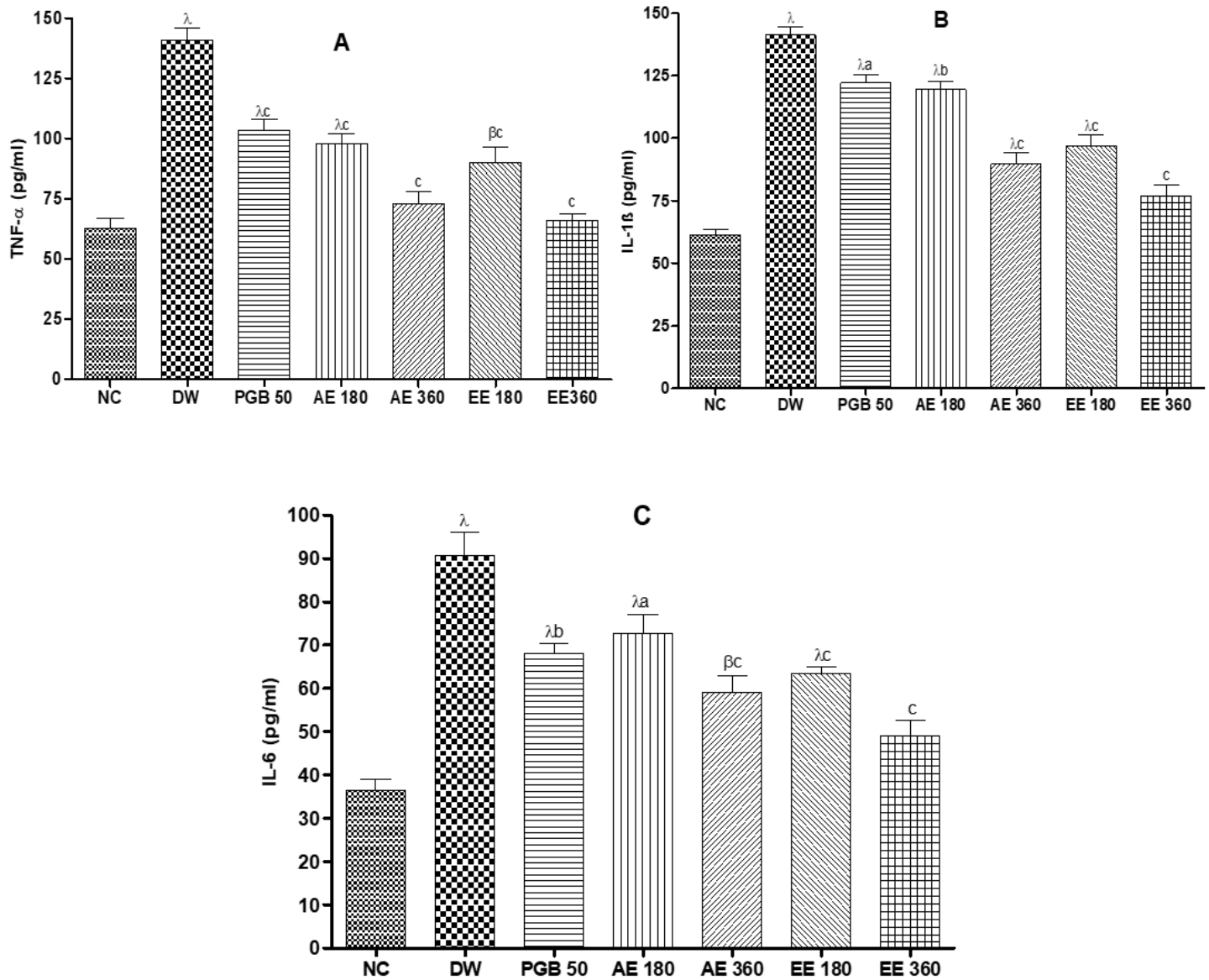


Figure 5

Effects of the aqueous (AE) and methanol (EE) extracts of *Cissus quadrangularis* on the levels of TNF- α (a), IL- β (b), and IL-6 (c) in the serum in vincristine-induced neuropathic pain. Data are expressed as the mean $\pm$ SEM of six animals per experimental group, compared by one-way analysis of variance followed by Tukey test; $\beta p < 0.01$ and $\lambda p < 0.001$ mean significantly different from the normal control (NC); and $a p < 0.05$, $b p < 0.01$, and $c p < 0.001$ mean significantly different from the distilled water (DW) group. PGB50: rat that received 50 mg/kg/day pregabalin, AE180: rat that received 180 mg/kg/day of aqueous extract, AE360: rat that received 360 mg/kg/day of aqueous extract, EE180: rat that received 180 mg/kg/day of ethanol extract, EE360: rat that received 360 mg/kg/day ethanol extract, TNF: tumour necrosis factor, IL: interleukin.

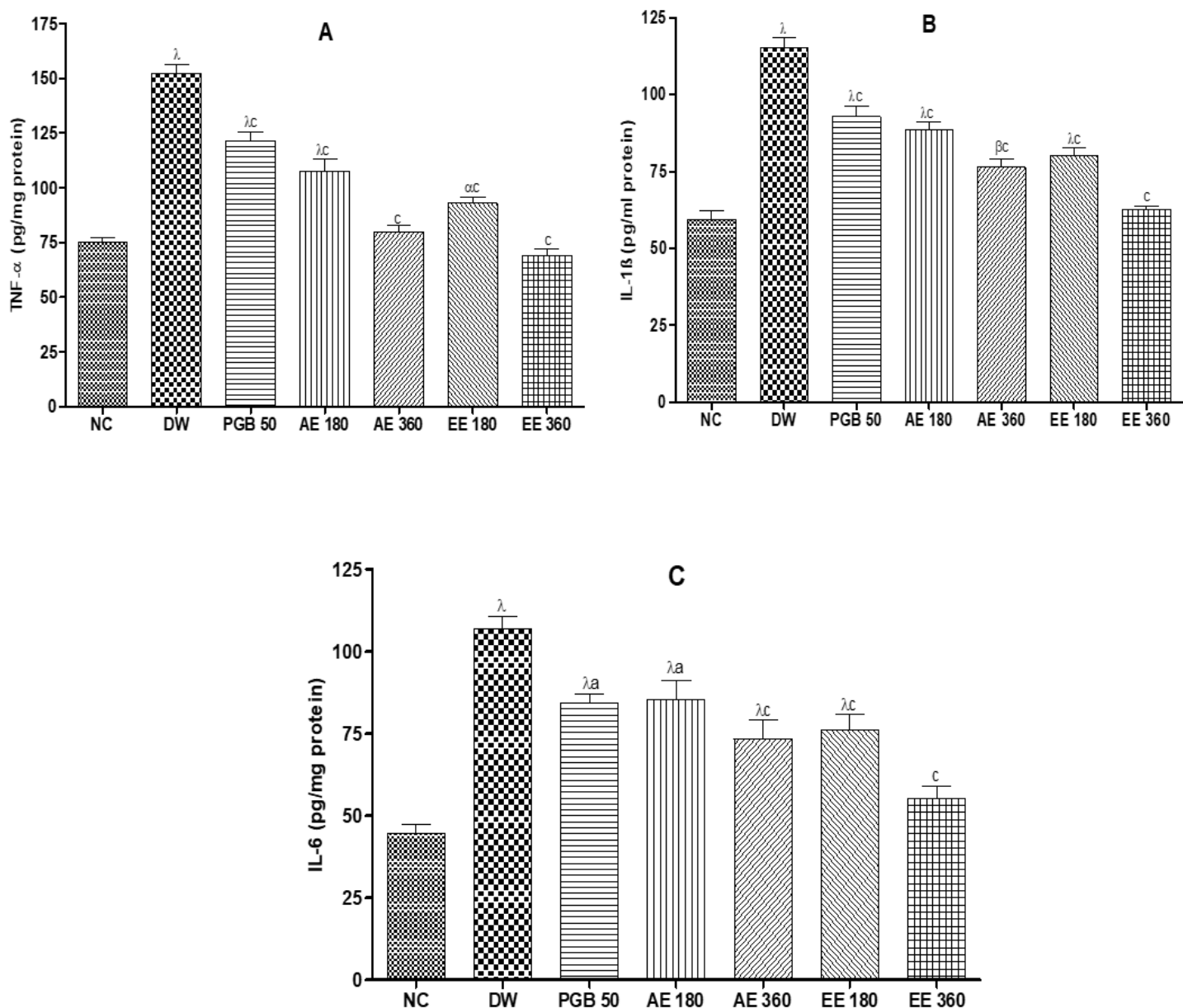


Figure 6

Effects of the aqueous (AE) and methanol (EE) extracts of *Cissus quadrangularis* on the levels of TNF- α (a), IL- β (b), and IL-6 (c) on sciatic nerves in vincristine-induced neuropathic pain. Data are expressed as the mean $\pm$ SEM of six animals per experimental group, compared by one-way analysis of variance followed by Tukey test; $^a p < 0.05$; $^b p < 0.01$ and $^c p < 0.001$ mean significantly different from the normal control (NC); and $^a p < 0.05$, $^b p < 0.01$, and $^c p < 0.001$ mean significantly different from the distilled water (DW) group. PGB50: rat that received 50 mg/kg/day pregabalin, AE180: rat that received 180 mg/kg/day of aqueous extract, AE360: rat that received 360 mg/kg/day of aqueous extract, EE180: rat that received 180

mg/kg/day of ethanol extract, EE360: rat that received 360 mg/kg/day ethanol extract, TNF: tumour necrosis factor, IL: interleukin.

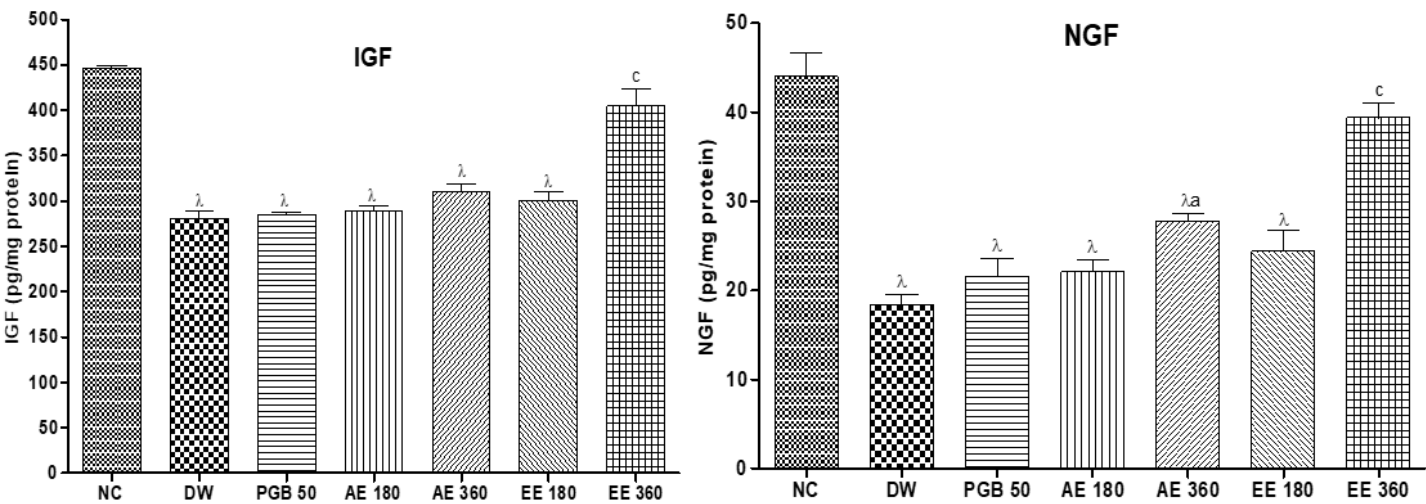


Figure 7

Effects of the aqueous (AE) and methanol (EE) extracts of *Cissus quadrangularis* on the levels of insulin-like growth factor (IGF) and nerve growth factor (NGF) on sciatic nerves in vincristine-induced neuropathic pain. Data are expressed as the mean $\pm$ SEM of six animals per experimental group, compared by one-way analysis of variance followed by Tukey test; ^λ $p < 0.001$ mean significantly different from the normal control (NC); and ^a $p < 0.05$ and ^c $p < 0.001$ mean significantly different from the distilled water (DW) group. PGB50: rat that received 50 mg/kg/day pregabalin, AE180: rat that received 180 mg/kg/day of aqueous extract, AE360: rat that received 360 mg/kg/day of aqueous extract, EE180: rat that received 180 mg/kg/day of ethanol extract, EE360: rat that received 360 mg/kg/day ethanol extract, TNF: tumour necrosis factor, IL: interleukin.