Tanzania Journal of Science 49(5): 930-942, 2023
ISSN 0856-1761, e-ISSN 2507-7961

Evaluation of the Modulatory Effects of *Capsicum Chinensis* Methanol Extract in Streptozotocin-Induced Diabetic Neuropathic Pain in Mice

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Received 19 Aug 2023, Revised 2 Jan 2024, Accepted 13 Jan 2024, Published Jan 2024

https://dx.doi.org/10.4314/tjs.v49i5.1

Abstract

Diabetes is one of the common causes of neuropathic pain. The first-line treatment drugs used in alleviating neuropathic pain can cause adverse effects. Therefore, the aim of this study is to evaluate the analgesic property of *Capsicum chinensis,* since the plant was reported to be used against chronic pain in traditional medicine. Streptozotocin-induced diabetes was adopted to model neuropathy in mice and the antihyperalgesic effect of the extract was evaluated using a hot plate test, cold allodynia test, and Randall- Selitto paw pressure test. The plasma level of pain-associated inflammatory biomarkers like interleukin-6 (IL-6) and tissue necrotic factor-α (TNF-α) was also measured. Treatment with varying doses of extract (1, 2.5, and 5 mg/kg) significantly ($p < 0.05$) increased the mean reaction time to thermal pain during hot and cold plate tests. During the Paw pressure test, reaction time was significantly prolonged in the treatment groups and the plasma level of TNF-α and IL-6 were reduced ($p < 0.05$). The extract showed a better antihyperalgesic effect than the positive control drug (gabapentin). The result obtained showed that *Capsicum chinensis* extract can alleviate diabetes neuropathic pain in mice models with a better analgesic effect thanthe control drug (gabapentin).

Keywords: Diabetes, *Capsicum chinensis,* analgesic, inflammation, neuropath

Introduction

Neuropathic pain arises from direct injury to the neural tissue leading to nociceptive pain that can last for months or years after the nerve injury has healed (Costigan et al. 2009, Moulin et al. 2014). Statistical studies have estimated that the prevalence of neuropathic pain within the global population, ranges from 1.5% to 8%, an equivalence of 100 million to 560 million people worldwide (Gilron and Flatters 2006, Torrance et al. 2006, Salter, 2014). Neuropathic pain can arise from traumatic nerve, spinal cord, or brain injury (including stroke) or can be associated with diabetes, human immunodeficiency virus/AIDS, post-herpetic neuropathies, multiple sclerosis, cancer, and toxic effects of chemotherapeutic agents

(Xiao et al. 2007, Treede et al. 2008, Schmidt et al. 2010). Peripheral, autonomic, digestive, proximal, and focal neuropathic pain can be classified based on common causes like accidents, infection, surgery, and disease. Chronic disease like diabetes is the most common cause of neuropathy. According to Boulton et al. (2005), diabetes is responsible for almost 30% of neuropathic pain in humans. Generally, peripheral neuropathy is characterized by tingling, burning, sharp, shooting, and lancinating or even electric shock sensations which affect the toes and distal foot, but slowly progress proximally to involve the feet and legs. It is also characterized by a progressive loss of nerve fibers affecting both the autonomic and somatic divisions, thereby leading to diabetic retinopathy and nephropathy (Bansal et al. 2006, Tesfaye et al. 2013). The pathophysiology of diabetic neuropathy is complex, but important pathogenesis like polyol pathway through aldose reductase (Oates, 2002), increased nerve tissue reactive oxygen species (Obrosova et al. 2005, Drel et al. 2007, Vareniuk et al. 2007), reduced myo-inositol/ATPase Na^+ /K^+ activity, infiltration and activation of inflammatory cells as well as pro-IC (pro-inflammatory cytokines) such as IL-1β, IL-6 and TNF-α (Boka et al. 1994, Jensen et al. 1995, Hirota et al. 1996) and increased non-enzymatic glycation/glycoxidation of proteins (Baynes 1991, Brownlee 2001, Thornalley 2002, Giacco and Brownlee 2010) have been linked with nerve damage. First-line treatment drugs for neuropathic pain include tricyclic antidepressants, serotonin/noradrenaline reuptake inhibitors, pregabalin, and gabapentin (Tanenberg et al. 2011). Tramadol and controlled-release opioids are recommended as second-line treatments and cannabinoids as third-line treatments. Fourth-line treatments include methadone, lamotrigine, lacosamide, tapentadol, and botulinum toxin (Mu et al. 2017). Other drugs in current clinical use include carbamazepine, lidocaine patch, capsaicin patch, and ziconotide (Zilliox and Russell 2011, Deli et al. 2013, Peltier et al. 2014). All these drugs are either anticonvulsants or antidepressants with serious toxic effects (Freeman et al. 2008). The mechanism of action of these drugs against diabetic neuropathic pain is not clear and none is a target of the important pathogenesis that was linked with this pain. The use of typical painkillers is not satisfactory in alleviating neuropathic pain and as a matter of fact, any pain that is opioid resistant is likely neuropathic. This necessitates the need for investigating new target agents with anti-nociceptive properties against diabetic neuropathy. Therefore, we designed this study to evaluate the analgesic properties of *Capsicum chinensis*. *Capsicum chinensis* fruit is well known to possess activities against rheumatism, sore throat, toothache, and fever in developing countries that are still practicing herbal/traditional medicine to a large extent.

The ripe fruits of *Capsicum chinensis* Linn. (African Chillies) belong to the family Solanaceae (Sofowora 1993) and contain capsicum in abundance. It is cultivated in tropical regions of India, Nigeria, Japan, Southern Europe, Mexico, and Sri Lanka. Phytochemical analysis has shown that the fruit contains oleoresin, carotenoids, capsico (a volatile alkaloid), volatile oil (1.5%) and ascorbic acid (0.2%) and pungent compounds like capsaicin (69%), dihydrocapsaicin (22%), norhihydrocapsaicin (7%), homocapsaicin (1%) and homodihydrocapsaicin (1%) (Pawar et al. 2011). The capsaicin component has been used externally as a stimulant, counter-irritant, and rubefacient in sore throat (Watt and Breyer-Brandwijk 2004). Drawing upon the historical utilization of *Capsicum chinensis* fruit in traditional practices, our study hypothesizes potential antihyperalgesic effects in mice models. This study aims to elucidate the potential impact of *Capsicum chinensis* extract on hyperalgesia, contributing to our understanding of its therapeutic potential in the context of pain management.

Materials and Methods

Drugs and chemicals

Methanol and Streptozotocin were obtained from Sigma-Aldrich Chemical Corporation (Sigma-Aldrich St Louis Missouri, USA), Citrate buffer, Gabapentin (TEVA)

Collection, identification, and extraction of plant materials

The fruits of *Capsicum chinensis* were purchased from the local market 'Shasha market' in the Akinyele Local Government area of Ibadan and identified in the Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan. The fruits were air-dried for 6-7 weeks and then pulverized with an electronic blender. 200g of the pulverized fruit was soaked in 70% methanol and left for 72 hours. The extract was filtered using Whatman 3mm filter paper fixed and subsequently concentrated using a rotary evaporator.

Experimental animals

Healthy Swiss mice (30) weighing between 20 g to 30 g were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Ibadan. The mice were fed with pelletized mouse chow and water liberally in well-ventilated cages. The mice were subjected to 12 hours of light and darkness daily. They were acclimatized for 7 days and received care in accordance with stipulated rules of animal experimentation as well as environmental enrichments to reduce pain and discomfort.

Induction and assessment of diabetes

The Swiss mice were administered a single dose of streptozotocin (STZ) (60 mg/kg) in citrate buffer (pH 4.4, 0.1 M) via the intraperitoneal route. The mice were given glucose solution (1 g/l) *ad libitum* after administration to ease the stress. Mice were considered diabetic at blood glucose levels above 200 mg/dl after 72 hours post-administration, using ACCU-CHECK^{R} blood sugar test strip.

Immediately after induction of diabetes, diabetic mice were randomly divided into five (5) groups of 5 animals *(n=5)* each in addition to normal control mice making six (6) groups in total. The six groups comprise normal control mice, diabetic-neuropathy control mice, diabetic-neuropathy treatment mice (1, 2.5, and 5.0 mg/kgMECC), and a positive control group (75 mg/kg gabapentin). Mice were treated with MECC and gabapentin for seven days after inducing type 1 diabetes before assessing the pain modulatory effect of the MECC and gabapentin post-treated mice. Modified doses of 1, 2.5, and 5.0 mg/kg were used to treat mice in this study based on the previous experimental research of Monica et al. (2016)

Assessment of the effects of methanol extract of C. chinensis using the 'hot plate' test method

The hot-plate test method used in this study was a modified protocol described by Ojewole (2007). The Ugobasile 35150 hot/cold plate apparatus with adjustable temperature was used. The temperature of the plate was regulated at 55±1 ^{o}C. Each mouse was placed in the glass beaker (on the hot plate) in order to obtain the animal's response to electrical heat-induced nociceptive pain (licking the forepaws and eventually jumping out of the glass beaker). Jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain. The time taken for each mouse to jump out of the beaker (reaction time) was noted and recorded. Capsicum extract was tested at doses of 1, 2.5, and 5.0 mg/kg i.p., respectively.

Assessment of the effects of methanol extract of C. chinensis using the cold allodynia test method

The Ugobasile 35150 hot/cold plate apparatus was set to and maintained at a temperature of 5 ±1^{o}C. Each mouse was placed on the plate in order to obtain the animal's response to cold pain behavior. In naive mice, the threshold for eliciting cold pain behavior is 5°C.

Assessment of the effects of methanol extract of C. chinensis using the Randall-Selitto paw pressure test

The nociceptive withdrawal threshold was assessed by using the Randall-Selitto

electronic analgesia meter. The 37215 Analgesy-meter is the classic device to perform Paw Pressure experiments according to the Randall-Selitto method. The force was applied to the animal paw, which was placed on a small plinth under a cone-shaped pusher with a rounded tip, without harming the animal.

Biochemical Assay

Immediately after the behavioral analysis, blood was collected via the retro-orbital plexus of the eye, and the plasma was separated by centrifugation at 800xg for 15min and kept at -80ºC for biochemical study. The serum level of tissue necrotic factor-α and interleukin-6 were measured according to Randox commercial ELISA kit protocol.

Statistical analysis

All the values were reported as Mean ±S.E.M (Standard error of the mean). The difference within the groups was compared using one-way analysis of variance (ANOVA) with post hoc test (Newman-Keul) for multiple comparisons where appropriate using Graph-Pad Prism software version 5. A level of $p<0.05$ was considered statistically significant for all tests at a 95% confidence interval level.

Results

Body weight of animals and fasting blood glucose of mice 72 hours post diabetic induction

Streptozotocin 60 mg/increased the fasting blood sugar significantly ($p < 0.05$) 72 hours after intra-peritoneal administration of MECC (Methanol extract of *Capsicum chinensis*), 1 and 5 mg/kg and GAB (Gabapentin), 75 mg/kg. There was a slight but insignificant decrease in body weight of the DNP (Diabetic neuropathy) group and MECC 5 mg/kg treated mice (Table 1).

Table 1: The body weight and fasting blood glucose of mice at 72 hours of streptozotocin-induced diabetes

Parameter	Normal control	Diabetic-neuropathy	MECC (1 mg/kg)	MECC (2.5 mg/kg)	MECC (5 mg/kg)	GAB (75 mg/kg)
Body Weight (g)	29 ± 1.9	26 ± 2.0	28 ± 1.0	28 ± 1.5	25 ± 1.0	28 ± 0.5
Fasting Blood Glucose (mg/dl)	75 ± 3.5	340 ± 8.8^{b}	469 ± 4.6^{e}	312 ± 6.2^{d}	410 ± 2.8^{e}	329 ± 2.4^{e}

Values were expressed as mean ± S.E.M for each group of five mice per group. The difference between the treated and the control group is significant at ($^{a}p < 0.05$, more significant at $^{b}p < 0.05$ and very significant at $^{c}p < 0.05$). The difference between the treated and the DNP untreated group is significant at ($^{d}p < 0.05$, more significant at $^{e}p < 0.05$, and very significant at $^{f}p < 0.05$).

Effect of MECC on latency period on the hot plate test method.

Capsicum chinensis methanol extract (1, 2.5, 5 mg/kg) produced a dose-dependent and significant ($p<0.05$) anti-hyperalgesic effect against thermal-induced nociceptive pain compared with the untreated group (negative control). The effect seen is also comparable with the standard drug (Gabapentin 75 mg/kg) GAB (Fig. 1).

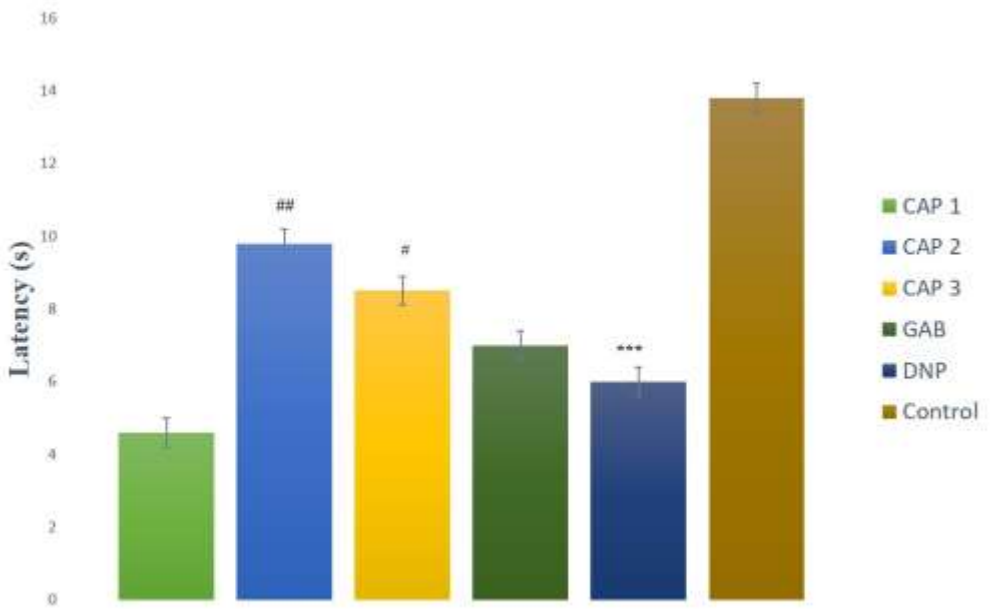

Figure 1: Showed the mean reaction time (in seconds) to thermal pain following treatment with MECC. Values were expressed as mean ± S.E.M;*n = 5*. The difference between the treated and the control group is significant at (*$p < 0.05$, more significant at **$p < 0.05$ and very significant at *$p < 0.05$). The difference between the treated and the DNP untreated group is significant at ($^{\#}p < 0.05$, more significant at $^{\#\#}p < 0.05$ and very significant at $^{\#\#\#}p < 0.05$). CAP – Capsaicin, GAB – Gabapentin 75 mg/kg, DNP – Diabetic Neuropathic Pain rats (negative control), CAP 1 (1 mg/kg), CAP 2 (2.5 mg/kg), CAP 3 (5 mg/kg).

Effect of MECC on reaction time using the cold plate method:

The effect of methanol extract on the reversal of allodynia was assessed. MECC significantly ($p < 0.05$) increased the reaction time on the cold plate compared to the negative control group. The result shows the group that received MECC (2.5 and 5 mg/kg) have a reaction time better than the positive control group (Fig. 2).

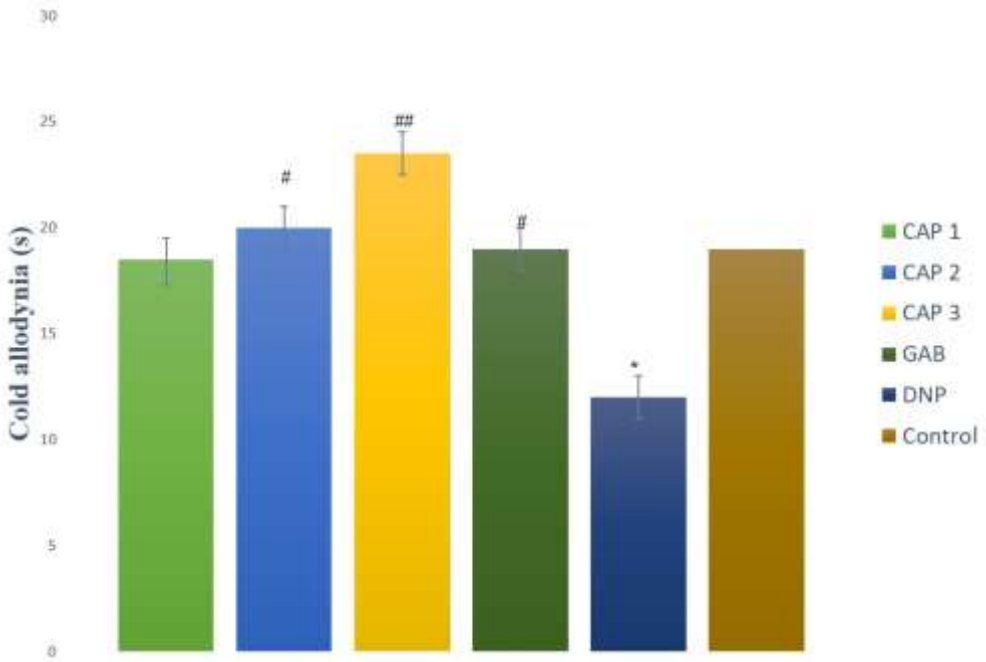

Figure 2: Effect of MECC on the mean reaction time on the cold plate. Values were expressed as mean ± S.E.M;n = 5. The difference between the treated and the control group is significant at (*$p < 0.05$, more significant at **$p < 0.05$ and very significant at *$p < 0.05$). The difference between the treated and the DNP untreated group is significant at ($^{\#}p < 0.05$, more significant at $^{\#\#}p < 0.05$ and very significant at $^{\#\#\#}p < 0.05$). CAP – Capsaicin, GAB – Gabapentin 75 mg/kg, DNP – Diabetic Neuropathic Pain mice (negative control), CAP 1 (1 mg/kg), CAP 2 (2.5 mg/kg), CAP 3 (5 mg/kg).

Effect of MECC on paw-pressure test

The effect of methanol extract on reducing hyperalgesia in diabetic neuropathic pain was evaluated using the Randall-Selitto paw pressure test. Methanol extract at a dose (5 mg/kg) showed a significant anti-hyperalgesic effect compared with the negative control and the standard drug (Fig. 3).

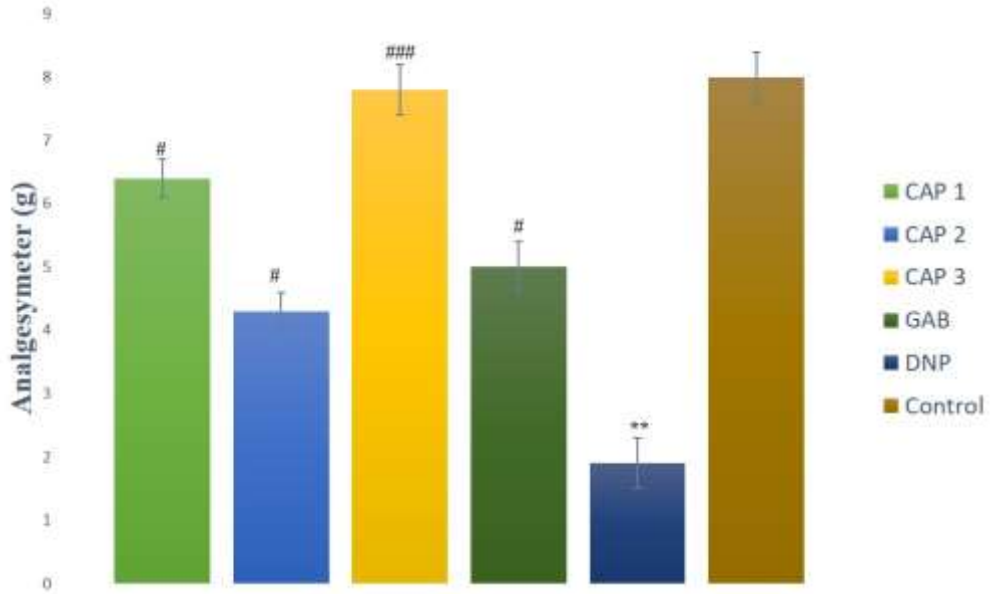

Figure 3: Effect of MECC on mechanical anti-hyperalgesic activity of paw pressure test. Values were expressed as mean ± S.E.M; n = 5. The difference between the treated and the control group is significant at (*$p < 0.05$, more significant at **$p < 0.05$ and very significant at *$p < 0.05$). The difference between the treated and the DNP untreated group is significant at ($^{\#}p < 0.05$, more significant at $^{\#\#}p < 0.05$ and very significant at $^{\#\#\#}p < 0.05$). CAP – Capsaicin, GAB – Gabapentin 75 mg/kg, DNP – Diabetic Neuropathic Pain mice (negative control), CAP 1 (1 mg/kg), CAP 2 (2.5 mg/kg), CAP 3 (5 mg/kg).

Effect of MECC on plasma level of tissue necrotic factor-α

Streptozotocin-induced diabetic neuropathy significantly produced a high level of TNF-α as shown in the negative control group compared to treatment groups receiving 2.5 and 5 mg/kg doses of MECC (fig. 4).

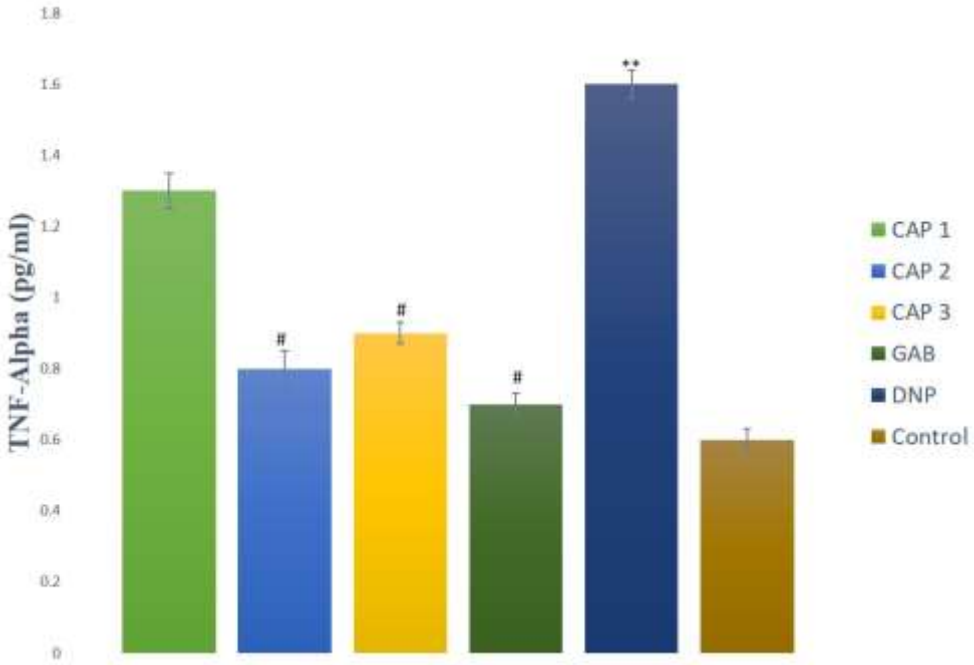

Figure 4: Effect of MECC on plasma level of tissue necrotic factor (TNF-α). Values were expressed as mean ± S.E.M; *n = 5*. The difference between the treated and the control group is significant at (*$p < 0.05$, more significant at **$p < 0.05$ and very significant at *$p < 0.05$). The difference between the treated and the DNP untreated group is significant at ($^{\#}p < 0.05$, more significant at $^{\#\#}p < 0.05$ and very significant at $^{\#\#\#}p < 0.05$). CAP – Capsaicin, GAB – Gabapentin 75 mg/kg, DNP – Diabetic Neuropathic Pain mice (negative control), CAP 1 (1 mg/kg), CAP 2 (2.5 mg/kg), CAP 3 (5 mg/kg).

Effect of MECC on plasma interleukine-6 level.

Diabetic neuropathy significantly increased the plasma level of interleukine-6 in the negative control group compared to groups receiving 2.5 mg/kg of MECC and gabapentin (Fig. 5).

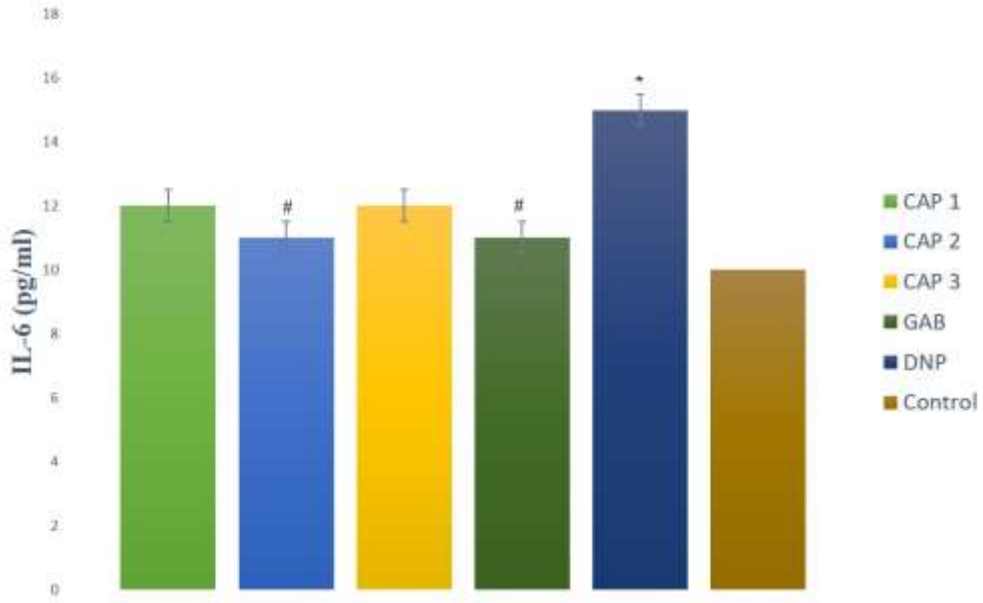

Figure 5: Effect of MECC on plasma level of interleukine-6 (IL-6). Values were expressed as mean ± S.E.M; *n = 5*. The difference between the treated and the control group is significant at (*p < 0.05, more significant at **p < 0.05 and very significant at *p < 0.05). The difference between the treated and the DNP untreated group is significant at ($^{\#}$p < 0.05, more significant at $^{\#\#}$p < 0.05 and very significant at $^{\#\#\#}$p < 0.05). CAP – Capsaicin, GAB – Gabapentin 75 mg/kg, DNP – Diabetic Neuropathic Pain mice (negative control), CAP 1 (1 mg/kg), CAP 2 (2.5 mg/kg), CAP 3 (5 mg/kg).

Discussion

Based on a statistical review, diabetes is the most common cause of neuropathy and is responsible for about 30% of neuropathic pain (Boulton et al. 2005). This evidence led to extensive studies on peripheral neuropathy using a streptozotocin-induced hyperglycemic model (Goud et al. 2015, Jorige and Annapurna 2015). In the current study, a streptozotocin-induced hyperglycemic model of peripheral neuropathy was used to evaluate the anti-hyperalgesic effect of capsaicin-containing natural fruit called *Capsicum chinensis*. The result obtained revealed a significant increase in the mean reaction time to hot plate-induced thermal pain and cold plate-induced allodynia in the treatment groups in a dose-dependent manner. Also, the methanol extract at 1, 2.5, and 5 mg/kg prolonged the reaction time of diabetic mice more than the positive control gabapentin (75 mg/kg). This suggests a potent analgesic effect of the methanol extract of *Capsicum chinensis* against diabetic neuropathy. The paw pressure test using an analgesymeter further confirms the anti-hyperalgesic effect of the extract. The extract also prolongs the nociceptive withdrawal threshold more than gabapentin when assessing their analgesic and anti-inflammatory effects with the Randall-Selitto method. The slow pain pathway transmits a

peripheral nociceptive signal through type C sensory nerve fibers via glutamate and substance P and transmits nociceptive information to laminar I and II (substantia gelatinosa). Laminar I zone is made up of interneurons that connect with other direct sensory output neurons to the higher center like the thalamus and cerebral cortex mainly through substance P. Laminar II which is subdivided into 3 zones transmits a nociceptive signal at the spinal cord level by glutamate, gamma-aminobutyric acid (GABA), and also partly by substance P (Todd 2010, Prescott et al. 2014, Peirs and Seal 2016). The positive control drug for this study (gabapentin) can bind to an allosteric site of the GABA receptor. The mechanisms of action of gabapentin and pregabalin are similar, they both selectively bind to pre-synaptic voltage-gated calcium channels containing the α2δ subunit in the brain and spinal cord, causing inhibition of the release of excitatory neurotransmitters. Although all primary afferent neurons are widely believed to be glutamatergic (West et al. 2015), it has been suggested that GABA-mediated inhibitory interactions may play a partial role in nociceptive processing at the level of the spinal cord [Du et al. 2017]. The partial role of GABA in nociceptive processing may be responsible for the lesser analgesic effect of gabapentin compared with the extract. Greater analgesic effect of MECC over gabapentin could be due to the abundant presence of capsaicin (69%), dihydrocapsaicin (22%), norhihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%) in the extract as reported by Pawar et al. (2011). Capsaicin is a pungent component of hot chili peppers (Moscone et al. 2007), with a greater affinity for vanilloid 1 receptors (Saleh et al. 2016). This receptor is a ligand-gated, non-selective cation channel, predominantly expressed on unmyelinated C nerve fibers which, after repeated exposure to topical capsicin, are depleted of their content of substance P and other neurotransmitters (Kulkantrakorn et al. 2013, Vinik et al. 2013). The C fibers depletion and desensitization reduce painful stimuli transmission from peripheral nerves to the central nervous system.

Apart from capsaicin derivatives, other reported phytochemicals in the *Capsicum chinensis* fruits include carotenoids (capsanthin, zeaxanthin, lutein, and beta-carotene) and xanthophylls (Kaulmann and Bohn 2014). The anti-inflammatory, antioxidant, and anti-apoptotic effects of carotenoids have been linked with their effects on transcription factors controlling gene expression and protein translation. Through the inhibition of nuclear factor κB translocation to the nucleus, carotenoids were able to interact with the nuclear factor κB pathway and thus inhibit the downstream production of inflammatory cytokines, such as interleukin-8 or prostaglandin E2 (Kaulmann and Bohn 2014). Decreased serum level of IL-6 and TNF-α observed in the MECC group suggests the attenuating effect of carotenoids in the extracton the genetic expression of IL-6 and TNF-α via the nuclear factor κB pathway. The anti-inflammatory results support the previous study of Zhang and Wang (2007), which showed the involvement of IL-6 and TNF-α in the process of pathological pain. The IL-6 has been shown to contribute to peripheral neuropathic pain after peripheral neuropathic injury (DeLeo, and Colburn 1995, Ramer et al. 1998). In addition, TNF-α has also been described to play an important role in neuropathic hyperalgesia and inflammation. Intra-plantar administration of TNF-α was shown to produce mechanical (Cunha et al. 1992) and thermal hyperalgesia (Perkins and Kelly 1994). It has been found that TNF-α injected into nerves induces Wallerian degeneration (Woolf et al. 1997, Creange et al. 1997) and generates the transient display of behaviors and endoneurial pathologies found in experimentally painful nerve injury (Wagner and Myers 1996).

Conclusion

Capsicum chinensis extract alleviates diabetes neuropathic pain associated with streptozotocin-induced diabetes in mice models via attenuation of inflammatory mediators.

Declarations

Conflict of interest: The authors declare no competing interests.

Ethical approval: All of the experiments were conducted according to the approved guidelines set by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), which is in agreement with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health. The ethical approval number assigned for animal use in this study by UI-ACUREC was UI-ACUREC/17/0097

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