ANTI-NOCICEPTIVE AND NEUROPROTECTIVE EFFECTS OF BROMELAIN IN CHRONIC CONSTRICTION INJURY-INDUCED NEUROPATHIC PAIN IN MALE WISTAR RATS

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APPROVAL PAGE

This thesis has been read and approved as having met the partial fulfilment of the requirement of the Department of Physiology and Postgraduate School, University of Ilorin, Ilorin, Nigeria, for the award of the degree of Doctor of Philosophy in Physiology.

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DECLARATION

I, Bakare Ahmed Olalekan (Matric Number 04/46KB077) hereby declare that this research was carried out under the supervision of Professor B.V. Owoyele in the Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria. The findings herein presented in this thesis emanated from my knowledge unless otherwise stated with appropriate referencing. This thesis has neither been submitted to any university nor is it before any other University for consideration for the award of a Doctor of Philosophy (PhD.) Degree in Physiology. Also, the research has been approved by the University of Ilorin Ethical Review Committee.

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DEDICATION

This project work is dedicated to my Mum, Mrs Bakare Afusat

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ABSTRACT

Neuropathic Pain (NP) is a burden in the society for which no effective treatment has been found. Bromelain is a group of endopeptidase found in pineapple that is commonly used for healing of wound and acute pain. The therapeutic effects of bromelain on NP remain unknown and its mechanisms of action are poorly understood. The study investigated the anti-nociceptive and neuroprotective effects of bromelain in chronic constriction injury (CCI)-induced NP in male Wistar rats. The objectives of the study were to: (i) determine the anti-nociceptive and neuroprotective effects of bromelain; (ii) investigate the effects of bromelain on comorbidities of NP; (iii) assess the anti-inflammatory and antioxidant effects of bromelain; and (iv) assess the effects of bromelain on sciatic nerve (SN) electrolytes level.

Sixty-four adult male Wistar rats randomly assigned into eight groups (n = 8) were used for the study. Three naïve groups (unligated, sham, and ligated) received 10.00 mg/kg per body weight (pbw) normal saline, reference group received 30.00 mg/kg pbw gabapentin. Two pretreatment (PrT) groups received 30 mg/kg pbw (low dose(LD)) and 50 mg/kg pbw (high dose(HD)) of bromelain. Likewise two post-treatment (PoT) groups received equivalent doses (LD and HD) of bromelain. CCI was used to induce peripheral neuropathy. Hotplate, tail-immersion and cold-plate test models were used to assess thermal hyperalgesia. Allodynia were assessed using von-Frey filaments test model while elevated-plus maze (EPM), and open-field test (OFT) models were used to assess anxiety-like behaviours. Oxidative-stress biomarkers, inflammatory-mediators, and electrolytes concentration were evaluated after twenty-one days of post-NP induction using spectrophotometry, Enzyme-Linked-Immunosorbent-Assay, and immunohistochemistry techniques. Data were analysed using both one-way and two-way analysis of variance, Bonferroni's and Tukey's *post-hoc* at $p < 0.05$.

The findings of the study were that:

i. There were increases ($p<0.05$) in the thermal threshold to hotplate stimulus in bromelain PrT (19.82 ± 0.43 vs 4.97 ± 0.37 sec.), bromelain PoT (6.32 ± 0.21 vs 1.63 ± 0.11 sec.) as well as cold-flick test in bromelain PrT and cold-plate test in bromelain PoT compared with ligated control group. Bromelain increased ($p<0.05$) the response-threshold to von Frey filament (47.25 ± 6.22 vs 5.25 ± 0.53 g);

ii. bromelain PrT increased ($p<0.05$) centre time duration (1.40 ± 0.34 vs 0.00 sec.) in the OFT. It increased the percentage of time duration (PTD) in open arms while it decreased PTD in close arms in the EPM test;

iii. bromelain increased ($p<0.05$) GSH, SOD and catalase enzymatic activities, reduced the concentration of nuclear factor kappa-B (15.92 ± 0.24 vs 20.28 ± 0.33 µg/ml/mg protein) and reduced the concentration of pro-inflammatory cytokines; and

iv. bromelain significantly ($p<0.05$) increased SN potassium (0.84 ± 0.02 vs 0.36 ± 0.05 mg/ml) and chloride (18.51 ± 0.43 vs 15.82 ± 0.23 mg/ml) ions concentration whereas it reduced sodium and calcium ions concentrations.

The study concluded that bromelain exhibited neuroprotective and antinociceptive effects in NP-induced *Wistar* rats via anti-inflammatory, antioxidant and electrolyte modulatory means. The study recommended that bromelain could be used for the treatment of NP and as a neuroprotective agent.