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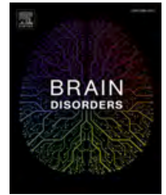

Effects of bromelain on striatal neuroinflammation in rat model of Parkinsonism

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ABSTRACT

Background: : Neuroinflammation alters the microenvironment in certain areas of the brain resulting in the development of Parkinson's disease (PD). Mechanisms involving cytokine and non-cytokine induced pathways are activated in the inflammatory response following injury. The proteolytic activity of bromelain appears to eliminate receptors on immune cells that respond to pro-inflammatory signals. This raises the question of whether manipulation of the inflammatory response pathways could lead to a beneficial intervention for PD. In this study, we investigated the effect of bromelain exposure on pro-inflammatory cytokine concentration and microglial activation in a parkinsonian rat model.

Methods: : Nigrostriatal dopamine neurons were lesioned by stereotaxic injection of the neurotoxin, 6-OHDA, into the medial forebrain bundle (MFB) of male Sprague-Dawley rats with weight ranging from 230- 250 g. The anti-inflammatory drug bromelain was used to treat a subset of the rats before or 24 h post 6-OHDA lesion. The systemic blood concentration of neutrophils and platelets was measured along with plasma and striatal concentrations of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α. In addition, the densities of CD11b and CD86 cells were differently quantified as a measure of glial cell activation.

Results: Pre-treatment with bromelain decreased the plasma concentration of neutrophils and platelets in 6-OHDA lesioned rats. The bromelain treatments (Pre and Post) decreased the plasma concentration of TNF-α and IL-1β. Pre-treatment with bromelain further resulted in the suppression of both systemic pro-inflammatory cytokines and microgliosis. Post-surgical bromelain treatment significantly resulted in the alleviation of systemic pro-inflammatory cytokines.

Conclusion: Early treatment with bromelain may slow the progression of PD by attenuating the inflammatory response associated with the disease. The present results suggest that bromelain may be considered for further clinical study and perhaps use as prophylactic treatment for patients with PD.

Introduction

Cascades of inflammatory reactions have been implicated in a variety of diseases affecting different organs of the body as well as the central nervous system [1,2]. During the inflammatory process, neutrophils express certain factors that promote the release of pro-inflammatory cytokines and activation of intrinsic pathways of coagulation that influence all aspects of thrombus formation including platelet activation and adhesion [3,4]. Although, the exact mechanism of the increase in platelet count is not known, it is however linked to inflammatory reactions and immune responses since the platelets contain a multitude of pro-inflammatory and immune-modulatory bioactive compounds [5]. Platelets were also reported to accumulate at an inflamed site and initiate an inflammatory cascade that attracts neutrophils by the local release of signal molecules [6]. In this view, neutrophils and platelets may represent an ideal and crucial link to explain inflammation as it occurs in a progressive neurodegenerative disease such as Parkinson's disease (PD).

Parkinson's disease is a neurodegenerative movement disorder characterized by the presence of oxidative stress, neuroinflammation and mitochondrial dysfunction [7]. The neuroinflammatory reactions in patients with PD and in MPTP-exposed animals have been linked to persistent microglial activation within the substantia nigra [8]. In the brains of patients diagnosed with PD, signs of inflammation were reported and were further characterized by glial activation as well as increased levels of proinflammatory cytokines [9,10]. Pro-inflammatory cytokines such as interleukin-1 β (IL-1β), interleukin-6 (IL-6) and tumour necrosis

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factor-alpha (TNF-α) were widely reported to mediate and facilitate both neural activities and inflammatory processes [10]. Proinflammatory cytokines like TNF-α and IL-6 were found to be increased in the substantia nigra pars compacta (SNpc) and in the cerebrospinal fluid of the patients with PD [11]. In addition, TNF-α, IL-1β and interferon-γ were reported to be increased along with activation of glial cells in a rodent model of PD [12]. The initial release of these pro-inflammatory cytokines was suggested to be primarily tissue protective but resulted in neuronal toxicity when the release became uncontrollable which was suspected to further exacerbate neurodegeneration [13]. Following these reports, treatment strategies targeting neuroinflammation may be beneficial to improve functional outcome in the progress of neurodegeneration in PD.

It has been shown that neuroinflammation is sustained by functionally activated microglia in both human and rat mid-brain sections containing substantia nigra [14] and microgliosis in turn enhances nigral destruction by promoting release of IL-1β and TNF-α [15,16]. Different therapeutic measures have been used as an alternative to exogenous administration of L-DOPA. However, these therapies were found to exacerbate the condition of the PD patients. Deep brain stimulation improved motor fluctuation and dyskinesia but was less effective in alleviating axial symptoms and dementia [17]. Radiofrequency lesioning as a measure of treatment for movement disorders as well as its limitations was as reported by Sinai at al. [18]. The reported incidence of significant gamma knife surgery and deep brain stimulation complications for PD vary considerably [18,19].

Immunotherapy is another curative option for PD which is yet to be fully explored. Neuroinflammation in PD is not responsive to dopamine repletion therapy but exacerbates dopaminergic neuron apoptosis [20, 21,22]. Bromelain is an anti-inflammatory drug containing cysteine proteases derived from pineapple stem that act through down-regulation of plasma kinninogen [23]. However, not much is known about the efficacy of bromelain in the treatment of neuroinflammation in neurodegenerative diseases such as PD. Therefore, for this study, we aimed to investigate the effect of timed bromelain treatment on levels of pro-inflammatory cytokines and microgliosis in a rat model of PD and sought to determine whether bromelain has potential as an early phase adjunct treatment for the disease.

Materials and methods

Animals and surgery

All animal experiments were performed according to the NIH guidelines for the care and use of laboratory animals and were approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (AREC/019/016D). Male Sprague-Dawley rats were housed under a 12 h light/dark cycle with free access to standard rat chow and water in the Biomedical Resource Unit of the University of KwaZulu-Natal. At postnatal day (PND) 51, the animals were divided into two major groups viz pre-surgery treatment with bromelain (40 mg/kg i/p), (Sigma-Aldrich, USA) for 7 days (n = 10) and presurgery saline treatment for 7 days (n = 30) animals. The dose of bromelain was chosen based on a previous report [24]. At PND 60, the rats were deeply anaesthetized with ketamine (90 mg/kg/i.p), (Bayer Pty Ltd, SA) and xylazine (5 mg/kg/i.p), (Intervet Pty Ltd, SA) was administered to stabilize systemic arterial pressure. The drugs were administered consecutively. Following testing to ensure that they were fully anaesthetised, the rats were positioned on a stereotaxic frame (Kopf Instruments, Tujunga, USA). The neurotoxin 6-OHDA (10 μg) was dissolved in normal saline (4 μL) containing 0.2% ascorbic acid (Sigma, St. Louis, MO, USA) was injected into the left medial forebrain bundle (MFB) (n= 30) using a Hamilton syringe and stereotaxic coordinates AP – 4.7, ML + 1.6, DV – 8.4 [25]. Control animals were injected with normal saline (4 μL) (n = 10). Following surgery, the animals were placed on a heating pad until recovery. This was followed by injection of Temgesic (0.05 mg/kg/s.c), (Reckitt Benckiser Ltd, UK) for pain relief. The animals were further subdivided into four groups viz: presurgery saline treatment followed by intra-MFB saline injection and post-surgery saline treatment (**Control**), presurgery saline treatment followed by intra-MFB 6-OHDA injection and post-surgery saline treatment (**6-OHDA**), presurgery bromelain treatment followed by intra-MFB 6-OHDA injection and post-surgical saline treatment (**Pre-Br**) and presurgery saline treatment followed by intra-MFB 6-OHDA injection and post-surgery bromelain daily treatment starting from 24 h after surgery for 7 days (**Post-Br**) (Table 1).

Animal sacrifice and tissue collection

A subset of the animals (6 per group) was randomly selected and sacrificed by decapitation 24 h after the last treatment with either bromelain or saline. A portion of the blood was collected in heparinized tubes and kept for further analysis while the remaining trunk blood was collected in separate heparinized tubes and centrifuged for 15 min at 1000 × g in a 4°C refrigerated centrifuge (HERMLE LABTECH, Germany) after which plasma was pipetted into eppendorff tubes. The brain was also removed immediately after decapitation and placed in a frozen 0.9% saline slush so as to suppress the degradation of brain structures during dissection. The striatum was dissected out, weighed and placed in eppendorff tubes. Then, both the plasma and the striatal tissues which were already placed in eppendorff tubes were further snap-frozen in liquid nitrogen before being stored in a -80°C bio-freezer.

The remaining subset of the animals (4 per group) were deeply anaesthetized with sodium pentobarbital (60 mg/kg, i.p.) before being transcardially perfused with cold PBS (1 ×) followed by 4% paraformaldehyde (PFA) freshly prepared in PBS (pH 7.4). The brains were removed from the skulls, post-fixed in 4% paraformaldehyde at 4°C for 24 h and then in 30% sucrose at 4°C for 48 h. This was followed by storage in a -80°C freezer until needed for CD11b and CD86 immunohistochemistry.

Determination of neutrophil and platelet counts

The total leukocyte and platelet count for each blood sample collected was determined by Coulter Counter and Analyzer ZM (Coulter Electronic Inc., Germany). A volume of 10 μL of blood was aliquoted from the heparinized blood samples and added to Isoton II diluent for erythrocyte lysis. The resultant cell volume was measured as the cells passed through the leukocyte aperture. Particles larger than 35 fL in the leukocyte apertures were counted as 'neutrohils' while the particles smaller than this were considered 'platelets' [26]. A Quality Assurance Program provided by Coulter Electronics showed that the measurements were valid.

TNF-α, IL-1β and IL-6 analysis

In order to ascertain the neurochemical basis for inflammatory reactions in both the brain and systemic environment, markers of neuroinflammation such as TNF-α, IL-1β and IL-6 were analysed in plasma and striatal tissue using a Sandwich-ELISA method (Elabscience Biotech. Texas, USA). The analysis protocol for TNF-α, IL-1β and IL-6 consisted of both extraction and quantification procedures. Both steps were conducted on the same day. The micro ELISA plate provided with each kit was pre-coated with antibody specific to TNF-α, IL-1β or IL-6 only in the samples. The striatal tissues were removed from the bio-freezer and allowed to thaw at room temperature. The tissues were rinsed and minced in ice cold PBS (1 mg of tissue/4 mL) and further homogenized in a sonicator (CML-4, Fischer, USA) before being centrifuged at 1160 × g for 10 min at 4°C. The supernatant was pipetted into new eppendorff tubes. The standard, control and the tissue samples were respectively pipetted into each well of the respective micro ELISA plate. The corresponding antiserum (100 μL) was added to each well and incubated for 1 h at room temperature. Following this, was the addition of corresponding conjugates (100 μL) to each well and incubation for 30 min at

Table 1.
Treatment of the four groups: pre-surgery saline treatment, saline injected with saline post-surgical treated rats (**control**); pre-surgery saline treatment, 6-OHDA injected with saline post-surgical treated rats (**6-OHDA**); pre-surgery bromelain treatment followed by 6-OHDA injection with saline post-surgical treated rats (**Pre-Br**); and pre-surgery saline treatment, 6-OHDA injected with post-surgical bromelain treatment starting from 24h after the surgery (Post-Br). n=10 per group.

Groups	Pre-surgical Bromelain Treatment	6-OHDA (10 μg/4 μL) Lesion	Post-surgical Bromelain Treatment
Control	-	-	-
6-OHDA	-	+	-
Pre-Br	+	+	-
Post-Br	-	+	+

room temperature. This was then followed by the addition of respective substrates (90 μL) to each well. The microplate was then incubated again at room temperature for 15 min. Following this incubation, the corresponding stop solution (50 μL) was added. The absorbance of each pro-inflammatory cytokine was quantified using a microtitre plate reader (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany) at a wavelength of 450 nm ± 2 nm within 10 min as per the manufacturer's protocol. All samples, standard and control were analysed in triplicate. The assay guidelines provided by the manufacturer were followed (Catalogue No: E-EL-R0012, R0017 and R0019).

Immunohistochemical staining

Sets of serial coronal sections (8 μm thickness) capturing the entire striatum were cut at -25°C on a cryostat (Leica VT 1000S; Leica Microsystems, Nussloch, Germany). Each slice containing the striatum was incubated in a well containing PBS (0.01 M, pH 7.4) for 10 min at room temperature. Following this, the brain slices were incubated in blocking buffer containing 5% BSA, 0.5% TritonTM X-100 for 1 h. The brain sections were immunolabelled with primary antibody for microglial activation using mouse anti-rat CD11b and CD86 (dil 1:100) (Biorad Lab. Inc.) before incubation at 4 °C for 24 h. After three rinses in PBS (10 min each), the sections were incubated for 1h at room temperature in secondary antibody (Goat anti-mouse) (1:200) (Biorad Lab. Inc.). After three rinses in PBS, the sections were incubated for 15 min at room temp in 0.04% peroxide ($H_2O_{2.}$ 30%) after which the sections were washed twice in PBS. For the colour reaction, the sections were put in a medium containing 0.05% 3, 3'-diaminobenzidine tetrachloride (Sigma, St Louis, MO, USA). Positive signals for glial cells were presented as brown granular masses. The transected rats' striatal slices were examined in a microscope Leica DM500 equipped with a Leica ICC50 HD digital-charge coupled device camera using Leica DC viewer software. The brain slices with the most striatal lesion were selected. The density of both CD 11b and CD 86 cells in each slice was measured using Image J software 1.42q (Wayne Rasband National Institutes of Health, USA). The mean cell density was calculated for each animal. The calculated mean was compared with that of the 6-OHDA injected and saline treated rats (6-OHDA). Sections from four animals were used for the analysis.

Statistical analysis

Data are displayed as mean ± SEM. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., USA). Data normality was assessed by the Kolmogorov-Smirnov test, and where the data met the requirement, the obtained outcomes were compared with the 6-OHDA injected rats that received saline post-surgical treatment (6-OHDA) using one factor analysis of variance followed by Bonferroni's post hoc test. Data that were not normally distributed were analyzed using the Kruskal-Wallis test followed by Dunns multiple comparison posthoc test. Effects were considered statistically significant at p value < 0.05.

Results

Neutrophil and platelet counts

One-way ANOVA showed that there was a significant difference between the treatment groups in the percentage neutrophil count ($F_{(3, 20)} = 3.445$, $p = 0.0363$). There was no significant difference between rats that received the 6-OHDA injection and those that received the equivalent saline injection (Fig. 1a). However, the percentage neutrophil count in the 6-OHDA injected rats that received pre-surgical bromelain treatment was significantly different from that of rats that received saline treatment (Pre-Br vs. 6-OHDA, $p < 0.05$, Fig. 1a). There was also a significant difference in the percentage platelet counts between the groups ($F_{(3, 20)} = 3.644$, $p = 0.0326$). The percentage platelet count was significantly decreased by pre-surgical bromelain treatment compared to saline treatment in the 6-OHDA-injected rats (Pre-Br vs. 6-OHDA, $p < 0.05$, Fig. 1b).

Pro-inflammatory cytokine concentration

TNF-α and IL-1β levels were measured in the experimental groups (control, 6-OHDA, Pre-Br and Post-Br). There was a significant difference in the plasma concentration of TNF-α between the groups as shown by one-way ANOVA ($F_{(3, 20)} = 9.443$, $p = 0.0004$). The TNF-α level was significantly increased by 6-OHDA injection in comparison to the saline injection *(6-OHDA vs. control), $p < 0.05$, Fig. 2a. The increase in the plasma level of TNF-α due to 6-OHDA injection was significantly attenuated by treatment with bromelain prior to 6-OHDA injection compared to saline treatment *(6-OHDA vs. Pre-Br), $p < 0.05$, Fig. 2(a). Also, TNF-α was significantly reduced by bromelain treatment at 24 h following 6-OHDA injection compared to saline treatment *(6-OHDA vs. Post-Br), $p < 0.05$, Fig. 2a.

Similarly, one-way ANOVA showed that there was a significant difference in the plasma concentration of IL-1β between the groups ($F_{(3, 20)} = 5.801$, $p = 0.0051$). The plasma level of IL-1β was significantly increased by 6-OHDA injection compared to saline injection *(control vs. 6-OHDA), $p < 0.05$, Fig. 2b. Meanwhile, the 6-OHDA-induced increase in the plasma level of IL-1β was attenuated by bromelain treatment prior to 6-OHDA injection *(Pre-Br vs. 6-OHDA), $p < 0.05$, Fig. 2 (b). Bromelain treatment following 6-OHDA injection also exerted a suppressing effect on the plasma level of IL-1β compared to saline treatment *(Post-Br vs. 6-OHDA), $p < 0.05$, Fig. 2(b). However, there was no significant effect of 6-OHDA or treatment with bromelain on the striatal concentration of IL-6 or TNF-α (Fig. 3a,b).

Striatal Immunohistochemistry

Immunohistochemical examination of striatal tissue showed ramified glial cells with spherical cell bodies as shown by CD11b and CD86 staining density in the control animals which became less ramified with thicker and swollen cell bodies following 6-OHDA injection. A significant increase in the density of CD11b was observed following the neurotoxin injection ($H = 11.27$, $p = 0.0103$, Kruskal-Wallis test, Fig. 4b). Also, the CD86 density was significantly increased after the

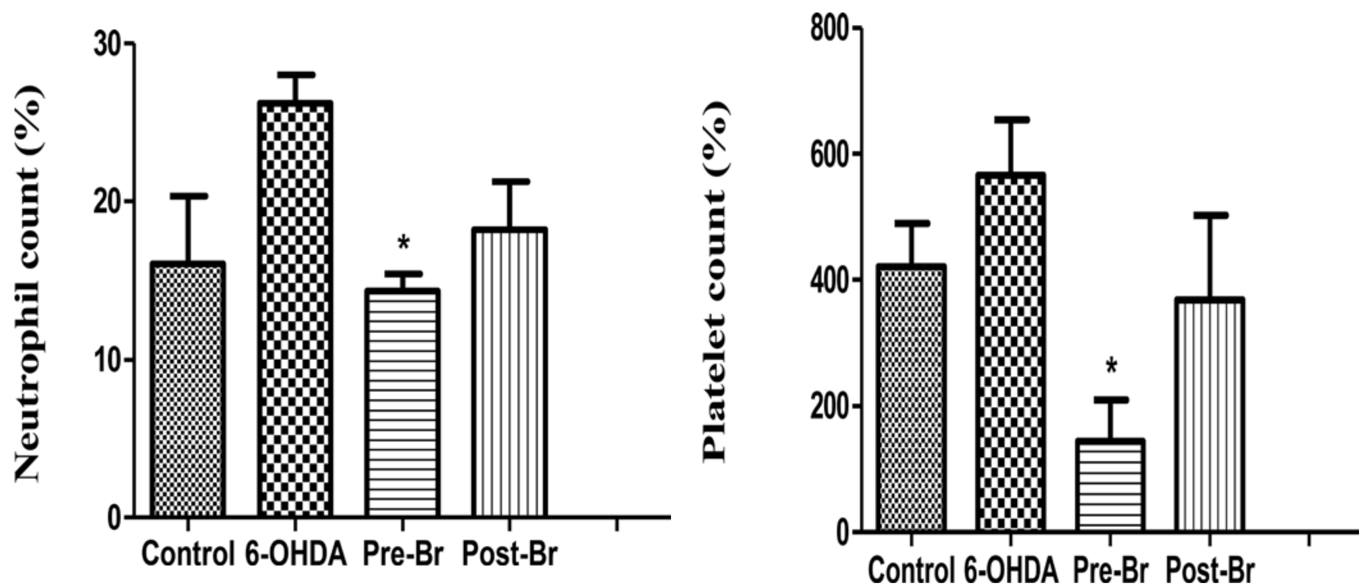

Fig. 1. a: Graph showing the % neutrophil count in the saline treated rats (control), pre-surgery saline treated, 6-OHDA injected rats (6-OHDA), bromelain pre-treated, 6-OHDA injected rats (Pre-Br) and 6-OHDA injected, post-surgery bromelain treated rats (Post-Br). $F_{(3, 20)} = 3.445$, $p = 0.0363$. *(6-OHDA vs. Pre-Br), $p < 0.05$. $n = 6$/group

Fig. 1b: Graph showing the % platelet count in the saline treated rats (Control), saline treated 6-OHDA injected rats (6-OHDA), bromelain pretreated, 6-OHDA injected rats (Pre-Br) and 6-OHDA injected, post-surgery bromelain treated rats (Post-Br). $F_{(3, 20)} = 3.644$, $p = 0.0326$.*(6-OHDA vs. Post-Br6), $p < 0.05$. $n = 6$/group.

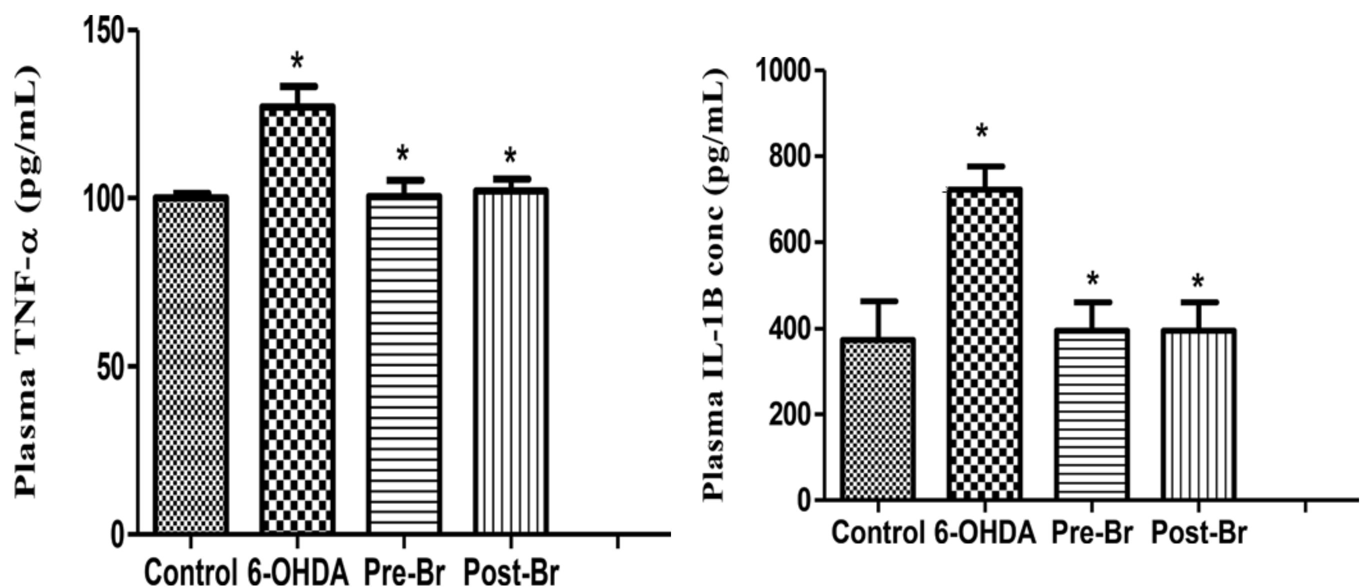

Fig. 2. a: Graph showing the effect of bromelain on plasma TNF-a conc. in the saline treated rats (Control), saline treated, 6-OHDA injected rats (6-OHDA), bromelain pretreated, 6-OHDA injected rats (Br6) and 6-OHDA injected, post-surgery bromelain treated rats (Pre-Br). $F_{(3, 20)} = 9.443$, $p = 0.0004$. *(Control vs. 6-OHDA) $p < 0.05$, *(6-OHDA vs. Pre-Br) $p < 0.05$, *(6-OHDA vs. Post-Br), $p < 0.05$. $n = 6$/group.

Fig. 2b: Graph showing the effect of bromelain on plasma IL-1b conc. in the saline treated rats (Control), saline treated, 6-OHDA injected rats (6-OHDA), bromelain pretreated, 6-OHDA injected rats (Pre-Br) and 6-OHDA injected, post-surgery bromelain treated rats (Post-Br). $F_{(3,20)} = 5.801$, $p = 0.0051$.*(Control vs. 6-OHDA), $p < 0.05$, *(6-OHDA vs. Pre-Br) $p < 0.05$, *(6-OHDA vs. Post-Br), $p < 0.05$. $n = 6$/group.

injection of 6-OHDA ($H = 10.88$, $p = 0.0124$, Kruskal-Wallis test, Fig. 4b) and observed to decrease in the pre-surgery bromelain treated rats in comparison with the saline injected rats, although not statistically significantly different (Pre-Br vs. 6-OHDA, $p = 0.056$, Fig. 4b).

Discussion

We investigated the effect of bromelain treatment on inflammatory markers in the plasma as well as markers of glial activation and neuroinflammation in the striatum following a unilateral 6-OHDA lesion in the rat medial forebrain bundle.

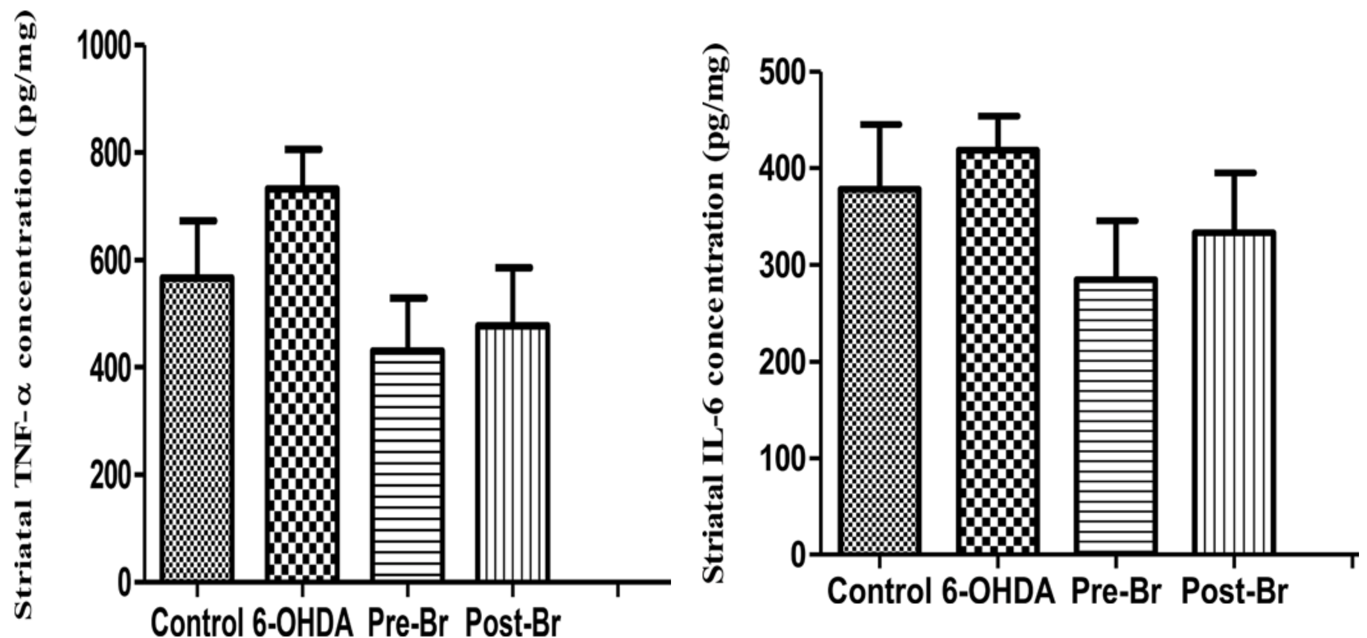

Fig. 3. a: The effects of bromelain on the striatal TNF-a conc. in the saline treated rats (Control), saline treated, 6-OHDA injected rats (6-OHDA), bromelain pretreated, 6-OHDA injected rats (Pre-Br) and 6-OHDA injected, post-surgery bromelain treated rats (Post-Br). $F_{(3, 20)} = 1.876$, $p = 0.1661$. n=6/group.
Fig. 3. b: The effect of bromelain on the striatal IL-6 conc. in the saline treated rats (Control), saline treated, 6-OHDA injected rats (6-OHDA), bromelain pretreated, 6-OHDA injected rats (Pre-Br) and 6-OHDA injected, post-surgery bromelain treated rats (Post-Br). $F_{(3, 20)} = 1.004$, $p = 0.5736$. n= 6/group.

Many studies showed that sustained inflammation plays an important role in the progression of PD [32]. Cytokines and chemokines, particularly TNF-α are considered the first line biomarkers that are activated concomitantly and may amplify systemic inflammation [27]. Also, TNF-α plays a key role in the inflammatory cytokine network, which can amplify the inflammatory reaction by activating the apoptotic pathway, promoting the synthesis and release of other cytokines which form a positive feedback [28]. Our study showed that plasma levels of TNF-α and IL-1β were increased in 6-OHDA injected rats compared with saline injected animals. This suggests that 6-OHDA can induce the synthesis and release of TNF-α and IL-1β in the systemic environment in the early stage of PD. The release of TNF-α and IL-1β are important mechanisms of neuropathogenesis of PD which is in close association with oxidative stress and suggested to be one of the consequences of the degeneration of dopaminergic neurons [29]. Oxidative stress via TNF-α signalling is accentuated and consequently initiates a vicious cycle of inflammation [30]. Inhibition of TNF-α yielded neuroprotection in rats [31]. Treatment with bromelain reduced plasma levels of TNF-α and IL-1β in the PD rat model. Thus, it is suggested that the beneficial effects of bromelain on the 6-OHDA induced PD rats are probably due, at least in part, to lowering the systemic levels of TNF-α and IL-1β. Perhaps, systemic up-regulation of TNF-α can technically be used as biomarkers for homeostatic disruption in PD since the pro-inflammatory cytokines are elevated during dopaminergic degeneration rather than waiting for completion of degeneration of dopaminergic nerves which would result in the manifestation of motor dysfunction at a later stage of the disease. In our study, the increase in plasma TNF-α may possibly involve the microglial signalling pathway to secrete pro-inflammatory cytokines. However, the systemic decrease in TNF-α with either pre-lesion bromelain treatment or post-lesion treatment may be considered to support the drug's ability to inhibit the synthesis and release of the proinflammatory cytokines at the early stage of PD.

Increase in neutrophil count contributes to oxidative stress, poor neurological outcomes and cognitive impairment and inflammation [32]. Platelets have been shown to adhere to neutrophils during the early phase of systemic inflammation [33]. Horvath and Ritz (2015) further provided evidence of an elevated granulocyte count in patients diagnosed with PD. The evidence suggested that there is a link between peripheral inflammation and PD in humans. This was further demonstrated in the animal model of PD. The administration of 6-OHDA caused an increase in the number of circulating neutrophil granulocytes [34] and the increase in the neutrophil count was attributed to the mobilization of immune cells from the lymphoid tissues and marginating pools. Our study showed that the percentage neutrophil count was reduced by bromelain treatment prior to 6-OHDA injection which suggests that the expected neutrophil recruitment and migration following the injection of the neurotoxin could have been inhibited by pre-surgical bromelain treatment as a result of the anti-inflammatory properties of the drug.

Cytokines and chemokines, particularly TNF-α, IL-6 and complement components after having passed through the blood brain barrier are considered the first line biomarkers that are activated concomitantly and may amplify systemic inflammation [35]. Along with the elevated production of the inflammatory mediators is the microglial activation which is also a key event in 6-OHDA-induced Parkinsonism in rats [36,37]. The neurotoxin (6-OHDA) accumulates in the brain where it is oxidized to generate reactive oxygen species and ultimately causes oxidative cytotoxicity [38]. Our result shows that the cell density of the glial markers (CD11b and CD86) is increased in the striatum after the 6-OHDA injection. However, the cells are observed thicker and swollen suggesting that the cells are may not have been totally activated since the expected features of glial cells were not totally observable. In contrast, the evidence provided by Blum at al. [39] showed that strong microglial reactivity can be observed in the rat's striatum in the lesioned hemisphere from 2 days after 6-OHDA injection. The larger size of the ramified cells in response to 6-OHDA injection in our result suggests that the 6-OHDA lesion rat model of PD allows for a progressive degeneration of the striatal neurons since the features of the observed glial cells are not in conformity with resting cells but rather portrays the PD pathogenesis.

Our findings show that the enhanced release of pro-inflammatory cytokines occurs concurrently with the onset of glial activation. Our results further show the up-regulation of systemic neutrophils, IL-1β and TNF-α with greater density of less ramified glia markers after 6-OHDA injection. Bromelain treatment after 6-OHDA injection reduces the density of glia marker (CD11b); although not statistically significant.

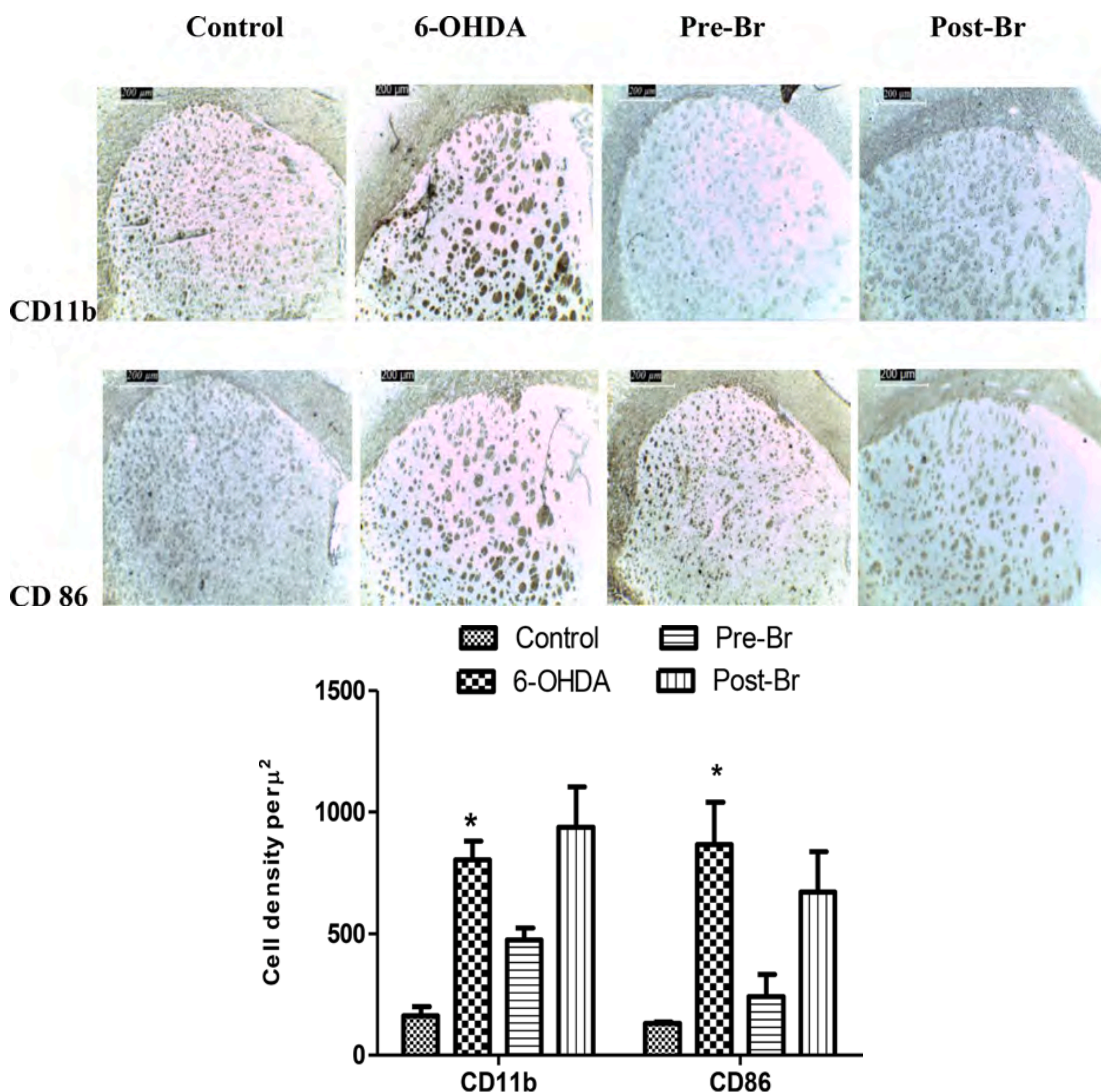

Fig. 4. a: Glial cell immunohistochemistry showing the striatal CD11B and CD86 in the pre-surgery saline treated, saline injected with saline post- surgery treatment (**Control),** pre-surgery saline treatment, followed by 6-OHDA injection with post-surgery saline treated rats **(6-OHDA),** pre-surgical daily bromelain treatment followed by 6-OHDA injection, with post-surgery saline treated rats (**Pre-Br**) and pre-surgery saline treated rats, with 6-OHDA injection and post-surgical bromelain daily treatment starting at 24hr after surgery (**Post-Br**). (Scale bars, 200 μm).

Fig. 4b: Graph showing the effect of bromelain on the density of CD11b and CD86 in the pre-surgery saline treated, saline injected with saline post-surgery treatment (control), pre-surgery saline treatment, followed by 6-OHDA injection with post-surgery saline treated rats (6-OHDA), pre-surgical daily bromelain treatment followed by 6-OHDA injection, with post-surgery saline treated rats (Pre-Br) and pre-surgery saline treated rats with 6-OHDA injection and post-surgical bromelain daily treatment starting at 24 hr after surgery (Post-Br). CD11b and CD86 cell densities were significantly increased by 6-OHDA injection (Kruskal-Wallis test, $F= 11.27$ followed by Dunn's post hoc test, $p = 0.0103$) and (Kruskal-Wallis test, $F = 10.88$ followed by Dunn's post hoc, $p = 0.0124$) respectively.

However, the treatment with bromelain prior to the injection of the neurotoxin significantly reduces the density of the glia marker (CD86). This further suggests that bromelain may provide prophylactic therapy for neuroinflammation in PD.

Conclusion

Changes in pro-inflammatory cytokines and phenotypic size of glial cells were demonstrated in rats following 6-OHDA lesion of the medial forebrain bundle. The increase in systemic levels of pro-inflammatory cytokines as a result of the lesion suggests neurotoxin induced inflammatory changes. Our study provides evidence to suggest that bromelain reverses the increase in cytokines and may exert its prophylactic effect by attenuating inflammation mediated neuronal degeneration in the 6-OHDA rat model of PD.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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