Proceding Paper

Modulatory Action of Phenolic-Enriched *Combretum paniculatum* Vent Ethanolic Extract on Oxidoinflammatory Anomalies in Experimental Animals †

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Abstract: Medicinal plants with favorable therapeutic effects have gained interest over conventional drugs in the treatment of oxidative stress and inflammatory-mediated diseases. The antioxidant and anti-inflammatory activities of *Combretum paniculatum* ethanolic extract (CPEE) were investigated in this study using *in vitro* and *in vivo* analyses. The results of phytochemical screening, recorded in mg/100 g, revealed that CPEE is phenolic-rich and also contains a high abundance of alkaloids, reducing sugars, and flavonoids. Terpenoids and tannins were recorded in moderate quantities. Our *in vitro* analysis revealed that CPEE inhibited nitric oxide, phospholipase A2, and thiobarbituric-acid-reactive substance activities, with half-maximal inhibitory concentration (IC50) values of 6.55, 361.1 and 2.28 µg/mL, respectively. Furthermore, the *in vivo* study showed that the implantation of cotton pellets elicited increases in granuloma tissue formation and the level of malondialdehyde (MDA) while decreasing the activities of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in the untreated groups compared to normal rats. Interestingly, the groups treated with 100 and 200 mg/kg of CPEE had decreased granuloma tissue, and MDA, with an increase in the activities of SOD, CAT, and GSH. These findings suggest that CPEE ameliorated chronic inflammatory-induced oxidative stress in the experimental animals. Thus, it could be applied as an effective remedy for the development of antioxidant and anti-inflammatory drugs.

Keywords: antioxidant; inflammation; medicinal plants; oxidative stress; phenols and phytochemicals

1. Introduction

Inflammation is a physiological process with the role of destroying injurious agents and harmful stimuli [1]. Based on the duration and mediators mobilized, inflammation can be acute or chronic. However, chronic inflammation leads to the unregulated release of activated mediators, cells, and oxidant species implicated in the pathogenesis of several diseases [2]. Consequently, several orthodox anti-inflammatory agents are prescribed in clinical settings to modulate inflammation [1]. Regrettably, most of these drugs relieve symptoms transiently and have severe side effects on the liver, gastrointestinal tract, and kidneys [3].

The current search for medicinal plants with favorable therapeutic effects in the treatment of oxidative stress and inflammatory-mediated diseases is due to their safety profiles, availability, biocompatibility, and multiple targeted approaches. *Combretum paniculatum* is a flowering plant in the *Combretaceae* family used as a treatment option for pain, dysentery, and enlarged liver, as well as an anti-cancer, antimicrobial, and anti-diarrhea agent [4]. This study investigated the extract’s efficacy in modulating oxidative stress and inflammatory anomalies using *in vitro* and *in vivo* approaches.
2. Materials and Methods

2.1. Plant Collection, Preparation, and Extraction

Fresh leaves of *C. paniculatum* were shade-dried, pulverized, and extracted with 2 L of ethanol (70%) for 72 h. The macerate was filtered with Whatman filter paper and concentrated with a rotary evaporator to obtain the *C. paniculatum* ethanol extract (CPEE) used for this research.

2.2. Chemicals and Reagents

In this study, we procured analytical-grade chemicals from the following companies: Sigma-Aldrich Inc., Gillingham, UK; Teco, Tampa, FL, USA, British Drug Houses (BDH), London, UK; and Evans Pharmaceutical, Newport, UK.

2.3. Phytochemical Screening, In Vitro Antioxidant and Anti-Inflammatory Activities

The amount of phytochemicals in the CPEE was estimated with the Harbone [5] method. The inhibitory effects of CPEE on nitric oxidescavenging activity, lipid peroxidation, and phospholipase A2 activity were investigated with the methods of Sreejayan and Rao [6], Banerjee et al. [7], and Vane [8], respectively.

2.4. Induction of Inflammation

This experiment was performed using twenty-five Wistar rats randomized into five groups (*n* = 5) according to the protocol described by Mosquera et al. [9]. The rats in group 1 served as the baseline (no induction or treatment); group 2 rats were subjected to implantation and given distilled water; the standard drug diclofenac sodium (100 mg/kg b.w.) was given to group 3; and groups 4–5 were treated with 100 and 200 mg/kg b.w. of CPEE, respectively. The treatment period lasted for seven days, and on the eighth day, the pellets were removed, and blood samples for the measurement of biochemical parameters were collected. Ethical approval of the study with the approval number UNN/FBS/EC/1082 was obtained from the Nigeria Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria.

2.5. Biochemical Parameters

The following biochemical parameters were measured in the serum: The MDA level, activities of SOD and CAT, and the levels of GSH and vitamins E and C using standard methods, as reported by Chukwuma et al. [10].

2.6. Statistical Analysis

Data were analyzed using GraphPad Prism version 6.5 (GraphPad Software, Inc., San Diego, CA, USA), and the results were presented as the mean ± S.D. Data were considered statistically significant when *p* < 0.05, **p** < 0.001, and ***p*** < 0.001.

3. Results and Discussion

3.1. Quantitative Phytochemical Screening of the CPEE

The use of plants as therapeutic agents started during human evolution due to the presence of phytochemicals that have significant biological and pharmaceutical actions [11–13]. This study recorded a high abundance of phenols, alkaloids, flavonoids, and reducing sugars with moderate amounts of tannins and terpenoids in CPEE. (Table 1). These phytochemicals that were identified in the extract have proven to be effective as antioxidant, anti-inflammatory, immunomodulatory, neuroprotective, and anti-diabetic agents [13]. Hence, their presence in the extract suggests that it could have a plethora of activities against the aforementioned diseases.
Quantitative phytochemical screening of the CPEE.

Table 1. Quantitative phytochemical screening of the CPEE.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemicals</th>
<th>Amount (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phenols</td>
<td>2711.02 ± 60.66</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>21.12 ± 0.41</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>49.00 ± 6.74</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>605.83 ± 10.10</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
<td>12.17 ± 0.55</td>
</tr>
<tr>
<td>7</td>
<td>Reducing sugar</td>
<td>57.03 ± 0.12</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>2.59 ± 0.82</td>
</tr>
</tbody>
</table>

Values are recorded as mean ± SD of triplicate experiments at the 95% confidence interval.

3.2. Antioxidant and Anti-Inflammatory Activities of CPEE

The results of the in vitro studies revealed that the extract demonstrated potent antioxidant and anti-inflammatory potential by inhibiting NO, TBARS, and PLA2, as shown in Figure 1. Natural products with antioxidant activity can mop up excess oxidant species and regulate inflammatory responses [2]. The high antioxidant and anti-inflammatory activities of CPEE indicate that it adopts a multi-therapeutic approach to avert cellular damage under oxidative stress and abrogated inflammatory responses.

3.3. Effect of CPEE on Wet and Dry Granuloma Tissue Weight

Cotton pellet implantation elicits the proliferation of neutrophils, fibroblasts, and macrophages, leading to granuloma tissue formation to ward off the external agent, i.e., the cotton pellet [1]. Herein, treatment with varied doses of CPEE (100 and 200 mg/kg b.w.) significantly inhibited granuloma tissue formation (p < 0.05) compared with the untreated group 2 (Figure 2). The inhibition of granuloma formation suggested that the extract might have reduced the angiogenesis, collagen synthesis, and excessive exudation of inflammatory cytokines, which limited the amount of granuloma formed [1].

3.4. Effects of CPEE on Lipid Peroxidation and Antioxidant Markers

Oxidative stress and inflammation are closely related pathophysiological processes implicated in the etiology and pathogenesis of several diseases. In this study, CPEE demonstrated an in vivo antioxidant effect by inhibiting the inflammatory-mediated peroxidation of the biomembrane, as evidenced by the significant (p < 0.06) decrease in the MDA level in the treated groups relative to group 2, given distilled water after cotton pellet implantation. Moreover, it restored the activities of SOD and CAT, as well as the levels of GSH and vitamins E and C (Table 2). This pharmaceutical activity validates the antioxidant potency of the extract. The inhibition of MDA and restoration of antioxidant markers are highly beneficial in preserving tissue and cellular integrity [10]. These actions could result from the high amounts of phenols and other antioxidant phytochemicals in the extract. Phenols
attenuate oxidative damage by scavenging and chelating radical species, activating the expression of endogenous antioxidants, and terminating the peroxidation reaction [12].

**Figure 2.** Effect of CPEE on wet and dry granuloma tissue weight. The values are presented as the mean ± S.D. (n = 5). Group 1 was subjected to implantation and given distilled water, and groups 2–4 were given diclofenac sodium (100 mg/kg b.w.) and 100 and 200 mg/kg b.w. of CPEE, respectively.

**Table 2.** Effects of CPEE on lipid peroxidation and antioxidant markers.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (mg/dL)</th>
<th>SOD (iU/L)</th>
<th>CAT (iU/L)</th>
<th>GSH (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.11 ± 0.45</td>
<td>11.22 ± 0.21</td>
<td>1.48 ± 0.24</td>
<td>0.60 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>4.81 ± 0.01</td>
<td>9.30 ± 0.12</td>
<td>0.19 ± 0.03</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.90 ± 0.07</td>
<td>11.36 ± 0.04</td>
<td>0.10 ± 0.10</td>
<td>0.47 ± 0.09</td>
</tr>
<tr>
<td>4</td>
<td>2.12 ± 0.15</td>
<td>11.16 ± 0.19</td>
<td>1.19 ± 0.28</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.96 ± 0.84</td>
<td>11.23 ± 0.02</td>
<td>1.24 ± 0.35</td>
<td>0.45 ± 0.01</td>
</tr>
</tbody>
</table>

The values are presented as the mean ± S.D. (n = 5). Group 1 was subjected to implantation and given distilled water, and groups 2–4 were given diclofenac sodium (100 mg/kg b.w.) and 100 and 200 mg/kg b.w. of CPEE, respectively.

4. Conclusions

The findings of this study show that CPEE ameliorated chronic inflammatory-induced oxidative stress in the experimental animals. This pharmaceutical action could be attributed to the rich phytoconstituents identified in the extract. Thus, the leaves of *C. paniculatum* could be employed as an effective remedy for developing antioxidant and anti-inflammatory drugs.

**Supplementary Materials:** The presentation material of this work is available online at https://www.mdpi.com/article/10.3390/ECB2023-14085/s1.

**Author Contributions:** Conceptualization, I.F.C., F.N.N. and V.O.A.; Methodology, I.F.C., F.N.N. and V.O.A.; Software, I.F.C. and V.O.A.; Validation, I.F.C.; Formal analysis I.F.C. and V.O.A.; Investigation, I.F.C., F.N.N. and V.O.A.; Data curation, I.F.C.; Writing—Original draft preparation, I.F.C., F.N.N. and V.O.A.; Writing—review and editing, I.F.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References


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