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A Single-Dose Treatment of *Alstonia boonei* Stem Bark Extract Elicits Anti-inflammatory Effect *in vivo*

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INTRODUCTION & AIM

- Although beneficial to maintaining normal physiologic processes, inflammation could trigger or aggravate the course of many disease conditions when prolonged.
- Natural products possess significant pharmacological activities and minimal toxicity, establishing them as highly promising resources for therapeutic applications (Deng et al., 2022).
- Numerous studies have demonstrated the anti-inflammatory properties of *Alstonia boonei* (Enechi et al., 2013; Okoye et al., 2014; Olanlokun et al., 2021); however, most of these investigations employed multiple dosing regimens.
- This study aimed to evaluate the impact of a single dose of *Alstonia boonei* stem bark on lipopolysaccharide-induced inflammation in Wistar albino rats.

METHOD

Extraction of the Plant Sample
The air-dried and pulverized *A. boonei* stem bark was defatted using n-hexane and then extracted with methanol using a Soxhlet extractor. The resulting filtrate was evaporated *in vacuo* at 20°C to obtain the crude methanol extract.

Induction of Inflammation
Wister albino rats (70-100 g) were held in standard conditions in the Animal Facility of the Department of Biochemistry, University of Nigeria, Nsukka and were provided with standard laboratory food and water *ad libitum*. Induction of inflammation was done following the protocol of Abdallah *et al*. (2020) with some modifications. Twenty Wistar albino rats were divided into 5 subgroups of 4 rats each. Inflammation was induced in groups 1-4 by an intraperitoneal injection of (10 mg/kg b.wt.) lipopolysaccharide (LPS) solution.

Group 1 (AB_{250}): LPS + 250 mg/kg b.w. of *A. boonei* extract.
Group 2 (AB_{500}): LPS + 500 mg/kg b.w. of *A. boonei* extract.
Group 3: LPS + 100 mg/kg b.w. Ibuprofen (Standard control).
Group 4: LPS + 0.5 ml distilled water.
Group 5: Normal control (No inflammation).

After six (6) hours of LPS injection, blood was collected from the animals for biochemical analyses.

Biochemical Analysis
The quantitative sandwich enzyme-linked immunosorbent Assay (ELISA) technique was used to determine levels of the inflammatory markers (IL-1β, IL-6, and NFKB-p65) in the blood samples using the corresponding ELISA test kits (Elabscience Biotechnology Co. Ltd., USA).

Statistical Analysis
The data obtained were analyzed using the Statistical Product and Service Solutions (SPSS version 23.0) and expressed as mean ± SEM values. The statistical significance of the difference in mean values was determined using a one-way analysis of variance (ANOVA) with Duncan's multiple comparison test for post-hoc analysis. A probability value of $p < 0.05$ was used as the criterion for statistical significance.

RESULTS & DISCUSSION

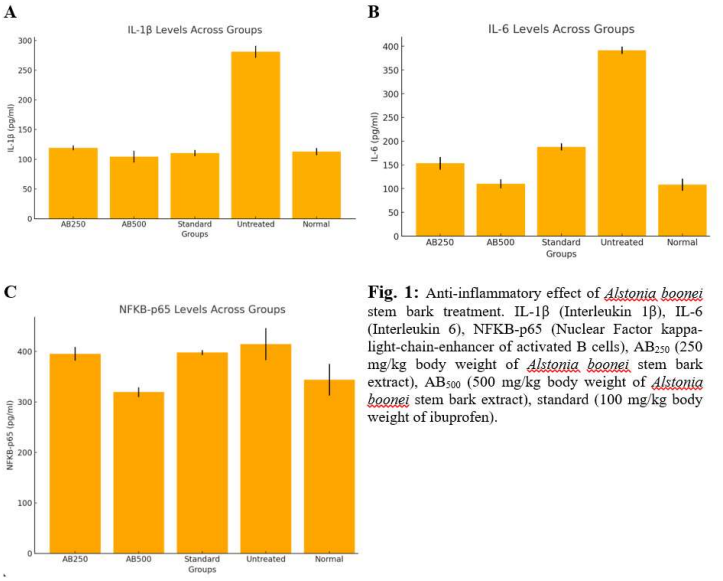

Fig. 1: Anti-inflammatory effect of *Alstonia boonei* stem bark treatment. IL-1β (Interleukin 1β), IL-6 (Interleukin 6), NFKB-p65 (Nuclear Factor kappa-light-chain-enhancer of activated B cells), AB_{250} (250 mg/kg body weight of *Alstonia boonei* stem bark extract), AB_{500} (500 mg/kg body weight of *Alstonia boonei* stem bark extract), standard (100 mg/kg body weight of ibuprofen).

The results presented in Figure 1 illustrate the impact of *A. boonei* stem bark treatment on rats induced with LPS. The findings reveal that LPS induction caused significant ($p < 0.05$) increases in the levels of IL-1β, IL-6, and NFKB-p65 in the untreated group compared to the normal control. LPS is a well-established inducer of inflammation through the activation of Toll-like receptor 4 (TLR4), which triggers downstream signaling pathways, including the nuclear factor kappa B (NF-κB) pathway. This activation leads to the production of pro-inflammatory cytokines such as IL-1β and IL-6, which amplify the inflammatory response (Akira et al., 2006). The significant elevation of IL-1β, IL-6, and NFKB-p65 in the untreated group confirms the successful induction of an inflammatory state by LPS.

In contrast, treatment with the extract resulted in significant ($p < 0.05$) reductions in the levels of IL-1β and IL-6 in the treatment groups, although there was no significant change observed in NFKB-p65 levels compared to the untreated group. The observed reductions in IL-1β and IL-6 suggest that the single dose of the extract has potent anti-inflammatory properties, likely targeting immediate cytokine production. These results are consistent with studies showing rapid cytokine modulation following the administration of plant-based anti-inflammatory agents (Williamson et al., 2013; Xu et al., 2018).
The unchanged NF-κB-p65 levels indicate that the extract's mechanism may bypass upstream transcription factors and directly target cytokine release.

CONCLUSION

- The results demonstrate that a single dose of *A. boonei* stem bark extract effectively reduces acute inflammation markers like IL-1β and IL-6. However, the lack of significant impact on NF-κB-p65 levels suggests that multiple dosing regimens may be required to achieve broader anti-inflammatory effects, particularly in chronic conditions.

FUTURE WORK / REFERENCES

- Xu, H., Zhou, Y., & Fu, X. (2018). Inflammasome activation and IL-1β release in inflammatory diseases: Molecular mechanisms and therapeutic strategies. *Journal of Immunology Research*, 2018.
- Williamson, E. M., Lorenc, A., Booker, A., & Robinson, N. (2013). The rise of traditional Chinese medicine and the need for evidence-based evaluation. *British Journal of Clinical Pharmacology*, 75(5), 949-963.
- Enechi, O., Odo, C., & Onyekwelu, O. (2013). Inhibition of leukocyte migration: A mechanism of the anti-inflammatory effect of the ethanol extract of the stem bark of *Alstonia boonei* in Wistar rats. *Journal of Pharmacy Research*, *6*, 925-827.
- Olanlokun, J. O., Olowofolahan, A. O., Bodele, O., Adegbuyi, A. T., Prinsloo, G., Steenkamp, P., & Olorunsogo, O. O. (2021). Anti-inflammatory potentials of the n-hexane fraction of *Alstonia boonei* stem bark in lipopolysaccharide-induced inflammation in Wistar rats. *Journal of Inflammation Research*, *14*, 3905-3920.