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Immunomodulatory effects of *Sceletium tortuosum* (Trimesemine™) elucidated *in vitro*: Implications for chronic disease

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ABSTRACT

Ethnopharmacological relevance: *Sceletium tortuosum*, among other *Sceletium* species, was traditionally used by the Khoisan people of Southern Africa for relief of pain-related ailments. However, the commercial availability of this supplement has greatly expanded due to anecdotal claims of its mood-elevating and anxiolytic properties. Unrelated research has elucidated a significant link between cytokines and the mediation of depression. Therefore, the effect of *Sceletium* supplementation on immune cell functionality is of interest, since the efficacy of potential depression treatments could, at least in part, rely on downregulation of pro-inflammatory signalling.
Aim of the study: The current study evaluated the immunomodulatory effects of a *Sceletium* extract, both basally and in the context of acute endotoxin stimulation.
Materials and methods: Primary human monocytes were supplemented with either a 0.01 mg/ml or 1 mg/ml *Sceletium* extract dose, with or without E. *coli* LPS stimulation *in vitro,* for 24 h. Mitochondrial viability, as an indirect measure of cytotoxicity, and cytokine release in response to the treatment intervention were assessed.
Results: *Sceletium* extract treatment was associated with increased mitochondrial viability, as well as up-regulated IL-10 release under basal conditions. LPS exposure significantly decreased mitochondrial viability, but this was prevented completely under *Sceletium*-treated conditions. The acute inflammatory response to LPS stimulation was not negatively affected. *Sceletium* treatment conferred most significant effects at a dose of 0.01 mg/ml.
Conclusions: *Sceletium* exerts significant cytoprotective effects in the setting of endotoxin stimulation. Cytokine assessment indicated that *Sceletium* possesses mild anti-inflammatory properties, but does not hinder the mounting of an adequate immune response to acute immune challenge. These findings indicate that *Sceletium* may be beneficial for the attenuation of cytokine-induced depression, as well as in systemic low-grade inflammation.

1. Introduction

According to the latest World Health Organisation statistics (WHO, 2017), an estimated 4% of the global population is afflicted with depression. With this number ever increasing, the search for new and effective ways to address the symptoms, and modulate both central and peripheral maladaptations to stress-related conditions, remains one of the most important focus areas in research. Although medications are currently available for the management of depressive conditions, less than half (and in many developing countries, less than 10%) of the afflicted population receive such treatments.

In South Africa, as in many developing countries, general practitioners are largely outnumbered by traditional healers. Therefore, it is not surprising that a tendency exists for patients to turn to traditional plant and herbal remedies for the treatment of ailments and disorders (Morris, 2001). Thus, the effectiveness of natural medicines with potential anti-stress capacity has been the focus of several research groups for some time, and one candidate which has shown great potential in this setting is *Sceletium tortuosum*.

Traditionally, *Sceletium tortuosum,* among other *Sceletium* species, was used by the Khoisan people of Southern Africa for the relief of toothache and stomach pain (Harvey et al., 2011; Loria et al., 2014; Murbach et al., 2014; Patnala and Kanfer, 2013), and the commercial availability of this herbal supplement has increased considerably as a result of anecdotal claims of its mood-elevating and anxiolytic properties (Shikanga et al., 2012). However, this commercial expansion is

Abbreviations: 5-HT, 5-Hydroxytryptamine; ANOVA, Analysis of Variance; DPBS, Dulbecco's Phosphate Buffered Saline; ELISA, Enzyme-Linked Immunosorbent Assay; FBS, Foetal Bovine Serum; HBSS, Hank's Balanced Salt Solution; IDO, Indoleamine 2,3 dioxygenase; IFN-γ, Interferon-Gamma; IL-1, IL-6 etc., Interleukin-1, interleukin-6 Etc.; LPS, Lipopolysaccharide; MCP-1, Monocyte Chemotactic Protein-1;; PBMCs, Peripheral Blood Mononuclear Cell(s); RPMI media, Roswell Park Memorial Institute media; SEMs, Standard Error of the Mean(s); TNF-α, Tumour Necrosis Factor-Alpha; Tri, Trimesemine™; XTT, 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl] − 2H-tetrazolium-5-carboxanilide

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associated with increasing concerns relating to the quality of, and consistency across available *Sceletium* products.

It is important to note that the alkaloidal composition of a supplement is a key determinant of both its beneficial and adverse effects (Nell et al., 2013). This being said, the alkaloidal composition of raw *Sceletium* is complex, and further complicated by post-harvesting interventions which have shown to alter plant composition across the majority of herbal supplements (Smith, 2011). *Sceletium tortuosum* is widely available as an herbal extract for daily supplementation, but little information is available regarding the phytochemical contents, which is necessary for quality control.

In terms of its effectiveness in the context of stress, *Sceletium* extract has been shown to have both effectiveness as monoamine releasing agent and selective serotonin reuptake inhibitor (Coetzee et al., 2016), and to limit glucocorticoid production via inhibition of specific adrenal p450 enzymes (Swart and Smith, 2016). In addition, rats subjected to experimental restraint stress after being supplemented with crude *Sceletium tortuosum* extract, did exhibit less anxiety behaviour. However, they also presented with increased circulating levels of the pro-inflammatory cytokine, interleukin-1-beta (IL-1β) (Smith, 2011). Although the unrefined nature of the product used may have been responsible for this result, this potentially undesired effect of *Sceletium* supplementation may have far-reaching implications for consumers.

According to the Cytokine Hypothesis of Depression, a significant link exists between cytokines and the mediation of depression (Anisman and Merali, 2002; Maes et al., 1995; Schiepers et al., 2005). It has furthermore been suggested that cytokine inhibitors, aside from their anti-inflammatory effects, may be capable of offsetting depressive symptoms that accompany chronic inflammation (Dantzer, 2004; Yirmiya, 1996). Therefore, the effect of *Sceletium* supplementation on immune cell functionality is an important area of study, as the efficacy of potential treatments for depression may rely on their ability to down-regulate pro-inflammatory cytokine production.

In the current study, it was hypothesised that the anti-depressive and anxiolytic properties claimed for *Sceletium* are exerted via immunomodulation - specifically anti-inflammatory effects. Our aims were therefore firstly to determine the effects of a commercially available high-mesembrine *Sceletium* extract, Trimesemine™, on primary human monocyte viability, both basally and in the presence of an acute pro-inflammatory stimulus (*Escherichia coli* lipopolysaccharide, LPS), the latter to simulate severe acute inflammatory challenge. The second aim of this study was to investigate the functional capacity of these immune cells, following treatment with Trimesemine™.

2. Materials and methods

2.1. Ethical considerations

Human primary isolated monocytes were employed in this study. Ethical clearance for blood collection was obtained from Stellenbosch University Subcommittee C Human Research Ethics Committee (reference # ×15/05/013). Monocytes were isolated from peripheral blood buffy coats obtained from healthy donors between the ages of 18 and 25 years old, which were provided by Western Province Blood Transfusion Services (South Africa).

2.2. Cell culture

2.2.1. Preparation of intervention media

A lyophilised extract (Trimesemine™ (Tri)), prepared from a proprietary hybrid (DV17) of *S. tortuosum* (L.) N.E. Br. and *S. expansum* (L.) L. Bolus (family Aizoaceae) using a proprietary method, was obtained from Botanical Resource Holdings Pty (Ltd) affiliate Verve Dynamics (Somerset West, South Africa) (Lot #BTRMA:001/024, manufacturing reference# DV SCIET: E 028/024 (24123) (refer to Swart and Smith (2016) for the certificate of analysis and quality control data).

We have previously shown a 0.01 mg/ml solution to be most beneficial in an *in vitro* setting (Coetzee et al., 2016), thus a 0.01 mg/ml Trimesemine™ solution was prepared in serum-free Roswell Park Memorial Institute (RPMI) media. The mixture was vortexed for two minutes and filtered through a 0.22 μm syringe filter. As representative of a supra-physiological dose, a 1 mg/ml solution was also included. For the inflammatory challenge, a 1 mg/ml LPS (Sigma Aldrich, L4391) stock solution was made in Hank's Balanced Salt Solution (HBSS), as per manufacturer's instructions.

2.2.2. Cell propagation

Human peripheral blood mononuclear cells (PBMCs) were separated from the buffy coat using a Histopaque (Sigma Aldrich, 10771) density gradient. Following centrifugation, the PBMCs were collected, and centrifuged over PercollPLUS (Sigma Aldrich, E0414) density gradients. The resulting monocyte-rich layers were then collected and re-suspended in complete RPMI (RPMI 1640 media containing 10% Foetal Bovine Serum (FBS), 1% Penicillin/streptomycin, 1% GlutaMAX (Gibco® by Life Technologies™, 35050-038) and β-mercaptoethanol (Sigma Aldrich, M3148)) (Menck et al., 2014).

The purified monocytes were seeded into a 48-well culture plate at a density of 1×10^5 cells/well in complete RPMI. Cells were incubated at 37 °C, 5% CO_2 and the media was refreshed every two days until 90% confluence was reached. It was observed that the cultured primary monocytes went through cycles of adherence and non-adherence to the culture plate surface, and thus media refreshment involved aspiration of the supernatant and centrifugation (400 × g, 10 min, without brake, room temperature), following which the supernatant was discarded, and the cell pellets re-suspended in fresh complete RPMI and returned to their respective wells.

2.3. Trimesemine™ treatment intervention

At desired confluence levels, supernatant was aspirated and placed in microfuge vials, and the wells washed once with Dulbecco's Phosphate Buffered Saline (DPBS). The DPBS was aspirated and added to the vials containing the media from each well, to avoid loss of non-adherent monocytes through the washing process. The microfuge vials were centrifuged as previously described, the supernatant removed, and the cell pellets re-suspended in either 0.01 mg/ml or 1 mg/ml Trimesemine™-containing media, or serum-free media (control groups), and returned to the respective wells.

After 30 min, the following were added to each well: (i) LPS stock solution to LPS-control and LPS-stimulated wells to achieve a final LPS concentration of 50 ng/ml (Ross et al., 2013), and (ii) LPS vehicle (HBSS) to the unstimulated wells. The cells were then incubated for a further 23.5 h under standard tissue culture conditions. All experiments were performed at least three times, in duplicate.

2.4. Viability testing

The XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl] − 2H-tetrazolium-5-carboxanilide) assay is a commonly used test method to indirectly measure cell viability, through assessment of mitochondrial viability (Wang et al., 2011). Following the 23.5-h incubation period, the supernatant was removed from each well and centrifuged. The resulting supernatant was aliquoted and stored at −80 °C for subsequent batch analysis, while the remaining cell pellet was re-suspended in XTT solution (1 mg/ml) and returned to their respective wells for incubation (4 h, 37 °C).

Following incubation, optical densities were determined at 490 nm, using a Universal Microplate Reader (EL800, Bio-Tek Instruments, Inc.) and analysed using *KCjunior for Windows Data Reduction Software (v1.41.3)*.