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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)*.

2.5. Cytokine measurement

Cell-free culture supernatant was analysed using a commercial Magnetic Bead Panel assay (custom-designed 6-plex Milliplex MAP Human Soluble Cytokine Receptor Panel, Merck Millipore), as well as an ELISA Kit (BioLegend, 438807), for concentrations of IL-1β, IL-4, IL-6, IL-10, tumour necrosis factor-alpha (TNF-α) and monocyte chemotactic protein-1 (MCP-1), respectively. Cytokine responses were expressed as absolute concentrations in cell culture supernatant.

Quantification of cytokine concentrations was performed based on a standard curve, derived from linear dilution of the manufacturer-supplied cytokine standards. The lower detection limit was 0.8 pg/ml for IL-1β, 4.5 pg/ml for IL-4, 0.9 pg/ml for IL-6, 1.1 pg/ml for IL-10, 0.7 pg/ml for TNF-α and 1.6 pg/ml for MCP-1.

To understand cytokine secretion in the context of relative viability, an index was calculated using the formula below, to correct the level of cytokines produced for the percentage of viable mitochondria:

Corrected cytokine secretion index $= log_{10}$*(cytokine concentration/mitochondrial viability (% control)* $\times 100$*)*

2.6. Statistical analysis

Results are presented as averages and standard error of the means (SEMs). Effects of Trimesemine™ and LPS stimulation were statistically analysed by assessing normality of data distribution, followed by a non-parametric 2-way ANOVA and LSD *post hoc* tests. If Levene's test for homogeneity of variances rejected the null hypothesis, the Games-Howell test was used as a *post hoc* test instead (Statistica v.12, StatSoft). Differences were considered significant at $p < 0.05$.

3. Results

The major findings of this study were that Trimesemine™ supplementation significantly increased monocyte viability in the setting of acute immune challenge. In addition, it was observed that Trimesemine™ exerted anti-inflammatory effects at basal level, through up-regulation of monocyte IL-10 secretion, but did not interfere with the cells' ability to mount an adequate inflammatory response following LPS exposure.

At both concentrations, Trimesemine™ treatment was not associated with cytotoxic effects on monocytes, as assessed by XTT (Fig. 1). In the absence of LPS, a significant increase in mitochondrial viability was observed in the low dose Trimesemine™ treated condition only, indicating a clear dose-response effect of Trimesemine™ supplementation. LPS exerted significant cytotoxic effects on the primary human monocytes at a concentration of 50 ng/ml, resulting in a 66% decrease in XTT viability, which was prevented completely by both Trimesemine™ doses.

In terms of pro-inflammatory cytokine secretion, monocytes produced detectable absolute levels of IL-1β, IL-6 and TNF-α under basal conditions - a response significantly upregulated for all three cytokines

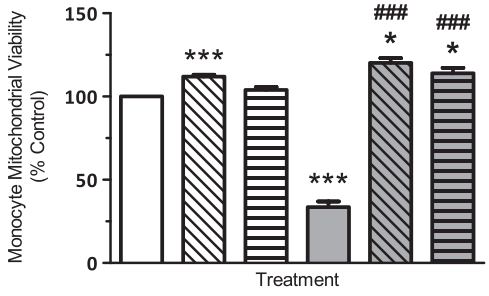

Fig. 1. Dose-dependent primary monocyte mitochondrial viability after exposure to Trimesemine™ with/without LPS for 24 h. * = $p < 0.05$ (compared to Ctrl); *** = $p < 0.0001$ (compared to Ctrl); ### = $p < 0.000001$ (compared to Ctrl (+ LPS)).

in response to LPS stimulation (Fig. 2A-C). Basal secretion levels for MCP-1 was relatively higher than for the other cytokines – which is consistent with the literature (Muenster et al., 2015; Ross et al., 2013; Weldon et al., 2007) – with a dampening effect evident for the high dose Trimesemine™ treatment. Similar to the other cytokines, a significant elevation was seen in response to LPS treatment (Fig. 2D).

In comparison to the LPS-stimulated condition, and when considering that the average basal levels for cytokines are very close to the lower detection limits for these assays, monocyte cytokine secretion in response to Trimesemine™ does not give any indication that this extract exerts pro-inflammatory effects under basal conditions. This interpretation is further supported by the corrected cytokine indices for the pro-inflammatory cytokines (Fig. 3A-E; please note that the Y-axes have a log scale).

Under LPS-challenged conditions, neither dose of Trimesemine™ seemed to inhibit absolute pro-inflammatory cytokine concentrations, except for a lower IL-6 response after high dose treatment. However, when correcting cytokine levels according to relative mitochondrial viability, a consistent dose effect emerges, with Trimesemine™ treatment lowering the pro-inflammatory response to LPS challenge (Fig. 3A-D). However, although dampened, the response mounted was still significant relative to basal conditions.

Turning to the anti-inflammatory cytokines, IL-4 secretion was below the lowest detectable limit across all treatment groups (data not shown). In contrast, basal IL-10 (Figs. 2E1 and 3E) secretion was significantly increased by Trimesemine™ supplementation in a dose-dependent manner, with statistical significance reached after treatment with the higher dose. In contrast to basal levels, under LPS treated conditions, Trimesemine™ treatment was associated with lower IL-10 secretion, most probably as a result of the lower pro-inflammatory response to LPS in the treated groups.

4. Discussion

The current study presents a novel approach to the elucidation of *Sceletium's* potential immunomodulatory properties, firstly through the simultaneous investigation of pro- and anti-inflammatory cytokine production, which to our knowledge has not been performed in other immune cell dose-response ethnopharmacological studies in the context of *Sceletium*. This was motivated by the fact that individual cytokines have overlapping regulatory actions which may be synergistic, additive or antagonistic (Balkwill and Burke, 1989; Cavaillon, 1994), emphasising the importance of studying a broad range of cytokines. Secondly, this study elucidated the effects of a mesembrine-rich commercial *Sceletium* product on leukocyte basal and acute stimulus-induced inflammatory cytokine production, which to our knowledge has not been previously reported in the literature.

The rationale for the use of monocytes as a model was that they are a major source of cytokines, and are characterised by maximal cytokine synthesis within hours of activation (Beutler, 2004; Cavaillon, 1994). It has been observed that these cells are capable of production and release of an impressive panel of cytokines, including, but not limited to, IL-1α, IL-β, IL-6, IL-8, IL-12, TNF-α, interferon-gamma (IFN-γ) and MCP-1 (Cavaillon, 1994).

The fact that monocyte mitochondrial viability decreased significantly after LPS challenge validates the sensitivity of the model to reflect changes in cell viability. Current data indicates that Trimesemine™ did not exert cytotoxic effects at either dosage under basal conditions.

Furthermore, and most importantly contributing to the literature evidence for another major physiological effect of *Sceletium*, Trimesemine™ treatment conferred cytoprotective effects onto immune cells in the presence of acute endotoxin stimulation, thus maintaining XTT viability at control levels. The significance of this result is considerable. Firstly, LPS is known to activate immune cells such as monocytes primarily by binding to Toll-like receptor 4, resulting in

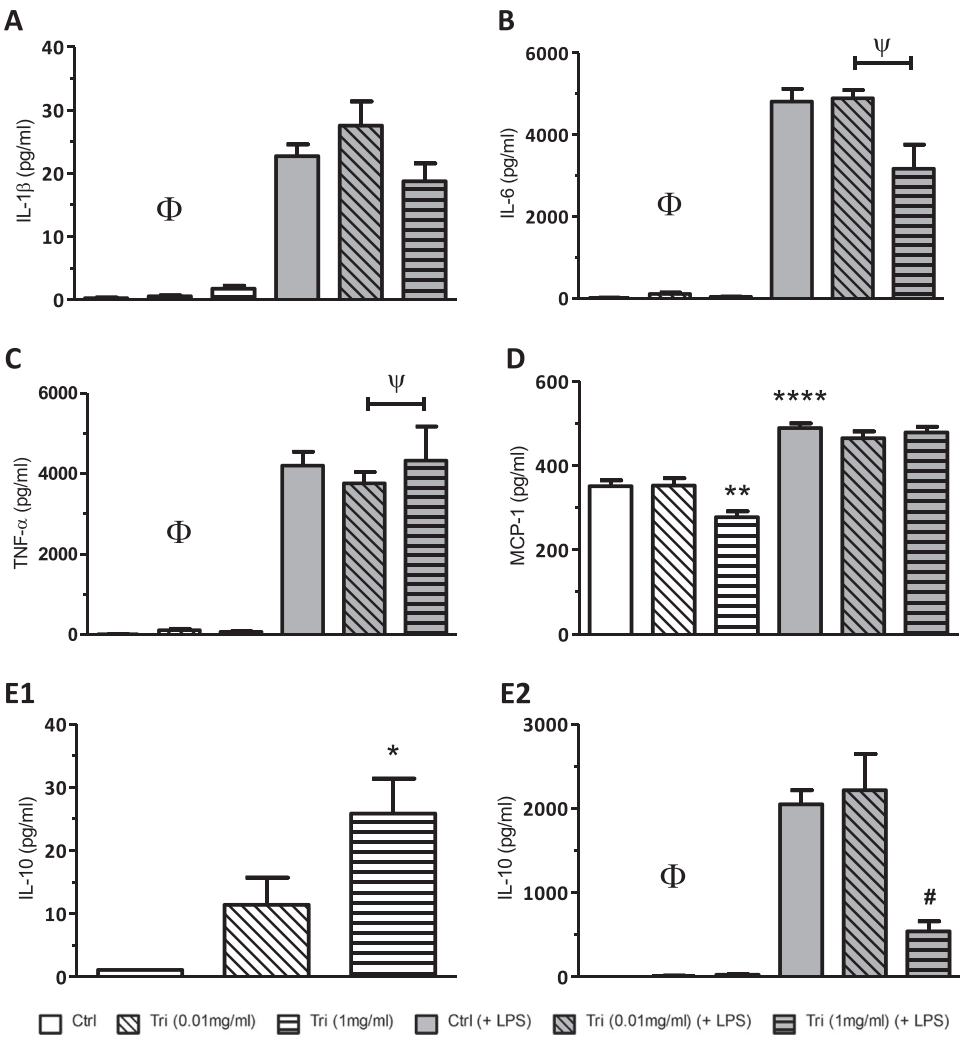

Fig. 2. Effect of Trimesemine™ treatment with/without LPS stimulation on pro-inflammatory (A, B, C and D) and anti-inflammatory (basal only on different axes for clarity, E1 and all conditions, E2) cytokine production by primary human monocytes, as compared to the control. Φ = Main effect of LPS treatment, $p < 0.01$; * = $p < 0.05$ (compared to Ctrl); ** = $p < 0.005$ (compared to Ctrl); **** = $p < 0.000001$ (compared to Ctrl); # = $p < 0.001$ (compared to Ctrl (+ LPS)); ψ = $p < 0.05$.

activation of intracellular kinases and transcription factors, such as mitogen-activated protein (MAP) kinases and NF-κB, respectively (Lu et al., 2010). The resultant immune response is characterised by release of pro-inflammatory cytokines, nitric oxide and eicosanoids, all of which upregulates the immune response required for removal of the pathogen. However, it is known that normal inflammatory processes results in significant secondary damage to host cells themselves (Smith et al., 2008), as indeed evidenced by the significant drop in XTT viability in the LPS-control cells.

Current data thus suggests that *Sceletium* may protect host immune cells from this secondary damage, thereby effectively enabling the immune system to more efficiently rid the host of the pathogen, with less severe clinical symptoms. Secondly, the fact that the immune cells are protected from the nitric oxide (a free radical) that they secrete in response to LPS, also have implications in the context of sterile inflammation. It is known that many chronic diseases are characterised by increased levels of oxidative stress and low-grade inflammation. Thus, the potential for *Sceletium* to also have antioxidant capacity in this context, in combination with its illustrated anti-stress and anti-anxiety effects (Smith, 2011; Terburg et al., 2013), suggests that this plant medicine may be the magic bullet with which to counter the effects of many modern lifestyle-associated diseases. In further support of this notion, the striking cytoprotective effect of *Sceletium* was associated with significant desired modulatory effects in the context of inflammatory signalling.

The fact that Trimesemine™ up-regulated basal IL-10 secretion, may indicate that this *Sceletium* extract possesses mild anti-inflammatory potential. Importantly, despite this effect, the monocytes could mount an adequate response to LPS stimulation in the presence of Trimesemine™, with a significant up-regulation of IL-10 secretion in response to increased IL-6 production induced by LPS. This result is positive, as it provides evidence to suggest that although a mild modulation by Trimesemine™ under basal conditions may have significance in terms of longer term clinical outcome, its consumption will not hinder the immune response to an acute, pathogenic challenge.

Interestingly, when considering absolute cytokine levels, high-dose Trimesemine™ appeared to result in a smaller IL-6 response to LPS, which indirectly resulted also in decreased monocyte IL-10 secretion. However, when corrected for mitochondrial viability, this dose-effect was not maintained. This suggests that even small (statistically insignificant) differences in mitochondrial viability may affect the cell's functional capacity to produce and/or secrete pro-inflammatory cytokines significantly. The more limited inflammatory cytokine response to LPS in the presence of higher dose Trimesemine™ may be interpreted in two ways. Firstly, the higher dose may limit the undesired symptomatic effects of the inflammatory hyper-response to LPS, which is a favourable clinical outcome. Secondly however, it is possible that this reduced response may be the result of detrimental immunosuppression. However, this seems unlikely, since the cytokine response was still significantly increased in response to LPS when compared to control. Rather, since the high dose employed in the current study represents a supraphysiological dose at least 100-fold higher than suggested daily

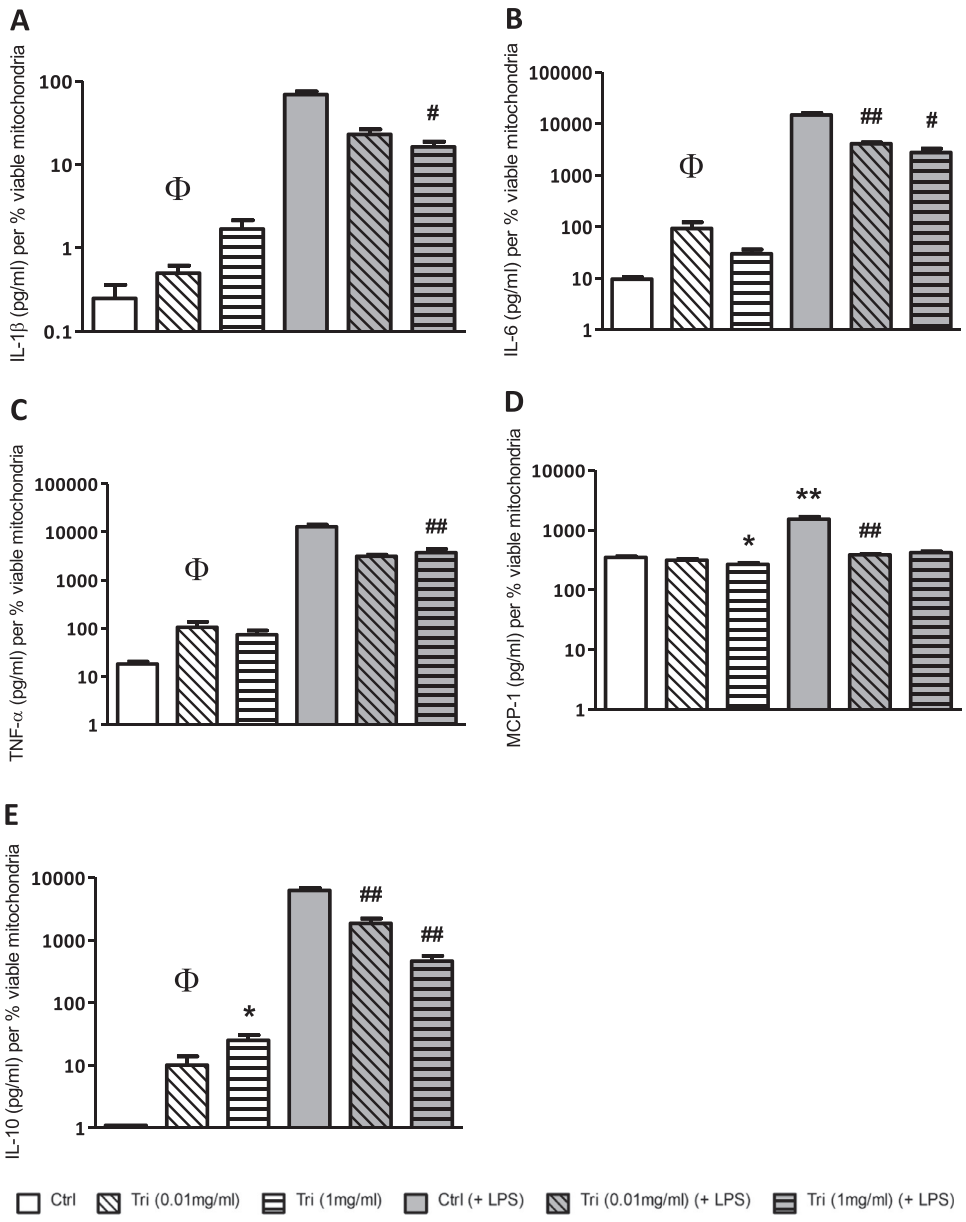

Fig. 3. The monocyte cytokine-viability index per treatment group on a logarithmic scale (base-10). Φ = Main effect of LPS treatment, $p < 0.01$; * = $p < 0.05$ (compared to Ctrl); ** = $p < 0.005$ (compared to Ctrl)); # = $p < 0.001$ (compared to Ctrl (+ LPS)); ## = $p < 0.005$ (compared to Ctrl (+ LPS)).

consumption, this data suggest low risk of overdose, at least in terms of immune capacity and resistance to pathogenic infection.

In terms of the low levels of IL-4 in the current study, a relatively short serum half-life of approximately 20 min was reported for IL-4 following intravenous administration (Conlon et al., 1990). This could account for the undetectable levels of IL-4 across all treatment groups in the current study, where cytokine assessments were carried out only after a 24-h intervention. This finding necessitates a need for more time points during the treatment period, to more comprehensively elucidate the secretion peaks for each relevant cytokine. This also indicates that IL-10 serves as a more viable marker for anti-inflammatory profile, as it provides more conclusive results when assessment of fewer time points apply.

To place current results in context with other physiological effects reported for *Sceletium*, exposure to psychological stress has been implicated in defects in production of pro-inflammatory cytokines (Anisman and Merali, 2002; Maes et al., 1995; Schiepers et al., 2005). It is hypothesised that immune activation may be related to depression-related neurotransmission defects, as pro-inflammatory cytokines themselves have been implicated in alterations in norepinephrine and serotonin/5-hydroxytryptamine (5-HT) turnover in brain regions known to be involved in depression, including the amygdala, hypothalamus, hippocampus and prefrontal cortex (Anderson and Maes, 2015; Dantzer, 2004; Godbout et al., 2005; Poon et al., 2015).

Cytokines are responsible for the lowered activity of presynaptic 5-HT neurons, also due to their interference with serotonin metabolism. Reduced serotonin availability is a result of one or more of the following: (i) the serotonin precursor, tryptophan, is preferentially used for leukocyte activation and synthesis of acute phase proteins, instead of for 5-HT synthesis; (ii) indoleamine 2,3 dioxygenase (IDO), an important enzyme in tryptophan catabolism, is induced by cytokines such as IFN-γ and IL-6; and (iii) low levels of serum albumin, which is essential for tryptophan transport to the blood-brain barrier (Godbout et al., 2005; Maes et al., 1997). In conjunction with reduced availability of 5-HT, upregulation of post-synaptic cleft serotonin receptors has also been observed in the setting of chronic inflammation (Maes et al., 2011).

In addition to their effects on neurotransmitters, cytokines may also influence depression development through their stimulatory actions on the hypothalamic-pituitary-adrenal axis and downregulation of

glucocorticoid receptors, resulting in hypercortisolaemia (Maes et al., 1997).

In this context, one can appreciate the beneficial potential of a supplement a combination of selective serotonin-reuptake inhibition, monoamine releasing, anti-oxidant and mild anti-inflammatory properties. *Sceletium tortuosum* is already known to be an effective mood-elevating natural product (Harvey et al., 2011; Loria et al., 2014; Smith, 2011), but this current study has revealed a second positive effect of *Sceletium* supplementation – namely that it may directly and beneficially target immune cells in the peripheral compartment. These findings indicate that *Sceletium* may act as a double-edged sword in attenuation of cytokine-induced depression, as well as in psychologically-induced systemic low-grade inflammation. Importantly, the current study also provides evidence that the beneficial physiological effects of this plant medicine extends beyond its psychoactive effects.

The current study design did not allow for the elucidation of specific plant constituents responsible for these effects. This warrants further investigation in purpose-designed experiments and *in vivo* supplementation studies. In our opinion, in addition to the different alkaloids generally accepted to be the active constituents, anti-oxidant profiling should also be performed, to further elucidate physiological mechanisms of action of this promising plant medicine.

5. Conclusion

In conclusion, data presented here suggests that a high-mesembrine *Sceletium* extract, Trimesemine™, beneficially modulates the basal inflammatory cytokine profile without hindering an acute response to pathogenic challenge. Furthermore, data suggests that the extract may affect anti-oxidant activity conferring significant cytoprotection from endotoxin-associated oxidative stress damage. Given the recent reports illustrating increased LPS levels not only in pathogenic infection, but also in chronic diseases such as Alzheimer's disease (Zhao et al., 2017), arthritis (Azzouz and Silverman, 2017) and depression (Zhou et al., 2018), data suggests a potential broader role for Trimesemine™ in the sphere of preventative supplementation.

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Declaration of Interest

All authors declare no conflict of interest.

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