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Treatment with *Sutherlandia frutescens* ssp. *microphylla* alters the corticosterone response to chronic intermittent immobilization stress in rats

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Much anecdotal evidence suggests a stress-relieving effect of the *Sutherlandia frutescens* herb. We investigated this in a model of chronic intermittent immobilization stress in 40 adult male Wistar rats. A warm water extract of *Sutherlandia* leaves (4 mg/ml) was used as treatment and isotonic saline as placebo, both injected intraperitoneally. The four experimental groups were: a) control + treatment (CS); b) control + placebo (CP); c) immobilization + treatment (IS) and d) immobilization + placebo (IP). After 28 days, resting blood corticosterone, testosterone, interleukin (IL)-6 and tumor necrosis factor (TNF)-α concentrations were measured. Data were analysed with two-way ANOVA. Immobilization stress resulted in significantly increased corticosterone concentrations in IP vs CP (81 ± 11 vs 22 ± 7 ng/ml, $P < 0.001$), whereas corticosterone concentrations were significantly decreased in IS (43 ± 14 ng/ml, $P < 0.05$) compared to IP. The two *Sutherlandia*-treated groups did not differ (CS 57 ± 11 ng/ml). Neither testosterone nor IL-6 and TNF-α concentrations were significantly different among groups. In summary, our data show that *Sutherlandia frutescens* treatment effectively decreased the corticosterone response to chronic stress, thereby scientifically confirming the indigenous wisdom.

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Introduction

Two of the most severe and common chronic diseases, cancer and HIV/AIDS, result in heightened activation of the hypothalamic–pituitary–adrenal (HPA) axis and therefore chronically increased blood cortisol concentration.[1–3] The long-term effects of raised cortisol include decreased capacity of the cellular immune response,[4] chronic inflammation due to glucocorticoid insensitivity,[5] and increased muscle catabolism.[6,7] All these effects result in chronically decreased quality of life. Considering the high cost of most commercial (synthetic) medicines prescribed for symptomatic treatment, it is of great importance to find a less expensive treatment for these debilitating symptoms. A natural remedy, *Sutherlandia frutescens,* has been used in traditional medicine in South Africa for many decades, for the treatment of stress-related illnesses.[8,9]

Some of the components of the *Sutherlandia frutescens* plant, identified through chemical analyses,[10–13] include L-canavanine, gamma-aminobutyric acid (GABA) and pinitol. The individual effects of these compounds have been illustrated in previous studies on rats: L-canavanine had anti-viral[14] and anti-cancer actions,[15,16] and was proved to be a selective inhibitor of inducible nitric oxide synthase,[17] which has been used effectively to treat endotoxic shock in rats.[11] GABA is an inhibitory neurotransmitter and has mood-elevating properties. Pinitol has anti-inflammatory properties.[18] Although the individual actions of these substances are known, their combined palliative effects in one compound have not been investigated before. Nevertheless, a toxicology study in vervet monkeys[19,20] has shown that *Sutherlandia* leaves, even at nine times the recommended dose for humans on an equivalent per mass basis, had no toxic or any other side effects with regard to haematological and biochemical parameters measured.

The native words for this herb, *motlepelo* (Sotho for 'bringing back the heart'), *insiswa* (an ancient Zulu word meaning 'the one which dispels darkness') and *unwele* (Zulu for 'hair' — alluding to the fact that the plant stops people from 'pulling out their hair' with distress) are indicative of the main effect claimed — to relieve symptoms such as irritability, anxiety and depression. Although much anecdotal evidence exists to validate this herb as having medicinal properties,[8,9] to our knowledge, no scientific proof exists of its action.

The aim of this study was therefore to determine the effects of daily treatment with an extract of *Sutherlandia frutescens* ssp. *microphylla* on selected serum hormone (both anabolic and catabolic) and cytokine levels, in a rat model of chronic immobilization stress.

Methods

Experimental groups. Forty adult male Wistar rats (average body mass of 376 ± 40 g at start of intervention protocol) were used in this study. All rats were housed in groups of 4 in standard, plastic-bottomed wire mesh cages with a 12 h:12 h light:dark cycle. The animals were fed rat chow and tap water *ad libitum.* All rats were allowed 4 weeks to acclimatize after transport to the animal house, before the start of the stress intervention and supplementation protocols.

Rats were separated into four mass-matched groups (10 animals in each group). Two groups represented the controls. One of these received treatment with *Sutherlandia* extract (CS), while the other received placebo (isotonic saline)(CP) for the same period. The third and fourth groups were subjected to chronic immobilization stress in addition to receiving either *Sutherlandia* extract (IS) or placebo (IP).

Interventions. All rats were weighed and handled once a day for 3 weeks before the start of the immobilization protocol to accustom them to humans, preventing stress caused by (unaccustomed) handling being a confounding factor in this study. Immobilization was achieved by placing rats individually into small Perspex cages ($8 \times 6 \times 18$ cm) designed for this purpose, which did not allow free movement. The duration of immobilization sessions was 2 h, once daily, for 28 consecutive days.

Commercially available *Sutherlandia* is sold as 700-mg tablets, each containing 300 mg *Sutherlandia* leaf powder. Manufacturers of these tablets[22] recommend a dose of one tablet twice daily. This recommended dose of the commercial product equals 9 mg per kg body mass for humans. This corresponds to a daily dose of about 3.4 mg per rat, which was rounded up to 4 mg per rat, to allow for weight gain during the study period. Specimens of *Sutherlandia* were harvested in the vicinity of Murraysburg, Western Cape province, South Africa, by W. Grobler. The plants were identified as *Sutherlandia frutescens* subspecies *microphylla* by B-E. van Wyk of the Botany Department, Rand Afrikaans University [voucher specimen from W. Grobler: C. Albrecht s.n sub. B-E. van Wyk 4126 (JRAU)]. Since the traditional way of administration is in the form of a herbal infusion, a warm water extract was prepared: boiling water was added to dried *Sutherlandia* leaves (8 mg/ml) and left to infuse overnight at room temperature. The infusion was not filtered, as this might have resulted in removal of an active component, but poured through a sieve to remove any relatively big leaf particles. Thereafter, the extract was diluted 1:2 with 1.7% saline to produce an extract of 4 mg/ml in isotonic saline. Placebo consisted of a solution of sterile 0.85% saline. Since the *Sutherlandia* extract has a bitter taste, rats will not voluntarily ingest it, and forced oral administration would represent an uncontrolled additional stressor. Therefore, all rats were subjected to intraperitoneal injection twice per day, of either 0.5 ml of 0.85% saline (CP and IP) or 0.5 ml of *Sutherlandia* extract (CS and IS).

Sample collection. At the end of the protocol, all rats were sacrificed between 11:00 and 13:00 by decapitation. The animals were taken from the housing cage one at a time, placed into a weighing basket and carried to another room, where they were weighed and then decapitated. (The sacrifice process, from removal from the cage to decapitation took less than one minute per rat.) Whole blood, retrieved by exsanguination from the

Table 1. Effects of stress, *Sutherlandia* treatment and interaction on concentrations of parameters measured.

	Corticosterone	Testosterone	C:T ratio*	IL-6
Treatment	n.s.	n.s.	n.s.	n.s.
Stress	$P < 0.05$	$P < 0.05$[†]	$P < 0.01$	n.s.
Stress × treatment	$P < 0.005$	n.s.	n.s.	n.s.

*Corticosterone:testosterone ratio.
[†]*P*-values were obtained by two-way ANOVA analysis.

aorta, was deposited into SST blood collection tubes (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, U.K.). Blood was allowed to clot at room temperature for 10 min, after which it was centrifuged at 3000 rpm for 10 min at 4°C, and the serum aliquoted and frozen at –80°C until subsequent analysis. Between consecutive sacrifices, all traces of blood and animal waste were removed from the room, and the working area disinfected with the chemical routinely used to clean housing cages, to prevent acute stress in rats just prior to decapitation.

Analysis: Serum samples were analysed for corticosterone concentration by radio-immunoassay (Biotrak RPA 548, Amersham), testosterone concentration by immunoassay (Advia Centaur, Bayer Diagnostics) and IL-6 and TNF-α concentrations by ELISA (Biotrak RPN 2742 and RPN 2734, Amersham). Data were analysed using two-way ANOVA with Fisher *post hoc* tests to assess differences between subgroups. Relationships between variables were assessed using Pearson correlations.

Results

The effects of *Sutherlandia* treatment, stress and interaction of supplementation and stress on blood properties are illustrated in Table 1 and Fig. 1a–c. Two-way ANOVA indicated no significant main effect of *Sutherlandia* treatment alone. There was, however, a significant primary effect of stress on both corticosterone ($P < 0.05$) and testosterone ($P < 0.05$) concentrations, associated with a significant change in corticosterone:testosterone ratio ($P < 0.01$), as well as a significant effect of interaction of stress and *Sutherlandia* treatment on serum corticosterone concentration ($P < 0.005$) (Table 1).

Fisher's *post hoc* analysis indicated significantly increased corticosterone concentration in IP vs CP ($P < 0.001$), and significantly reduced corticosterone concentration in IS vs IP ($P < 0.05$; Fig. 1a). Despite the (conservative) ANOVA result of no effect of *Sutherlandia* treatment alone on corticosterone concentration, which was probably the result of large inter-individual variations in all four groups, *post hoc* analysis indicated that corticosterone concentration was significantly higher in CS vs CP ($P < 0.05$; Fig. 1a). Both CP and CS had significantly higher testosterone concentrations than IS ($P < 0.01$ and $P < 0.05$; Fig. 1b), whereas the corticosterone:testosterone ratio were significantly higher in IP vs CP ($P < 0.05$; Fig. 1c).

IL-6 concentration showed a high level of inter-individual variation and was not significantly different from the others in any group (Fig. 2). TNF-α concentration was below detectable levels (<10 pg/ml) in all samples.

Discussion

Intermittent immobilization of rats is commonly used as a means of inducing mild but physiologically significant stress.[23–26] Therefore, the finding in the current study that stress significantly increased basal serum corticosterone concentration ($P < 0.05$; Table 1) was expected. Also, the finding that stress resulted in decreased basal serum testosterone concentration ($P < 0.05$; Table 1) is in accordance with results from previous studies.[26–28]
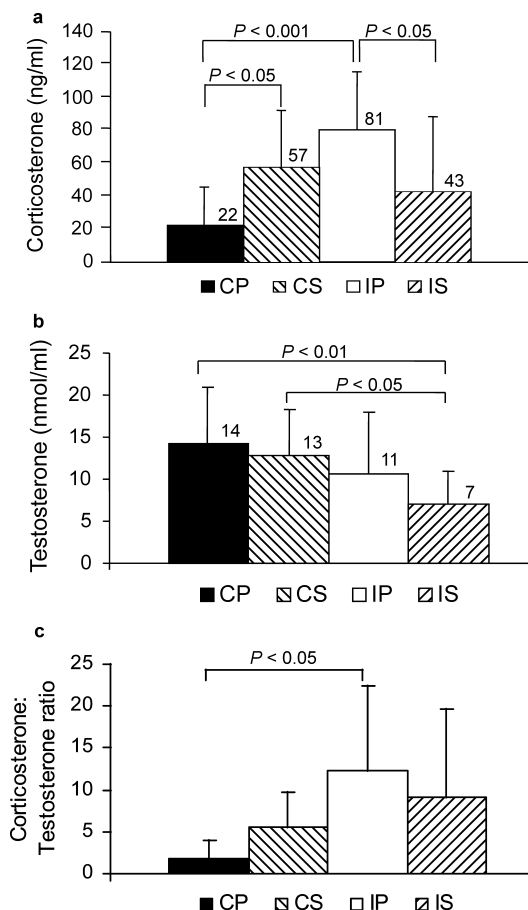
Fig. 1. Serum corticosterone concentration (**a**), testosterone concentration (**b**) and corticosterone:testosterone ratio (**c**). Error bars indicate standard deviations. *P*-values indicate significant differences obtained by Fisher *post hoc* analysis that followed two-way ANOVA.

In addition, we present two main novel findings: First, that *Sutherlandia* treatment decreased the corticosterone response to chronic intermittent immobilization stress, and second, that administration of the herb in control rats increased basal corticosterone concentrations in these animals when compared to placebo-supplemented rats as controls. The first main finding, of an attenuated corticosterone response to stress, supports the indigenous knowledge that *Sutherlandia* has stress-relieving properties. However, further investigation is necessary to establish the exact mechanism by which this is achieved. Possible target tissues for an anti-stress function of the herb include
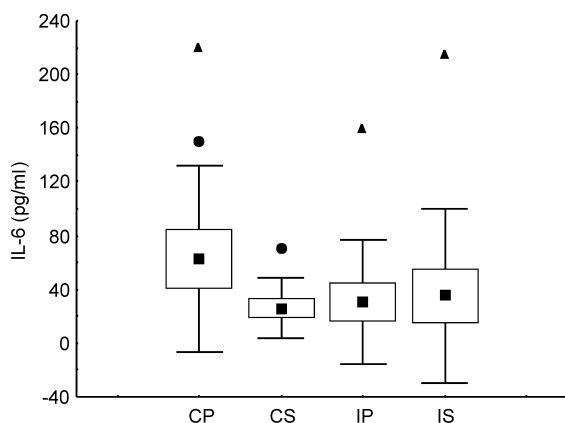

Fig. 2. Box-plot analysis of serum IL-6 concentrations. Boxes indicate mean ± s.e.m., error bars indicate standard deviations, circles indicate outliers and triangles indicate extreme outliers.

the hypocampus (down-regulated stress perception), the hypothalamus or pituitary gland (down-regulation of HPA-axis activation) and the adrenal gland (down-regulation of corticosterone production). Since the herb has not been fully characterized and the active ingredient(s) is not yet known, it is too early to speculate further on possible mechanisms.

The second main finding has more than one possible explanation. First, the herb was prepared in the traditional way, after which it was filtered through a coarse sieve (pore size 0.5 mm), but not sterilized, since sterilization at high temperature held the possibility that an as yet unknown active substance may have been inactivated. Similarly, filtration could possibly remove a large molecule from the extract. The *Sutherlandia* infusion was then administered by intraperitoneal injection, and not via the upper digestive tract, as it would traditionally be ingested (reasons discussed in methods section). The possibility therefore exists that the herb may have elicited an inflammatory response, resulting in increased basal corticosterone concentrations, compared to placebo-controls, which received sterile saline. However, IL-6 and TNF-α concentrations in the treated control rats were not significantly different from the other groups, arguing against a chronic inflammatory state at the time of sacrifice. A second, more likely explanation is that the *Sutherlandia* herb acts as an adaptogen (a substance increasing the body's ability to adapt and increase its resistance to stress and disease, changing the course of an illness into a favourable outcome by normalizing body functions), bringing about a more functional basal corticosterone concentration through allostasis. Allostasis is the term used to describe the process of maintaining homeostasis by means of multiple interacting adaptive processes[29] which may be achieved by combinations of mediators, produced by the immune system, autonomic nervous system and the HPA-axis.[30,31] The fact that the average basal corticosterone and IL-6 concentrations in the two *Sutherlandia* groups were similar, although one group was also subjected to immobilization stress, supports this idea.

While acute stress is known to increase both IL-6[32,33] and corticosterone levels,[33,34] these increases in IL-6 levels are inversely related to the raised glucocorticoid levels[35] and short-lived, due to the anti-inflammatory action of the enhanced circulating corticosterone produced during stress.[36] However, chronic stress is known to result in glucocorticoid insensitivity of monocytes, resulting in chronically increased IL-6 concentration.[5] Our result of no significant difference in IL-6 concentration between experimental groups seems to differ from the available literature. However, taking into account the high inter-individual variation reported for IL-6 in this study (Fig. 2) and in the literature,[37,38] the relatively small animal sample used in the current study may have masked effects of *Sutherlandia* treatment and chronic intermittent stress on secretion of IL-6. Further studies on the acute cytokine responses to *Sutherlandia* administration, possibly using larger sample numbers, are required to investigate this possibility. Furthermore, the phasic release of TNF-α secretion, as well as its down-regulation by both IL-6[33,39] and glucocorticoids,[40] may account for the non-detectable levels of TNF-α reported here.

The suppressive effect of stress alone on testosterone levels ($P < 0.05$; Table 1) and the resultant effect of increasing the corticosterone:testosterone ratio ($P < 0.01$; Table 1) may be due to inhibition of the nocturnal rise in testosterone levels by glucocorticoid action.[41] Although *Sutherlandia* treatment did not appear to have any direct effect on testosterone levels when compared to control rats (Table 1 and Fig. 1b), small sample size and large inter-individual variation may have masked such an

effect in the current study. This issue warrants further investigation, since it is of particular importance in chronic illness to be able to maintain testosterone levels, which is vital for tissue growth and recovery.[42,43]

Conclusion

Our data for the first time confirm scientifically the indigenous knowledge that the *Sutherlandia frutescens* herb has stress-relieving properties. In addition, our results indicate the necessity for further investigations of the effects of this herb, since it appears to have a complex mechanism of action and may therefore prove to be appropriate for other illnesses as well.

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