Anti-granuloma activity of Iraqi *Withania somnifera*

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The granuloma-tissue formation inhibiting activity of various fractions of an extract of the aerial parts of *Withania somnifera* were established using subcutaneous cotton-pellet implantation in rats. Antiinflammatory activity was retained in the methanolic fractions of the plant extract and was comparable to that of a 5 mg/kg dose of hydrocortisone sodium succinate. Activity was attributed to the high content of biologically active steroids in the plant, of which withaferin A is known to be a major component.

Key words: anti-inflammatory activity; anti-granuloma activity; *Withania somnifera*; withaferin A

Introduction

Usually inflammation is recognised by local heat, redness, swelling and tenderness and it can be initiated by various inciting means which may be radiant, mechanical, chemical, infectious or immunological (Bowman and Rand, 1980). At the microscopic level, the early inflammatory process is characterized by edema, fibrin deposition, capillary dilatation and migration of leukocytes. These symptoms are associated with capillary proliferation, fibroblast proliferation, deposition of collagen and eventually cicatrization (Haynes and Murad, 1985).

Adrenocorticotrophic hormone (ACTH) stimulates the release of cortisol and corticosterone from the human adrenal cortex. Cortisol and its synthetic analogs not only have the capacity to prevent or suppress the development of the inflammation symptoms, but also have physiological relevance in regard to the various stages of inflammation (Haynes and Murad, 1985). Administration of corticosteroids for their antiinflammatory effect have become an all too common practice in modern medicine (Nuki, 1987).

The plant *Withania somnifera* Dunal (family, Solanaceae) grows widely in Iraq. The steroidal contents of the aerial, root and fruit parts of the plant have been investigated and purification and identification of the major components attempted (Sour, 1980). Moreover, the antiinflammatory activity of an ethanolic extract of aerial parts of *W. somnifera* has been documented using carrageenan-induced rat paw edema (Al-Hindawi et al., 1989).

In the present work, the effect of various fractions of a 70% ethanol extract of the aerial parts of *W. somnifera* have been tested for their capacity to suppress the granulation-tissue formation induced by subcutaneous implantation of cotton pellets.

Materials and Methods

Plant source

The plant material (aerial parts of *W. somnifera*) was freshly collected from a farm near the city of Baghdad and identified by the Iraqi National Herbarium in Abu-Ghareeb, Baghdad, Iraq.

Animal stock

Inbred albino male Wistar rats weighing 120–160 g (Ministry of Health, Baghdad, Iraq.), were housed in groups of six at 22° ± 1°C with a light/dark cycle of 12:12 (lights from 8:00 to 20:00 h). The rats were maintained on standard rat chow (Ministry of Agriculture, Baghdad, Iraq) and water ad libitum.

Chemical sources

Commercially available hydrocortisone sodium succinate (Solu-Cortef®, Upjohn, Kalamazoo, USA) was purchased from a local pharmacy. All
other chemicals used in the present study were of analytical grade and obtained commercially.

Plant extraction

Prior to extraction, the plant material was air dried and powdered to pass through a 60-mesh sieve. The separation procedure of different fractions from the total extract of *W. somnifera* aerial parts was carried out according to that described previously (Sour, 1980) and is shown in Fig. 1.

From 100 g of plant material, 6.70 g of fraction F1, 11.05 g of fraction F2, 3.32 g of fraction F3, 8.40 g of fraction F4 and 2.46 g of fraction F5 were obtained. Prior to drug administration, the material to be dosed was dissolved or suspended in distilled water using minimum amounts of Tween 80.

Cotton-pellet granuloma

The assay for cotton-pellet granuloma was car-

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**Plant Material (60 Mesh)**

- Defat (with petroleum ether)
- Extraction with 70% methanol
- Marc
- Reextraction with 70% methanol
- Marc
- Evaporation to dryness
- (Fraction F1)
- Maceration with 5% acetic acid
- Extraction with 70% methanol
- Evaporation to dryness
- (Fraction F2)
- Dissolved in methanol:water (50:50)
- Filtration
- Filtrate
- Evaporation to dryness
- Addition of 10% lead acetate
- Filtration
- Filtrate
- Evaporation to dryness
- Addition of 10% lead acetate
- Filtration
- Filtrate
- Treatment with 2% sulphuric acid
- Filtration
- Filtrate
- Evaporation to dryness
- Evaporation to dryness
- (Fraction F5)
- (Fraction F4)

Fig. 1. Extraction scheme of steroids from aerial parts of *Withania somnifera*. For yields of fractions, see the text.
ried out as previously described by Lisciani et al. (1984). The plant extracts were administered intraperitoneally at a constant dosage volume of 10 mg/kg at a dose equivalent to one-tenth of the estimated LD_{50} of the total extract, as reported by Al-Hindawi et al. (1989). Doses of 5 and 20 mg/kg of hydrocortisone sodium succinate dissolved in distilled water were used as a reference. Dosage was given 1 h before pellet implantation and daily thereafter until the pellets were harvested on the 4th day.

Statistics
Data from all experiments were compared and elaborated using Dunnett's t-test for significant differences (Dunnett, 1964).

Results
The antiinflammatory activities of the different fractions of the aerial parts of *W. somnifera* were expressed as percentage of granuloma inhibition relative to control and are given in Table 1. The activities of fractions F1, F2 and F4 were significantly different from that of the control and were considered comparable to that of the 5 mg/kg dose of hydrocortisone sodium succinate. Fractions F3 and F5 showed little activity and did not significantly differ from the control value (Table 1).

All fractions significantly reduced the weight of adrenals, but did not affect that of the thymus or spleen. Hydrocortisone at the doses used simultaneously reduced the weights of the thymus and adrenals in a significant manner over that of the control (Table 1). Body weight reduction was not noticed with either hydrocortisone or with any fraction of the plant extract.

Discussion
Commonly, granuloma formation is very responsive to corticosteroid and rather insensitive to non-steroidal drugs (Dorfman and Dorfman, 1965). In the present work, fractions F1, F2 and F4 suppressed granulation-tissue formation in a significant manner, confirming the antiinflammatory activity of the ethanolic extract of the aerial parts of *W. somnifera* as reported (Al-Hindawi et al., 1989). This activity was accompanied by reduction in the weight of the adrenals, without affecting thymus weight (Table 1). Furthermore, extract-treated animals did not show the reductions in spleen and/or body weight that usually accompany steroid therapy (Silvestrini et al., 1967). This effect may be attributed to withaferin A previously isolated from the plant (Sour, 1980). This compound has been reported to show granuloma and adrenal inhibiting activity without affecting the spleen and body weight (Choda, 1976).

The present work supports the use of the aerial

**TABLE 1**

| GRANULOMA INHIBITION BY VARIOUS FRACTIONS OF AERIAL PARTS OF *W. SOMNIFERA* GROWN IN IRAQ |
|---|---|---|---|---|
| Treatment | Dose i.p. (mg/kg) | Granuloma weight (g) | Granuloma inhibition (%) | Weight relative to body weight (%) |
| | | Wet | Dry | Thymus | Spleen | Adrenals |
| Control | — | 0.294 ± 0.050 | 0.102 ± 0.042 | — | 0.270 ± 0.028 | 0.540 ± 0.251 | 0.032 ± 0.005 |
| Hydrocortisone sodium succinate | 5.0 | 0.282 ± 0.026 | 0.052** ± 0.013 | 49.0 | 0.151** ± 0.047 | 0.601 ± 0.091 | 0.022** ± 0.006 |
| Hydrocortisone sodium succinate | 20.0 | 0.249 ± 0.030 | 0.017** ± 0.024 | 83.4 | 0.225* ± 0.033 | 0.375 ± 0.099 | 0.023** ± 0.007 |
| Fraction F1 | 76.4 | 0.310 ± 0.044 | 0.059** ± 0.018 | 41.6 | 0.287 ± 0.078 | 0.703 ± 0.213 | 0.021** ± 0.005 |
| Fraction F2 | 76.4 | 0.300 ± 0.050 | 0.055** ± 0.008 | 45.9 | 0.261 ± 0.144 | 0.576 ± 0.117 | 0.017** ± 0.004 |
| Fraction F3 | 76.4 | 0.381 ± 0.071 | 0.086 ± 0.039 | 16.0 | 0.217 ± 0.042 | 0.758 ± 0.256 | 0.017** ± 0.005 |
| Fraction F4 | 76.4 | 0.291 ± 0.022 | 0.048** ± 0.020 | 52.6 | 0.254 ± 0.065 | 0.456 ± 0.141 | 0.020** ± 0.004 |
| Fraction F5 | 76.4 | 0.375 ± 0.073 | 0.087 ± 0.043 | 14.3 | 0.214 ± 0.094 | 0.641 ± 0.178 | 0.025** ± 0.006 |

Significance from control: *P < 0.05; **P < 0.001.
parts of *W. somnifera* as an antiinflammatory drug source. The mechanism of antiinflammatory action is as yet indefinite, but undoubtedly involves the pituitary-adrenal axis.

**References**


