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Effect of *Boophone disticha* on human neutrophils

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Abstract

Boophone disticha (Amaryllidaceae) are mainly used in Southern Africa for inflammatory conditions. It is also known for its toxic effects. Because of the putative effects on components of the immune system and inflammatory response the effects of extracts of the bulb of *Boophane disticha* were investigated on ATP production in isolated human neutrophils. Furthermore, one possible mechanism of *Boophone disticha*'s therapeutic properties might be its inhibition of superoxide release from neutrophils. The effect of the extracts on superoxide production of human neutrophils was also investigated.

Aqueous and ethanol extracts of the outer and inner scales of the bulb of *Boophone disticha* was investigated for their effect on human neutrophils. It was decided to test the dry other scales separately from the fleshy inner scales as the parts are also used separately by traditional healers for different applications.

ATP production was significantly decreased by ethanol extracts of the inner scales of the bulb. Superoxide production was significantly inhibited by aqueous extracts of the inner and outer scales of the bulb.

Keywords: *Boophane disticha*; Neutrophils; ATP extraction; Superoxide

1. Introduction

Boophone disticha (L) Herbert (Amaryllidaceae) (Archer et al., 2001) is well known for its toxic effects and therefore the English name poison bulb is not surprising. Plants of the family Amaryllidaceae are well known for a variety of reasons. Many of these plants have found use in the traditional practices of the indigenous people, while others are reaped for their economic use. These plants have become the focus of many pharmacological investigations, which have revealed that while many may be of pharmacological use, several plants are also highly toxic. Adverse effects included sedation, hallucinations, irrational behaviour and more seriously coma and death (Watt and Breyer-Brandwijk, 1932; Van Wyk et al., 1997; Du Plooy et al., 2001).

Traditionally, the dried scales of the bulb are used as an outer dressing for circumcision. Moistened scales are applied to boils, septic wounds and abscesses to alleviate pain and to draw out pus. Fresh scales are applied to burns and used to treat rashes and skin disorders including eczema. It is also used to relieve rheumatic pains, arthritic swelling, sprains, muscular strains, the pain of abrasions and inflammatory conditions.

The inner bulb is boiled and used as a hot compress in the treatment of oedema. Bulb decoctions are administered by mouth or as enemas to adults suffering from headaches, abdominal pain, weakness, sharp chest pains, persistent bladder pains and eye conditions. The bulb is also used in the treatment of varicose ulcers and for the relief of urticaria, as well as a treatment for cancer (Watt and Breyer-Brandwijk, 1932; Dyer, 1953; Watt and Beyer-Brandwijk, 1962; Munday, 1988; Van Wyk et al., 1997).

Bulbs are reported to have caused acute and fatal poisoning in humans, following medicinal administration (Du

Abbreviations: ATP, adenosine triphosphate; NH_4Cl, ammonium chloride; $KHCO_3$, potassium bicarbonate; PMA, phorbol myristate acetate

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Plooy et al., 2001). A group of alkaloids are the compounds responsible for most of the effects. *Boophone disticha* and other Amaryllidaceae contain extremely toxic alkaloids. The Amaryllidaceae alkaloids represent a still expanding group of isoquinoline alkaloids, which are found exclusively in plants belonging to this family (Viladomat et al., 1997). Alkaloids isolated from the bulb includes buphanamine, buphanidrine, buphanine buphanisine, haemanthamine, nerbowdine, undulatine, lycorine, crinamidine, crinine, 3-*O*-acetylnerbowdine, ambelline, buphacetine and distchamine (Raffauf, 1970; Viladomat et al., 1997). Other compounds that have also been isolated are a volatile oil, containing furfuraldehyde, acetovanillone, chelidonic acid, copper, laevulose, pentatriacontane, ipuranol and a mixture of free and combined fatty acids (Watt and Beyer-Brandwijk, 1962).

Buphanine is a strong basic amorphous alkaloid. Is mydratic, inhibits salivary secretion and paralyse the vagus terminations in the heart and leads to death due to respiratory failure. It has an action similar to that of scopolamine. Clinical features of a scopolamine overdose are hallucinations, delirium, coma, tachycardia, hypertension, hyperthermia and mydriasis (Mycek et al., 1997).

Because of the putative effects on components of the immune system and inflammatory response the effects of extracts of the bulb of *Boophone disticha* were investigated on ATP production in isolated human neutrophils. Furthermore, one possible mechanism of *Boophone disticha's* therapeutic properties might be its inhibition of superoxide release from neutrophils. The effect of the extracts on superoxide production of human neutrophils was also investigated. It was decided to test the dry other scales separately from the fleshy inner scales as the parts are also used separately by traditional healers for different applications as was mentioned above.

2. Materials and methods

2.1. Plant materials and extraction procedure

This bulbous plant occurs in grassland and rocky places in South Africa, but also in Kenya, the Congo, and Namibia (Van Wyk et al., 1997). The bulbs are 10–15 cm in diameter and are partly exposed above the ground. It has numerous papery scales around the fleshy part. (Du Plooy et al., 2001). Bulbs of *Boophone disticha* (Amaryllidaceae) were collected by Sr. L. Mathibe (Department of Pharmacology, Medical University of Southern Africa) from traditional leaders from the Waterberg area near Nylstroom, Northern Province, South Africa and taken to Prof. A.E. van Wyk (D.Sc.) (Plant Systems, Botany, Pretoria, University) for verification. The taxonomic identification of plant materials was confirmed by the National Botanical Institute, Pretoria, South Africa. A specimen is being kept in the herbarium in the Department of Pharmacology (indexed alphabetically). The name of William Herbert's Amaryllidaceous genera *Boophone* is spelled in four different ways, namely *Boophane*, *Buphane*, *Boophone* and *Buphone*. It was also known at times as *Buphane toxicaria*, *Haemanthus toxicarius*, *Amaryllis disticha* and *Boophone toxicarius* (Huttleston, 1960; Archer et al., 2001).

The roots and leaves of the plant were removed from the bulb. The outer scales were dry and were removed from the inner fleshy scales. They were tested separately. Twenty-five gram of each of the outer and inner scales of the bulb was separately placed in 500 ml water. The scales of the bulb placed in water were boiled for 5 min after which samples were collected at 5 min, 3 and 24 h. This we hoped reflected most accurately the way in which the concoctions are prepared traditionally. The same amount was placed in ethanol but was not boiled. Samples were also collected at 5 min, 3 and 24 h. In all cases the samples were centrifuged and the supernatant deep-frozen (−75 °C) until needed. Ethanol was chosen due to the fact that it is added during extraction processes and also to preserve plant extracts by the industry.

2.1.1. Neutrophil separation

Thirty milliliter of blood from each of seven healthy volunteers was collected in heparinized Vacutainer® tubes. The blood was then diluted, 1:1 (v/v) with RPMI-1640 medium and kept on wet ice (4 °C). Thirty milliliter of the diluted blood was layered onto 15 ml Percoll (1.088 g/ml), in 50 ml centrifuge tubes and then centrifuged at 2100 rpm (1000 g) for 30 min at 4 °C. The pellet was recovered and the remaining red blood cells in the recovered neutrophils were lysed for 5 min with a 4 °C solution made up of 155 mM NH_4Cl and 10 mM $KHCO_3$. The process was repeated if necessary. Following this the cells were re-suspended in 10 ml RPMI 1640 cell culture medium and a differential cell count was done on an aliquot of the cells (Technikon H1 system) to determine neutrophil purity. The cells were used within 3 h after isolation. All the chemicals used were obtained from Sigma-Aldrich, Atlasville, South Africa.

2.2. ATP production from human neutrophils and measurement

The Cytotoxicity and Cell Proliferation Kit (Aqualytic CC, Irene, South Africa) was used as specified by the manufacturer. The optimal working temperature for all reagents was 22 °C. All measurements were done at 22 °C.

Chemiluminescence was measured before and after production of ATP. The sample was treated with 100 μl somalyze for 5 min to facilitate the release of ATP from the neutrophils. Twenty microliters ATP monitoring reagent was added and the production of ATP measured immediately (Bio Orbit 1250). Each cuvette had a final volume of 500 μl. These values served as the control values. One-hundred microliters of bulb extracts obtained after the different extraction times was added to aliquots of neutrophils and incubated for 30 min after which the 5 min production procedure followed.

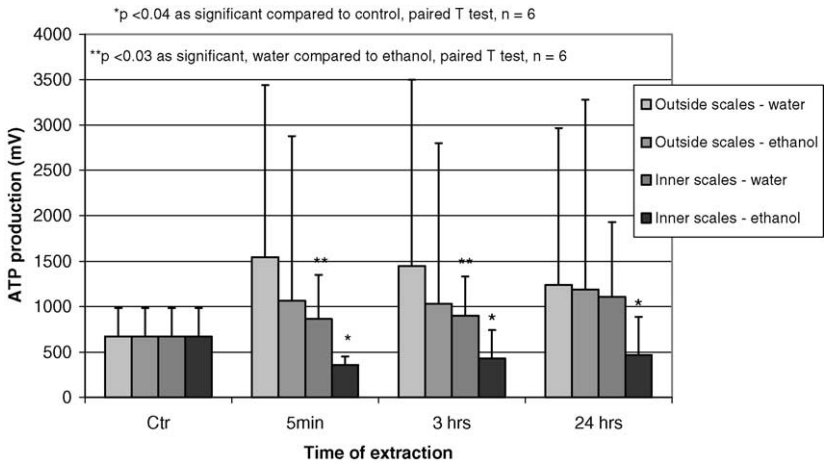

Fig. 1. The effect of aqueous and ethanol extracts of the outer and inner scales of the bulb of *Boophane disticha* on ATP production in human neutrophils (values are means ±S.D., $n = 6$, 10^6 cells).

Results were expressed as mean of maximum ATP production (mV) ±S.D., $n = 6$, for 10^6 neutrophils. The paired *T* test was used to determine statistical significance.

2.2.1. *Determination of superoxide production of human neutrophils*

Phorbol myristate acetate (PMA 2 ng/ml) was used to stimulate neutrophils to release superoxides. Isolated neutrophils (500 μl in RPMI-1640 medium) were incubated for 30 min with 100 μl plant extracts in water or ethanol for the different extraction times. Two hundred μl luminol (10^{-4} M) was added followed by 300 μl PMA. Chemiluminescence was measured immediately. All the measurements were done at 37 °C. Results were expressed as mean of maximum superoxide production (mV) ±S.D., $n = 6$, 10^6 cells. Because certain investigators have stressed the importance of adjusting the results to accommodate differences in leukocyte numbers (Knyszynski and Fischer, 1981; Heberer et al., 1982). Paired *T* test was used for statistical evaluation with $n = 6$. All the chemicals used in the method were obtained from Sigma–Aldrich, Atlasville, South Africa.

3. Results

A slight but non-significant increase in ATP production in human neutrophils was found after incubation with aqueous and ethanol extracts of the outer scales of the bulb. The same effect was seen with the aqueous extracts of the inner scales of the bulb. However, the ethanol extracts of the inner scales significantly decreased ATP production (Fig. 1).

Superoxide production was significantly inhibited by the aqueous extracts of both inner and outer scales of the bulb. Though the ethanol extracts showed the same tendency the inhibition was not significant (Fig. 2). Aqueous extracts showed significantly lower superoxide values than ethanol extracts at 3 h. There was also a significant difference between ethanol extracts when the outer scales were

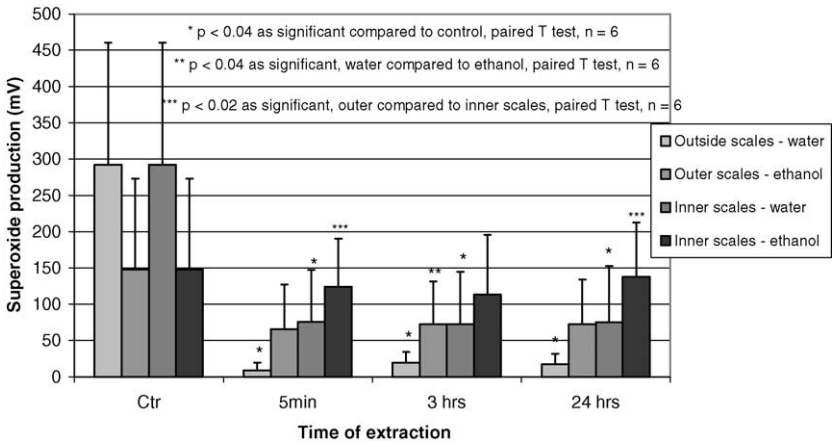

Fig. 2. The effect of aqueous and ethanol extracts of the outer scales and inner scales of the bulb of *Boophane disticha* on superoxide production of human neutrophils (values are means ±S.D., $n = 6$, 10^6 cells).

compared with the inner scales for ethanol extracts at 5 min and 24 h.

4. Discussion

The first assay reflects the intracellular ATP concentration of isolated human neutrophils before and after incubation with the different *Boophone disticha* bulb extracts. A decrease in intracellular ATP reflects possible cell injury due to toxicity to neutrophils or when oxygen substrate depletion occurred (Crouch et al., 1993).

ATP production was increased for aqueous extracts and for ethanol extracts of the outer scales. Only the inner scales showed reduced ATP production. This could be due to a substance in the fleshly inner scales that do not occur in the outer dry scales that could be responsible for toxic effects. Time had no effect on the concentration of this substance. It seems to have been extracted immediately and was available from 5 min at the concentration that caused a decrease in ATP production.

Cell injury or stimulants of the inflammatory process would cause the release of superoxides. Aqueous extracts of both the inner and outer scales could inhibit superoxide production. The fact that the outer scales produced more inhibition could be explained by the fact that 25 g of each was taken. That means that more dry scales must be taken to reach the weight and the active substance could be more concentrated in the drier scales than in the fleshy inner scales. Ethanol extracts showed a tendency to inhibit superoxide production, but the inhibition was not significant. In this instance the inhibitory effect of ethanol (Nilsson et al., 1992) could have interfered with the results, as ethanol controls were also lower than aqueous controls. It was interesting to discover that here also there was no difference between 5 min, 3 and 24 h. It seems that the substance causing superoxide inhibition was also extracted immediately with possible higher concentrations after boiling in water than leaving in ethanol.

The effect of *Boophone disticha* bulb extracts on superoxide production in human neutrophils could explain why the extracts are used to relieve rheumatic pains, arthritic swellings, sprains, muscular strains, the pain of abrasions and inflammatory conditions (Watt and Breyer-Brandwijk, 1932; Dyer, 1953; Watt and Beyer-Brandwijk, 1962; Munday, 1988; Van Wyk et al., 1997).

To conclude, possible toxicity was only observed for ethanol extracts of the inner scales of *Boophone disticha*. None of the other extracts showed effects on ATP production. It would also seem that the aqueous extract of the outer and inner scales of *Boophone disticha* have significant antioxidant properties explaining their traditional use. Ethanol addition could limit these effects.

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