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Effect of Cadmium Accumulation on Anti-Inflammatory Activity in Two *Eucomis* Species

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Abstract *Eucomis* species (Hyacinthaceae) are widely used in South Africa as traditional medicine. The bulbs are used to alleviate a variety of symptoms including pain and inflammation. High levels of cyclooxygenase-1 and -2 (COX-1 and COX-2) inhibitory activity have been associated with certain *Eucomis* species. The aim of this study was to quantify cadmium (Cd) accumulation and examine its effect on COX-1 and COX-2 anti-inflammatory activity in *Eucomis autumnalis* and *Eucomis humilis*. Cadmium application at 2 mg L^{-1} over a 6 week period revealed a substantial difference in total Cd accumulation in *E. autumnalis* and *E. humilis* (40.2 and 15.3 mg Cd kg^{-1}, respectively). When supplied with Cd at 2 mg L^{-1}, *E. humilis* bulbous extracts showed lower inhibitory activity than the control for both COX-1 and COX-2. *E. autumnalis* bulbous extracts had greater COX-1 activity compared to the control. While COX-2 activity was suppressed. Researchers should be aware of the effect of environmental contaminants when reporting on biological activity of crude plant extracts.

Keywords Cadmium accumulation · Cyclooxygenase inhibitors · Medicinal plants · South Africa

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It is estimated that approximately 28 million South Africans depend on over 1,000 medicinal plant species for their health care needs (Mander 1998). The medicinal plants are commonly collected from wild populations (Zschocke et al. 2000) which causes not only a threat to medicinal plant biodiversity but also raises concerns about potential contamination of the plant material due to anthropogenic activities polluting the environment. Heavy metal contamination of South African air, water and soils has been reported by Von Schirnding and Fuggle (1996), Bell et al. (2001) and Okonkwo and Mothiba (2005). To date, there is mounting global concern about cadmium (Cd) as one of the most problematic ecotoxic metals in the environment (Kabata-Pendias 2001), particularly due to high solubility in water (Barazani et al. 2004).

Many South African medicinal plants have been screened for biological activity. In many cases, merely reporting on biological activity of crude plant extracts is considered sufficient (Jäger and Van Staden 2005) and validates the use of these plants in traditional medicine (Taylor et al. 2001). However, recent reports have demonstrated the effect of environmental pollutants on biological activity including the effect of heavy metals on secondary metabolite production (Murch et al. 2003; Narula et al. 2005; Rai et al. 2005). Elgorashi et al. (2004) revealed that the mutagenic effect of medicinally used *Cyrtanthus suaveolens* Schönland was derived not from the plant extract but from a commercially available pesticide, Captan, found in the plant. Such effects could have serious implications on the quality, safety and efficacy of natural products prepared from medicinal plant species.

Eucomis (Hyacinthaceae) is a small genus consisting of bulbous geophytes which are extensively distributed in South Africa (Taylor and Van Staden 2001; Koorbanally et al. 2006). *Eucomis* species are widely used in South

Africa as traditional medicine and one of the top selling bulbs in South Africa, *Eucomis autumnalis* (Mill.) Chitt, is traded at around 73 tons per annum (Mander 1998). The bulbs of *Eucomis* are used to alleviate a variety of symptoms (Hutchings et al. 1996) including pain and inflammation (Taylor et al. 2001). Enzyme assays are useful to assess the effects of herbal remedies for inflammation and pain associated production of prostaglandins (Taylor and Van Staden 2001). Cyclooxygenase, a key enzyme in the biosynthesis of prostaglandins from arachidonic acid, exists in three isoforms, namely cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and cyclooxygenase-3 (COX-3) (Jäger and Van Staden 2005). High levels of COX-1 and COX-2 activity have been associated with *Eucomis* species, in particular *E. autumnalis* and *Eucomis humilis* Baker (Taylor and Van Staden 2002).

Despite the wide use of *Eucomis* sp. in traditional medicine, there is no information regarding Cd accumulation and the effect of Cd on the biological activity. The aim of this study was to quantify Cd accumulation in *E. autumnalis* and *E. humilis* and to determine the effect of Cd accumulation on COX-1 and COX-2 anti-inflammatory activity.

Materials and Methods

Stock plants raised in the shade house at the University of KwaZulu-Natal Botanical Gardens, Pietermaritzburg Campus (29°37.55′S; 30°24.13′E), were used for the experiment. *Eucomis* plants from both species (bulb diameter: 5.0 ± 1.0 cm; n = 5) were transferred into 12 cm pots containing sterilized, acid-washed quartz sand. The pots were placed in a greenhouse in a randomized block design. Hoagland's 50% nutrient solution (Hoagland and Snyder 1933) supplemented with $CdCl_2$ at 2 mg L^{-1} was added weekly (100 mL per pot). Hoagland's nutrient solution (without Cd) served as control. Additional watering of plants (200 mL per pot) took place every alternate day.

After 6 weeks, the plants were harvested, cut into small pieces and placed in a drying oven at 50°C for ±72 h. Once dry, the individual plant parts (leaves, bulbs and roots) were ground into fine powders (<0.5 mm) using an IKA A11 (IKA Works, Inc.) analytical mill. The powders were placed in air-tight containers and stored in the dark at room temperature until analysis.

Borosilicate glass digestion tubes, containing 500 mg of homogenized plant material and 10 mL HNO_3-HCl-H_2O_2 (8:1:1 v/v/v) were placed on a heating block with increasing temperature up to 120°C over 3 h. All reagents (55% HNO_3, 32% HCl, 30% H_2O_2), supplied by Merck (Germany), were of analytical grade. Reagent blanks were used throughout the procedure. Elemental analysis was performed using Inductively Coupled Plasma-Optical Emission Spectrophotometry (Varian 720-ES, Varian Inc., Palo Alto, CA, USA). The detection limit was 0.02 mg Cd kg^{-1}. A certified reference material (NCS DC 73349 – bush branches and leaves) was used to verify the analytical procedure (Certified value 0.14 ± 0.06 mg Cd kg^{-1}; determined value 0.14 ± 0.01 mg Cd kg^{-1}).

Previous work on *Eucomis* species concluded that ethanolic bulb extracts gave the highest inhibitory activity in the COX-1 and COX-2 anti-inflammatory bioassays (Taylor and Van Staden 2002). Therefore, ethanol extracts were prepared where 500 mg of powdered bulbs in 5 mL ethanol were sonicated (Julabo Labortechnik, Seelbach, Germany) for 30 min. The bulb extract was then filtered under vacuum through Whatman No. 1 filter paper using a Büchner funnel, dried under vacuum using a rotary evaporator and stored at 5°C. Bulb extract residues were resuspended in ethanol to a concentration of 10 mg mL^{-1}. The cyclooxygenase-1 and -2 assays were performed as described by Noreen et al. (1998) with slight modifications (Zschocke and Van Staden 2000). The COX-1 and COX-2 enzymes were purchased from Sigma–Aldrich. The enzyme (10 μL) was activated with 50 μL cofactor solution (0.9 mM L-epinephrine, 0.49 mM glutathione and 1 μM hematin in 0.1 M Tris buffer, at pH 8). The enzyme solution (60 μL) and the sample solution (2.5 μL dissolved plant extract applied to 17.5 μL distilled water) were incubated for five mins at room temperature. The reaction started with the addition of 20 μL [^{14}C]arachidonic acid (16 Ci/mol, 30 μM) to each of the samples. The samples were incubated for 10 min at 37°C before the reaction was terminated by adding 10 μL of 2 N HCl. About 4 μL of a 0.2 mg mL^{-1} carrier solution of unlabelled prostaglandins (PGE_2:PGF_2 1:1 v/v) was added. The ^{14}C-labelled prostaglandins synthesized during the assay were separated from the unmetabolized [^{14}C]arachidonic acid by column chromatography. Silica gel in hexane:dioxane:acetic acid 350:50:1 v/v/v) (eluent 1) was packed to a height of 3 cm in Pasteur pipettes. About 1 mL of eluent 1 was added to each of the assay mixtures and this mixture applied to separate columns. The arachidonic acid was eluted from the column with 4 mL of eluent 1 and discarded. The labeled prostaglandins were subsequently eluted with 3 mL ethyl acetate:methanol 85:15 v/v (eluent 2) into scintillation vials. Scintillation fluid (4 mL) was added to each vial and the radioactivity measured using a Beckman LS 6 000LL scintillation counter. The percentage inhibition of the test solutions was calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank. IC_{50} was calculated based on 5 readings, using Grafit Version 5 (Erithacus Software Ltd., UK) at a starting concentration of 250 μg mL^{-1}. Indomethacin (Fluka BioChemika) was included as a control. The experiment was performed in duplicate.

Results and Discussion

No visible symptoms or growth abnormalities were observed for either *Eucomis* species after the 6 week Cd (2 mg L^{-1}) treatment (results not shown). By comparing different plant organs, it was observed that when plants supplied with Cd at 2 mg L^{-1}, *E. autumnalis* stored more Cd in the leaves (8.3 mg kg^{-1}), bulbs (4.9 mg kg^{-1}) and roots (26.7 mg kg^{-1}) than *E. humilis* (0.99 mg kg^{-1}, 1.3 mg kg^{-1} and 13 mg kg^{-1}, respectively; Fig. 1). Total Cd concentration in *E. autumnalis* was more than double that of *E. humilis* (40.2 and 15.3 mg kg^{-1}, respectively).

According to WHO (1998), heavy metal contamination of medicinal plants should be monitored to ensure safety. Bulbs of *E. autumnalis* contained 16 times more Cd than the WHO guideline of 0.3 mg kg^{-1} when supplied with Cd at 2 mg L^{-1}. The bulbs are widely used in decoctions administered as enemas or emetics to treat a variety of symptoms and are taken during pregnancy to facilitate labor (Hutchings et al. 1996). Kuriwaki et al. (2005) studied the effects of Cd exposure on pregnant rats. In addition to the Cd detected in the foetal liver, it was suggested that Cd may inhibit Ca, Cu, Na and K uptake and transportation across the placenta. Similarly, Fe and Zn transportation from the placenta to the foetus may be negatively affected. The study concluded that Cd exposure decreases the elemental concentration in the foetal liver and kidneys, which may in turn influence foetal development and metabolism. Thus, caution is urged, especially for pregnant users, as a large number of these *Eucomis* bulbs are used in traditional medicine. Taylor and Van Staden (2002) revealed higher COX-2 activity in the leaves and roots of *E. autumnalis* compared with the bulbs and

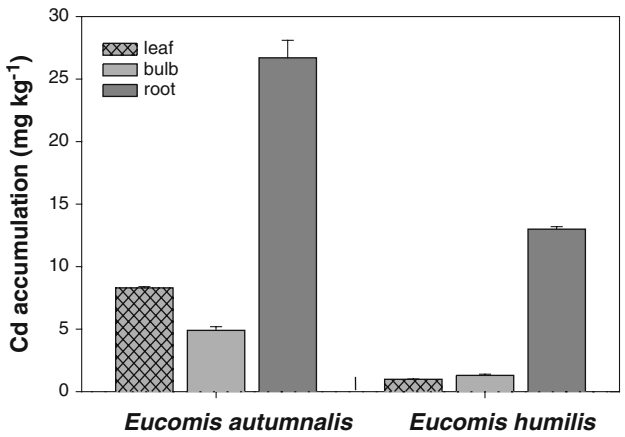

Fig. 1 Cadmium accumulation (mg kg^{-1}) in leaves, bulbs and roots of *Eucomis autumnalis* and *Eucomis humilis* after 6 weeks. Hoagland's 50% nutrient solution supplemented with $CdCl_2$ at 2 mg L^{-1} was added weekly (100 mL per pot). Hoagland's nutrient solution without Cd was the control. Cadmium was undetected in the control plants (data not shown on the graph). *Error bars* indicate SE

recommended the leaves as a more sustainable alternative to harvesting the bulbs. However, the finding of this study show that the leaves of *E. autumnalis* store considerably more Cd than the bulbs (8.3 mg Cd kg^{-1} and 4.9 mg Cd kg^{-1}, respectively) and thus may be a potential exposure route for users of South African traditional medicine, especially considering their ability to accumulate Cd.

Both the COX-1 and the COX-2 assays follow the same basic protocol, which facilitate comparisons between activities of the extracts on the two enzymes (Zschocke and Van Staden 2000). It is clear from the results that compared to control plants, *E. autumnalis*, supplied with Cd at 2 mg L^{-1}, had higher COX-1 inhibitory activity (Table 1). However, compared with the control, Cd-treated plants had reduced COX-2 inhibitory activity (Table 1). The Cd-treated *E. humilis* bulbs showed lower inhibitory activity than the control for both COX-1 and COX-2, however, it was more pronounced in COX-1. In general, *E. humilis* bulbs, which accumulated less Cd than *E. autumnalis* (1.3 and 4.9 mg kg^{-1}, respectively; Fig. 1) were less affected by the Cd treatment for inhibition of both COX-1 and COX-2 activity.

The Cd in the bulb extract can be affecting the anti-inflammatory (COX-1 and COX-2) activity of both *E. autumnalis* and *E. humilis* in two ways. First of all, the presence of Cd in the crude plant extract may affect the COX-1 and COX-2 enzyme activity in the bioassay. To corroborate this, one would need to determine the activity of Cd. Conversely, testing pure Cd would not necessarily be valid owing to the presence of other extracted substances which may well interact with the Cd and alter its form. Secondly, the occurrence of Cd as an environmental stress may influence (increase or decrease) secondary metabolite production. To authenticate this, one would need to quantify the effect of Cd on the biosynthesis of the biologically active compound. However, in the case of *Eucomis* sp. the active compound is not yet identified, in spite of its wide use in South African traditional medicine.

When screening South African medicinal plants for biological activity, the plants are often obtained from

Table 1 Prostaglandin synthesis inhibition expressed as IC_{50} (μg mL^{-1} ± SD) of *Eucomis autumnalis* and *Eucomis humilis* bulbous ethanolic extracts following Cd treatment

Plant species	Treatment (Cd mg L^{-1})	IC_{50} (μg mL^{-1})	
		COX-1	COX-2
E. autumnalis	0	76.5 ± 16	81.5 ± 15
	2	26.7 ± 6.3	223 ± 9.9
E. humilis	0	47.7 ± 2.8	90.6 ± 4.4
	2	58.8 ± 1.4	97.3 ± 8.1
Indomethacin (μM)		2.2 ± 0.18	135.4 ± 7.6

informal medicinal markets. Informal markets are supplied by plant gatherers that collect the plants from undisclosed locations. Owing to the fact that elevated levels of heavy metals have been detected in medicinal plants from informal street markets (Street et al. 2008), it is interesting to note that little attention has been given to the effect of such environmental contaminants on biological activity of crude plant extracts. Although reporting on biological activity is deemed sufficient to validate the traditional use of the medicinal plants (Jäger and Van Staden 2005), researchers cannot rule out the possibility of environmental pollutants skewing reported data.

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