

# The effects of orally administered crude alcohol and aqueous extracts of African potato (*Hypoxis hemerocallidea*) corm on the morphometry of viscera of suckling rats

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## Abstract

The litter from six Sprague–Dawley rats was used to study the short-term effects of African potato (AP) corm extracts in suckling rats. Ten days after birth, the pups in each litter were assigned to treatment groups and received alcohol (AL) or aqueous (AQ) extracts of AP (50 mg kg<sup>-1</sup> b.w. in 0.9% saline, 10 ml kg<sup>-1</sup> b.w., and a high dose 200 mg kg<sup>-1</sup> b.w. in 0.9% saline, 10 ml kg<sup>-1</sup> b.w.) via a stomach tube, for 5 consecutive days. A fifth group (control) received 0.9% saline (10 ml kg<sup>-1</sup> b.w.). Between gavage, the pups were kept with their dams. The pups were then killed and the viscera removed for gross and microscopic morphometric measurements.

The low dose of AQ and AL extracts of AP significantly increased ( $P < 0.01$ , ANOVA) the mean weight gain. The high dose of AQ significantly increased ( $P < 0.05$ , ANOVA) the weight of the caeca whilst the low dose of the AL extract reduced pancreas weight compared to the control and low dose AQ groups. All other morphometric parameters of the viscera measured did not differ significantly between the groups. The small intestinal villi and crypts did not reveal any signs of pathology.

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**Keywords:** *Hypoxis hemerocallidea*; Suckling rats; Viscera; Morphometry

## 1. Introduction

The corm of the African potato plant (*Hypoxis hemerocallidea*) is widely used in traditional African medicine for the treatment of many general ailments, e.g., allergies, ulcers, arthritis, hypercholesterolaemia and infertility (Mills et al., 2005). Aqueous extracts of African potato corm have been shown to have anti-inflammatory, anti-diabetic and antinociceptive properties in rats and mice (Ojewole, 2006). Early reports on its ability to boost immunity in Human Immunodeficiency Virus (HIV) infected patients

and its treatment of cancers (Nicoletti et al., 1992) captivated scientists and the general public. However, more recently, The Medicines Control Council of South Africa prematurely terminated a study on the safety and efficacy of African potato extracts in HIV-positive patients because of bone marrow suppression (van Niekerk, 2003). Additionally, the *Hypoxis*-induced immune suppression has been confirmed experimentally in the feline immunodeficiency virus model (van Niekerk, 2003).

African Potato is high in sterols and sterolins which are thought to be the main mediators of its curative actions (Bouic et al., 2001). It also contains lectins which affect bacterial adhesion and interactions with epithelial cells in the gastrointestinal tract (GIT) (Naughton, 2000). Lectins from red kidney beans have been shown to induce precocious maturation of the GIT and pancreas in suckling rats (Kruszewska et al., 2003; Linderoth et al., 2000). The

Abbreviations: AL, alcohol; AP, African potato; AQ, aqueous; Cl, chloride; GIT, gastrointestinal tract; Na, sodium.

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interaction of lectins with enterocytes and lymphocytes facilitates the translocation of dietary and gut-derived pathogenic antigens to peripheral tissues causing persistent immunological stimulation (Cordain et al., 2000).

The route of administration of extracts from the African potato is normally *per os*, thus their first contact with the body is with the gastrointestinal tract. The GIT is a major source of peptides that regulate metabolic and digestive functions. Dietary manipulations during the peri-natal suckling period have long lasting and apparently irreversible effects on some transport mechanisms in the GIT (Pacha, 2000). With increased use of African potato in human populations in sub-Saharan Africa mainly for medicinal effects, mothers may be tempted to give the plant extracts to their suckling babies, there is thus need to further study its effects on the GIT especially since the traditional extracts are made from raw, potato which could result in the possibility of toxic constituents such as lectins being present in the extracts. The suckling rat is an ideal model for neonatal studies.

The objective of this study was to investigate the effects of orally administered aqueous and alcohol extracts of African potato corms on the weight gain and viscera of suckling rats.

## 2. Materials and methods

The experimental protocol used in this study was approved by the Animal Ethics screening Committee of the University of the Witwatersrand (AESC number 2004/46/03).

### 2.1. Animals and experimental design

Six Sprague–Dawley rats (Central Animal Services, University of the Witwatersrand, Johannesburg, South Africa) that had given birth to a total of 68 suckling pups were used in the study. The dams were individually housed in plastic cages with beddings of hard wood shavings. The dams were kept with their own pups and supplied with standard rat cubes and tap water *ad libitum*. They were placed on a 12 h light–dark cycle (lights on 07.00–19.00) and environmental temperature of 22–24 °C.

The pups in each litter were treated in a split litter manner. The birth date of the pups was designated day zero. On the day 10, the pups in each litter were randomly assigned to five treatment groups. Namely two groups ( $n = 14$  per group) that received a preparation of African potato extract (ethanol extract) suspended in a saline solution at a low dose of dried extract ( $50 \text{ mg kg}^{-1}$  body weight in 0.9% NaCl at  $10 \text{ ml kg}^{-1}$  b.w.) and a high dose of dried extract ( $200 \text{ mg kg}^{-1}$  b.w. in 0.9% NaCl at  $10 \text{ ml kg}^{-1}$  b.w.) via a stomach tube, daily for 5 consecutive days (starting at day 10). The third and fourth groups ( $n = 14$  per group) received a preparation of African potato (water extract) in the same low and high doses as the groups administered alcohol extracts of AP. The fifth group of suckling rats (control group,  $n = 12$ ) received saline (0.9% NaCl at  $10 \text{ ml kg}^{-1}$  b.w.). Between gavage, the pups were kept with their dams and allowed to nurse freely.

The pups were weighed daily. One day after the last treatment, the pups were killed by intra-peritoneal injection of sodium pentobarbitone and the viscera removed for gross and microscopic morphometric measurements. Lengths of the GIT were taken by meticulously dissecting out the intestines and stretching them out on a cooled board. The viscera were also weighed. Sections of the small intestine (proximal and distal halves) were preserved in 10% phosphate-buffered formalin and then embedded in paraffin, sectioned and stained with haematoxylin and eosin for microscopic examination of the intestinal morphology.

### 2.2. Preparation of plant extracts

Fresh corms of African potato were chopped finely and then 675 ml distilled water (for aqueous extract) or 675 ml ethanol (alcohol extract) was added and the mixture homogenized for 30 min using an ultra turrax homogenizer, the mixture was then placed in a shaking incubator at room temperature for 24 h. The suspension was then filtered through Whatmann No. 1 filter paper and the filtrate was dried in a convection oven at 40 °C. The dried extracts were reconstituted in 0.9% saline for administration to the rats.

### 2.3. Statistical analyses

All data are presented as mean  $\pm$  SD. A one way analysis of variance (ANOVA) was used to investigate differences in the parameters measured between the groups. The level of significance was set at  $P < 0.05$ .

## 3. Results

### 3.1. Body weight

The rats administered the low doses of the aqueous and alcohol extracts of the African potato had a significantly ( $P < 0.01$ , ANOVA) higher absolute and relative (percentage of starting weight) weight gain compared to the other groups of rats (Table 1).

### 3.2. Morphometric measurements

The caeca of the suckling rats fed the high dose of alcohol extracts had a significantly higher ( $P < 0.05$ , ANOVA) absolute and relative weight compared to the other groups (Table 1). The rats given a low dose of the alcohol extract had a significantly attenuated pancreatic weight compared with the control and low dose aqueous extract groups respectively. All other morphometric parameters of the viscera measured did not differ significantly between the groups.

Microscopic examination of the small intestinal villi and crypts did not reveal any signs of pathology. Although the mean height of the villi from the distal segments of the small intestine of the rats fed the alcohol extracts was greater than for the other groups, it did not differ significantly ( $P > 0.05$ , ANOVA) from the other groups (Table 2).

## 4. Discussion

Apart from the effects on body weight gain, the pancreatic weight and the weight of the caeca, the administration of African potato extracts at the two doses used did not show any significant effects on the parameters measured for the other visceral organs of suckling rats.

The determination of organ weights is a long established practice in toxicological studies (Baltrop and Brueton, 1990). Generally practitioners of traditional medicine do not have standard fixed doses and as a consequence patients may be subjected to much higher doses than the ones we used. In a study with orally administered aqueous extracts of African potato, Ojewole (2006) found

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