

Full Length Research Paper

Effect of erythraline alkaloids from *Erythrina lysistemon* on human recombinant caspase-3**Shanta Armwood¹, Bernard F. Juma², Japheth O. Ombito², Runner R. T. Majinda^{2*} and Ephraim T. Gwebu¹**

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Prostate cancer is a leading killer disease among men all over the world. Inducing apoptosis (programmed cell death) is a strategic chemotherapeutic approach. Caspase-3 is a key effector of apoptosis, and its activation promotes apoptosis. It was hypothesized that erythraline alkaloids activate caspase-3. The alkaloids were isolated from the flowers and pods of *Erythrina lysistemon*. Their effect on human recombinant caspase-3 was studied. This study reports that three erythraline alkaloids (+)-11 α -hydroxyerysotrine N-oxide (1), (+)-11 β -hydroxyerysotrine N-oxide (2) and (+)-11 β -methoxyerysotrine N-oxide (3) activated human recombinant caspase-3 in a dose-dependent manner. Compound 1 and 2 increased the activity by five-fold while compound 3 increased it by ten-fold. Erythraline alkaloids exhibit remarkable ability to activate caspase-3 and may be lead compounds as potential therapeutics for the treatment of cancer as inducers of apoptosis in cancer cells.

Key words: Erythraline alkaloids, human recombinant caspase-3, apoptosis, prostate cancer.

INTRODUCTION

There are over 110 species of the genus *Erythrina* found throughout the tropical and sub-tropical regions of the world existing as orange and red flowered trees, shrubs and herbaceous plants. Six species are found in South Africa (Fabian and Germishuizen, 1997). The *Erythrina lysistemon* species is a deciduous tree. Traditional medical practitioners use extracts of the leaves, roots, pods and stem bark of this plant to treat various ailments which have been validated through observed biological activities (NAPRALERT, 2016).

In the normal prostate gland, a unique balance between the rates of proliferation and apoptosis rates characterizes homeostasis in such a way that, neither overgrowth nor involution of the gland takes place (Kyprianou et al., 1988; Griffin et al., 2011; Parrish et al., 2013). The evasion of the normal homeostatic control mechanisms gives rise to the tumorigenic growth of prostate due to an increase in cell proliferation and a decrease in apoptotic death (Berges et al., 1995; Tu et al., 1996; Parrish et al., 2013). Enhancing the apoptotic

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process, therefore, is a significant therapeutic target for the effective elimination of androgen-dependent and androgen-independent prostate cancer cells (Nicholson et al., 1995; Bruckheimer et al., 2000; Griffin et al., 2011; Liew et al., 2014). Prostate cancer is only next to lung cancer, the second leading cause of cancer-related deaths of men in the United States (Jemal et al., 2004; Alam et al., 2014; Siegel et al., 2016). Anticancer effects of plant-based alkaloids against prostate cancer have been reported (Adhami et al., 2004; Griffin et al., 2011; Christodoulou et al., 2014; Liew et al., 2014). Activation of caspase-3, the apoptosis executioner/effector (Chang and Yang, 2000; Liew et al., 2014) could force cancer cells to undergo apoptosis. Indeed proteolytic activation of caspase-3 is a common event leading to apoptosis of prostate cancer (LNCaP) cells (Marcelli et al., 1999; Liew et al., 2016). Activation of caspase-3 may be a critical therapeutic target for prostate cancer treatment. Alkaloids activate cellular caspase-3-like activity and up-regulate expression of caspase-3 in various cancer cell lines (Fil'chenkov et al., 2006; Ganguly and Khar, 2002; Deng et al., 2006; Ito et al., 2006; Griffin et al., 2007; Griffin et al., 2011). To the best of our knowledge, the direct effect of alkaloids on human recombinant caspase-3 has not been reported, though the effect of alkaloids on prostate cancer cell lines (e.g. LNCaP, PC-3, Du-145 human prostate cancer lines) including over expression of caspase-3, has been reported (Liew et al., 2016; Christodoulou et al., 2014; Griffin et al., 2011). Potential anticancer extractives from *Erythrina* species including flavonoids (Kumar et al., 2013) and erythraline alkaloids (Mohammed et al., 2012) have also been reported. It was therefore hypothesized that erythraline alkaloids may directly activate human recombinant caspase-3.

MATERIALS AND METHODS

Extraction and isolation of erythraline alkaloids

The compounds tested were isolated from the flowers and pods of *Erythrina lysistemon*, obtained in July 2001, in Gaborone, Botswana. The flowers were crushed while still wet using a blender and extracted for 24 h three times with 1:1 CHCl₃/MeOH mixture at room temperature. The extract was concentrated *in vacuo* to give 65 g of a brown residue. The crude extract was suspended in water and partitioned successively between chloroform and *n*-butanol. The chloroform soluble fraction was chromatographed on silica gel and eluted using *n*-hexane/CHCl₃, CHCl₃, CHCl₃/MeOH mixtures with increasing polarities to afford ten fractions based on thin layer chromatography (TLC) analysis. Detailed isolation of the individual compounds from these fractions is described elsewhere (Juma and Majinda, 2004). Identification of these compounds was done by comparison of physical and spectral data with those published in the literature (Amer et al., 1991a, b; NAPR-ALERT, 2003), and authenticated by ¹H NMR and ¹³C NMR. A total of fourteen erythraline alkaloids were isolated and identified from the *E. lysistemon*. Four of these were new alkaloids, and they were (+)-11 α -hydroxyerysotrine N-oxide, (+)-11 β -hydroxyerysotrine N-oxide, (+)-11 β -hydroxyerysotramidine and (+)-11 β -methoxyerysotramidine (Juma and Majinda, 2004). Due to limited sample size only

compounds **1-3** were tested in the caspase-3 assay.

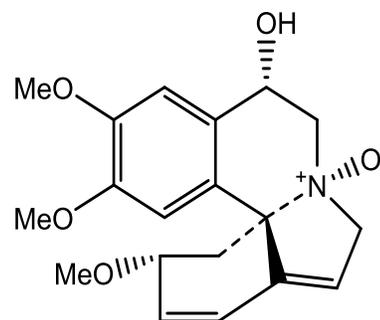
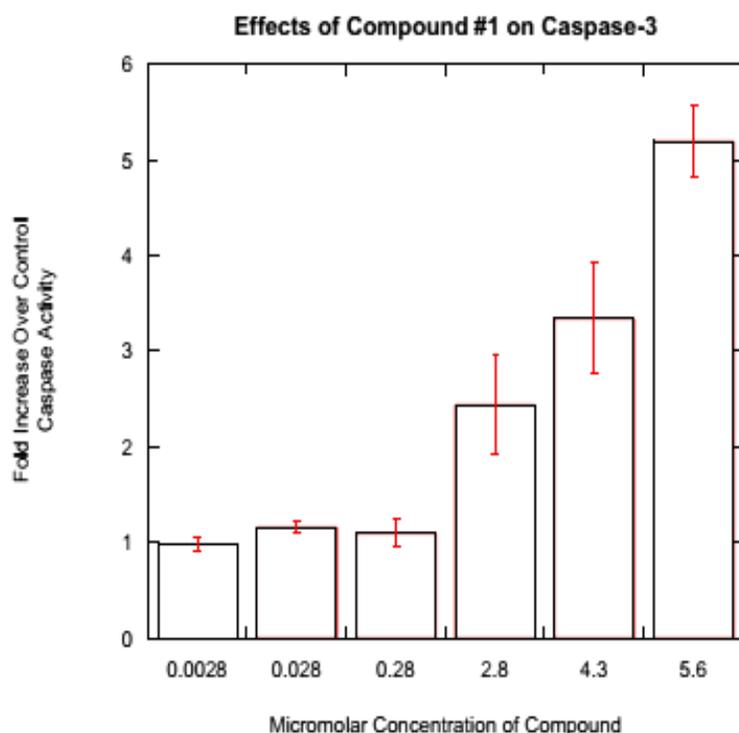
Caspase-3 assay

The compounds were screened for activity using CALBIOCHEM Caspase-3 Assay Kit. The assay solution contained assay buffer (100 mM NaCl, 50 mM HEPES, 10 mM DTT, 1 mM EDTA, 10% glycerol, 0.1% CHAPS, pH 7.4), caspase-3 (30 U) and substrate (200 μ M). The caspase-3 activity was assayed using a colorimetric assay kit purchased from Calbiochem® (www.calbiochem.com) as previously described (Jackson et al., 2002). The kit is designed to measure the protease activity of caspase-3. The enzyme is a human recombinant caspase-3 supplied as 100 units/ μ l. The colorimetric assay of caspase-3 activity is based on spectrophotometric detection of the chromophore, para-nitroanilide (pNA), with maximum absorbance at 405 nm upon a cleavage from the conjugated tetrapeptide substrate DEVD-pNA. The assay is performed in a 96-well benchmark microtiter plate format (BioRad). Stock solutions of each of the test compound were prepared as 1 mg/ml in dimethyl sulfoxide (DMSO) and diluted with assay buffer. The caspase-3 activity of each test sample was calculated according to the formula:

$$\text{Caspase-3 activation (Fold of Control)} = \frac{[\text{Absorbance of test sample} - \text{Absorbance of Blank}]}{[\text{Absorbance of Control} - \text{Absorbance of Blank}]}$$

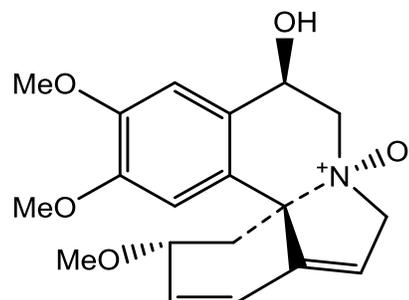
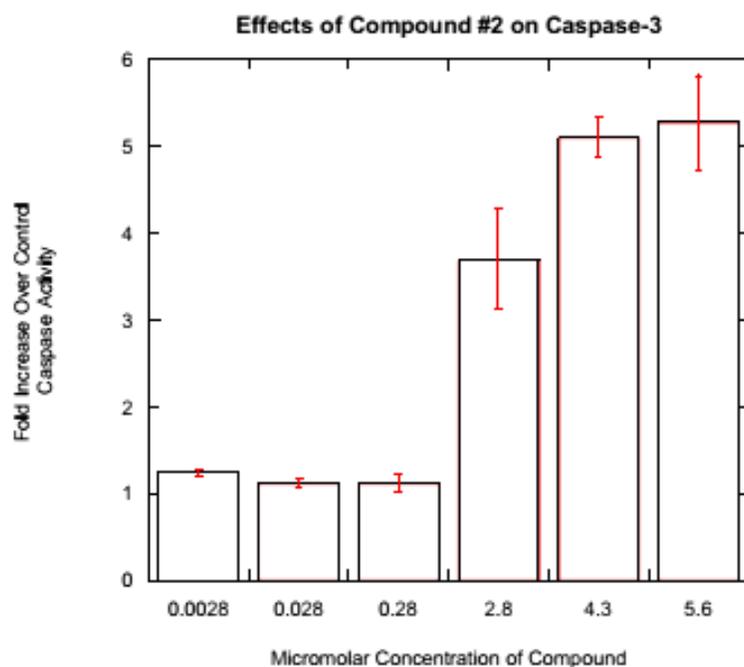
RESULTS AND DISCUSSION

Three structurally related alkaloids, **1** (Figure 1), **2** (Figure 2) and **3** (Figure 3) isolated from flowers and pods of *E. lysistemon* were screened for the activity of human recombinant caspase-3. The results are presented in the graphs shown in Figures 1 to 3, respectively. The hydrolysis of DEVD-pNA with absorbance at 405 nm was considered as the indicator for caspase-3 activity. The caspase-3 solution was incubated with test compound for 2 h at 30°C according to manufacturer's protocol. Compounds **1** and **2** which are diastereomeric and are C-11 epimers, both induced a five-fold increase in caspase-3 activity over the control, an observation that alludes to the fact that, for these compounds, the activation of caspase-3 does not appear to be dependant stereochemistry at C-11. Compound **3**, a methoxy derivative of compound **2** induced a ten-fold increase in caspase-3 activity. It appears conversion of a hydroxyl group to a methoxy derivative doubles the activation of caspase-3. It is interesting to note that other erythraline alkaloids, viz, erythraline, erysodine, erysotrine, 8-oxoerythraline and 11-methoxyerysodine have been shown to be cytotoxic against Hep-G2 (hepatocellular carcinoma) cell line with IC₅₀ values of 17.60, 11.80, 15.80, 3.89 and 11.40 μ g/ml and against HEP-2 (antinuclear antibody) cell line with IC₅₀ of 15.90, 19.90, 21.60, 18.50 and 11.50 μ g/ml respectively. Under the same conditions, the standard doxorubicin gave the IC₅₀ values of 3.64, 4.57, 4.89, 3.74, 2.97 and 3.96 μ g/ml respectively, for the same alkaloids (Mohammed et al., 2012). Based on the remarkable ability of Compounds **1**



(+)-11 α -hydroxyerysotrine N-oxide (**1**)

Figure 1. Effect of (+)-11 α -hydroxyerysotrine N-oxide on human recombinant caspase-3. Various concentrations of the alkaloids were incubated with the human recombinant caspase-3 for 2 h 30°C. Enzyme activity was determined by monitoring colorimetric absorbance at 405 nm resulting from the hydrolysis of the substrate DEVD-pNA. The results are fold increases over control experiments. For each concentration, n = 6.



(+)-11 β -hydroxyerysotrine N-oxide (**2**)

Figure 2. Effect of (+)-11 β -hydroxyerysotrine N-oxide on human recombinant caspase-3. Various concentrations of the alkaloids were incubated with the human recombinant caspase-3 for 2 h 30°C. Enzyme activity was determined by monitoring colorimetric absorbance at 405 nm resulting from the hydrolysis of the substrate DEVD-pNA. The results are fold increases over control experiments. For each concentration, n = 6.

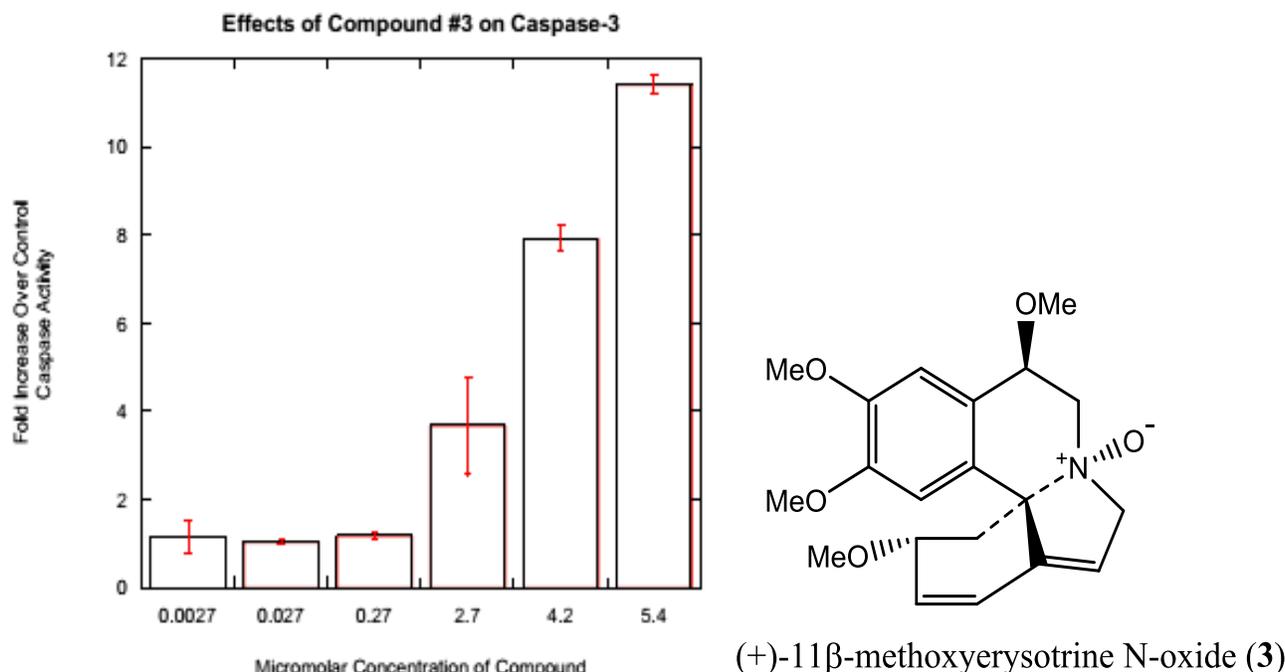


Figure 3. Effect of (+)-11β-methoxyerysotrine N-oxide on human recombinant caspase-3. Various concentrations of the alkaloids were incubated with the human recombinant caspase-3 for 2 h 30°C. Enzyme activity was determined by monitoring colorimetric absorbance at 405 nm resulting from the hydrolysis of the substrate DEVD-pNA. The results are fold increases over control experiments. For each concentration, n = 6.

to **3** to activate caspase-3, these compounds may be significant lead compounds as potential therapeutics for the treatment of cancer as inducers of apoptosis in cancer.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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