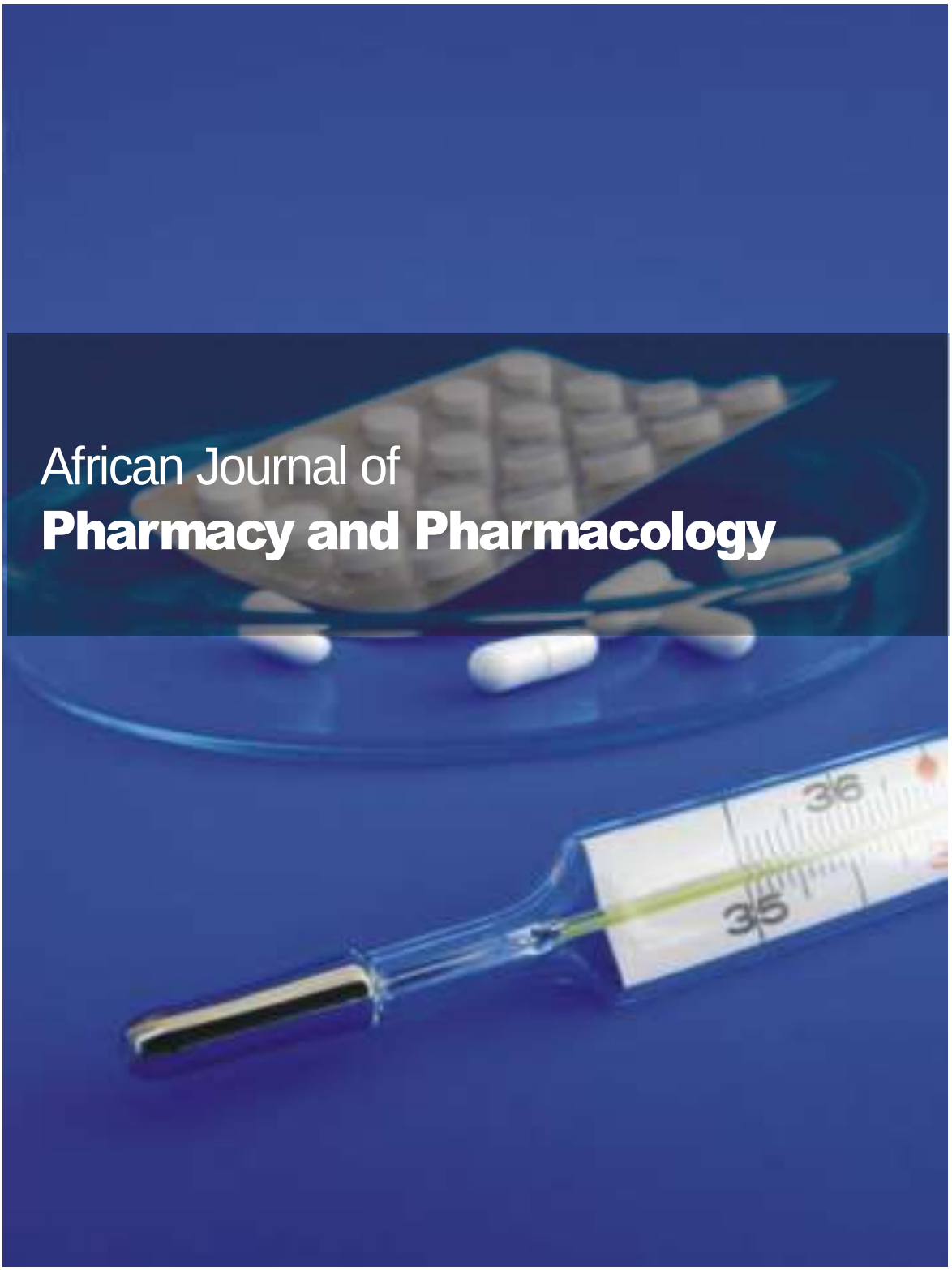
OPEN ACCESS



African Journal of Pharmacy and Pharmacology

22 April, 2018
ISSN 1996-0816
DOI: 10.5897/AJPP
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

ABOUT AJPP

The **African Journal of Pharmacy and Pharmacology (AJPP)** is published weekly (one volume per year) by Academic Journals.

African Journal of Pharmacy and Pharmacology (AJPP) is an open access journal that provides rapid publication (weekly) of articles in all areas of Pharmaceutical Science such as Pharmaceutical Microbiology, Pharmaceutical Raw Material Science, Formulations, Molecular modeling, Health sector Reforms, Drug Delivery, Pharmacokinetics and Pharmacodynamics, Pharmacognosy, Social and Administrative Pharmacy, Pharmaceutics and Pharmaceutical Microbiology, Herbal Medicines research, Pharmaceutical Raw Materials development/utilization, Novel drug delivery systems, Polymer/Cosmetic Science, Food/Drug Interaction, Herbal drugs evaluation, Physical Pharmaceutics, Medication management, Cosmetic Science, pharmaceuticals, pharmacology, pharmaceutical research etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in AJPP are peer-reviewed.

Contact Us

Editorial Office: ajpp@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJPP

Submit manuscript online http://ms.academicjournals.me/

Editorial Board

African Journal of Pharmacy and Pharmacology

Table of Contents: Volume 12 Number 15 22 April, 2018

ARTICLE

Effect of erythrinaline alkaloids from *Erythrina lysistemon* on human recombinant caspase-3 **183**
Shanta Armwood, Bernard F. Juma, Japheth O. Ombito, Runner R. T. Majinda and Ephraim T. Gwebu

academicJournals

Vol. 12(15), pp. 183-187, 22 April, 2018
DOI: 10.5897/AJPP2016.4628
Article Number: 8B98B4B56941
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Effect of erythrinaline alkaloids from *Erythrina lysistemon* on human recombinant caspase-3

Shanta Armwood[1], Bernard F. Juma[2], Japheth O. Ombito[2], Runner R. T. Majinda[2]* and Ephraim T. Gwebu[1]

[1]Department of Chemistry and Physics, Elizabeth City State University, 1704 Weeksville Road, Elizabeth City, North Carolina 27909, North Carolina, United States.
[2]Department of Chemistry, University of Botswana, Private Bag UB 00704, Gaborone, Botswana.

Received 14 July, 2016: Accepted 18 May, 2017

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 erythrinaline 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 erythrinaline alkaloids (+)-11α-hydroxyerysotrine N-oxide (1), (+)-11β-hydroxyerysotrine N-oxide (2) and (+)-11β-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. Erythrinaline 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: Erythrinaline 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 Germishhuizen, 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

*Corresponding author. E-mail: majindar@mopipi.ub.bw. Tel: + 267 335 2503, +267 72710520. Fax: + 267 355 2836.

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 prostrate cancer lines) including over expression of caspace-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 erythrinaline alkaloids (Mohammed et al., 2012) have also been reported. It was therefore hypothesized that erythrinaline alkaloids may directly activate human recombinant caspase-3.

MATERIALS AND METHODS

Extraction and isolation of erythrinaline 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 $CHCl_3$/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/$CHCl_3$, $CHCl_3$, $CHCl_3$/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 ^{1}H NMR and ^{13}C NMR. A total of fourteen erythrinaline alkaloids were isolated and identified from the *E. lysistemon*. Four of these were new alkaloids, and they were (+)-11α-hydroxyerysotrine N-oxide, (+)-11β-hydroxyerysotrine N-oxide, (+)-11β-hydroxyerysotramidine and (+)-11β-methoxyerysotramidine (Juma and Majinda, 2004). Due to limited sample size only compounds **1**-**3** were tested in the caspace-3 assay.

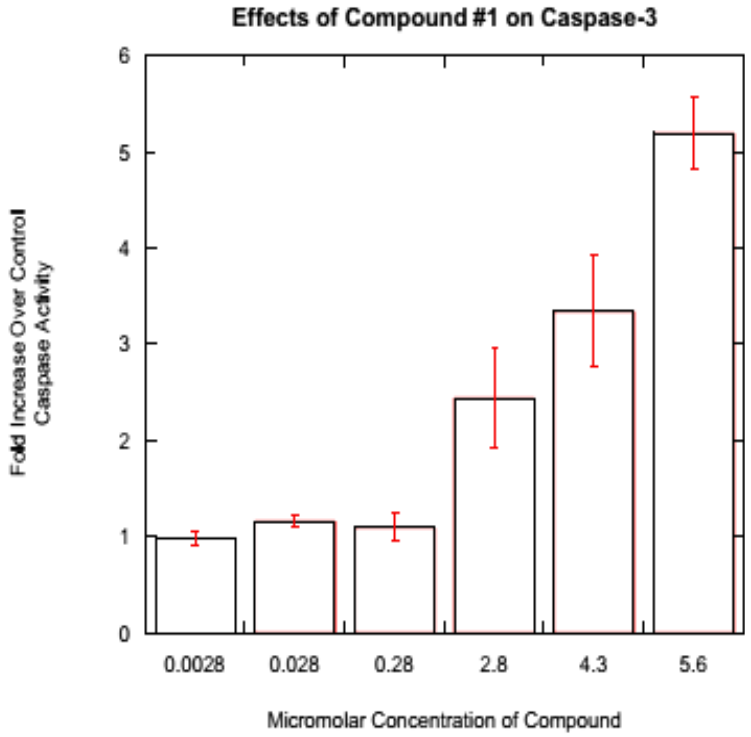
Caspase-3 assay

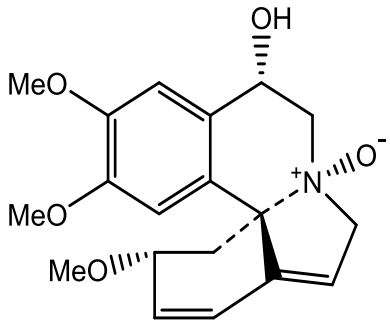
The compounds were screened for activity using CALBIOCHEM Caspase-3 Assay Kit. The assay solution contained assay buffer (100 mM NaCl, 50 mM HEPES, 10 mM DTT, 1 mM EDTA, 10% glycerol, 0.1% CHAPS, pH 7.4), caspase-3 (30 U) and substrate (200 µM). The caspase-3 activity was assayed using a colorimetric 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/µl. The calorimetric 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

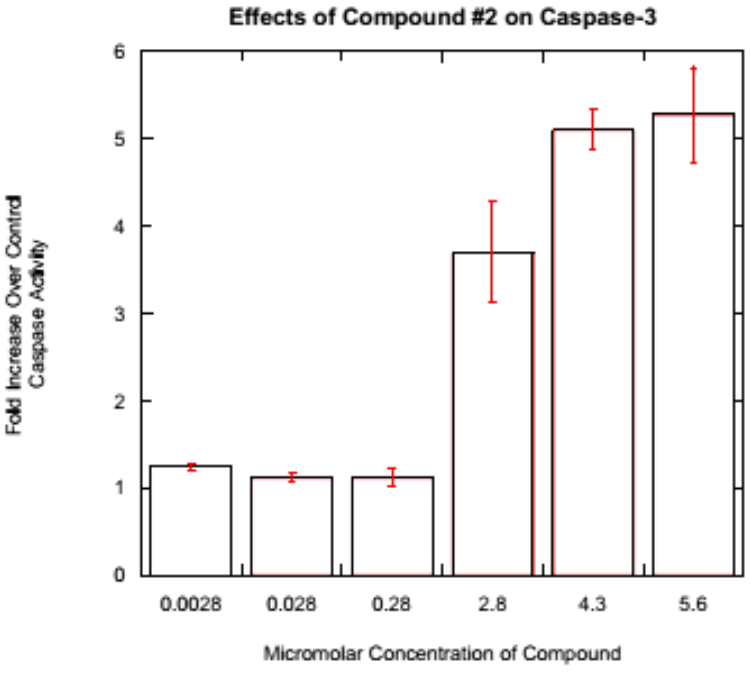
Three structurally related alkaloids, **1** (Figure 1), **2** (Figure 2) and **3** (Figure 3) isolated from flowers and pods of *E. lysistemon* were screened for the activity of human recombinant caspase-3. The results are presented in the graphs shown in Figures 1 to 3, respectively. The hydrolysis of DEVD-*p*NA 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 caspace-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 erythrinaline alkaloids, *viz*, erythraline, erysodine, erysotrine, 8-oxoerythraline and 11-methoxyerysodine have been shown to be cytotoxic against Hep-G2 (hepatocellular carcinoma) cell line with IC_{50} values of 17.60, 11.80, 15.80, 3.89 and 11.40 µg/ml and against HEP-2 (antinuclear antibody) cell line with IC_{50} of 15.90, 19.90, 21.60, 18.50 and 11.50 µg/ml respectively. Under the same conditions, the standard doxorubicin gave the IC_{50} values of 3.64, 4.57, 4.89, 3.74, 2.97 and 3.96 µg/ml respectively, for the same alkaloids (Mohammed et al., 2012). Based on the remarkable ability of Compounds **1**

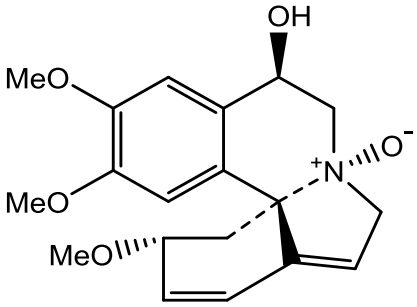




(+)-11α-hydroxyerysotrine N-oxide (**1**)

Figure 1. Effect of (+)-11α-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.





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

Figure 2. Effect of (+)-11β-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.

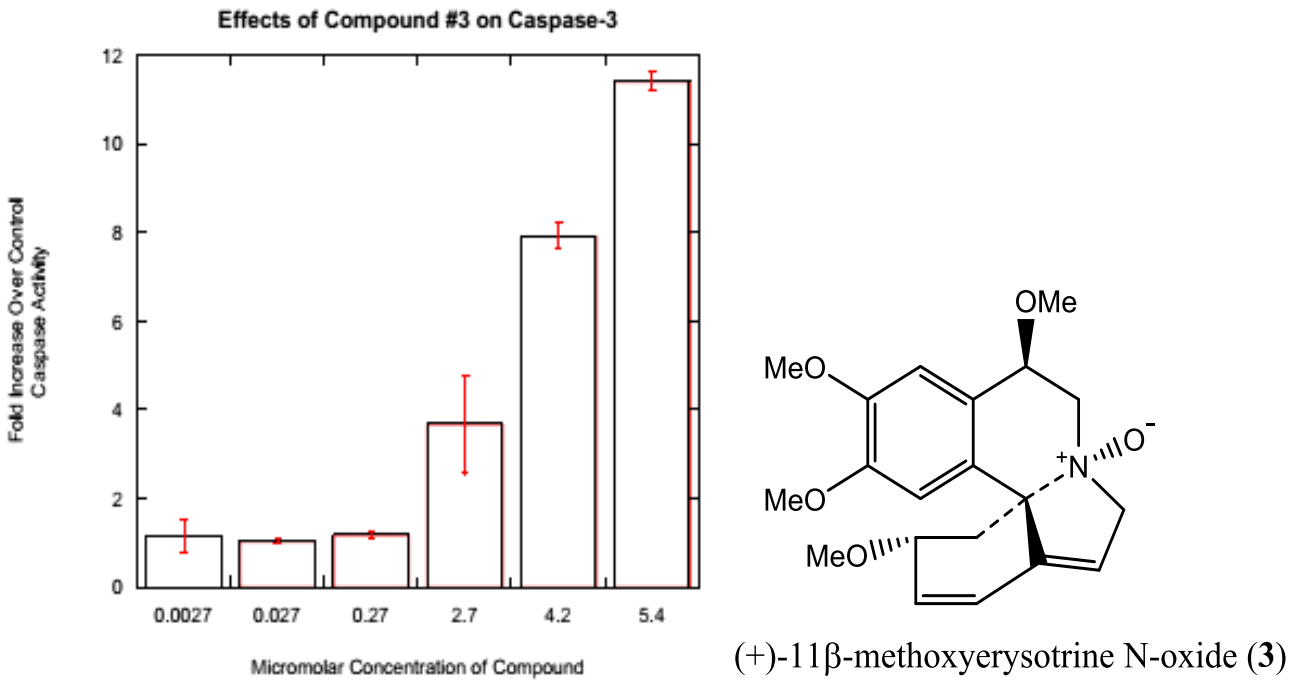


Figure 3. Effect of (+)-11β-methoxyerysotrine N-oxide on human recombinant caspase-3. Various concentrations of the alkaloids were incubated with the human recombinant caspase-3 for 2 h 30°C. Enzyme activity was determined by monitoring colorimetric absorbance at 405 nm resulting from the hydrolysis 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.

ACKNOWLEDGEMENTS

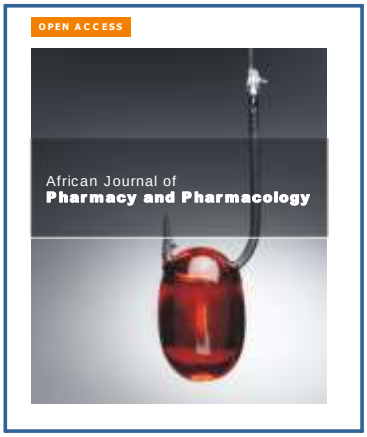
The study was funded in part by the National Center for Minority Health and Health Disparities of the National Institutes of Health (NCMHD/NIH Grant #137 MD001810, University of Botswana's Department of Chemistry and Elizabeth City State University.

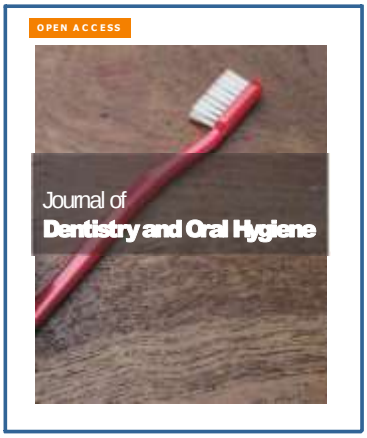
REFERENCES

Adhami VM, Aziz MH, Reagan-Shaw SR, Nihal M, Mukhtar H, Ahmad N (2004). Sanguinarine causes cell cycle blockade and apoptosis of human prostate carcinoma cells via modulation of cyclin kinase inhibitor-
cyclin-cyclin-dependent kinase machinery. Mol. Cancer Ther. 3(8):933-940.
Alam S, Satpathy P, Thosar A (2014). Plants and its parts as a source of anti-cancer compounds: a review. Int. Res. J. Pharm. 5:244-50.
Berges RR, Vukanovic J, Epstein JI, CarMichel M, Cisek L, Johnson DE, Isaacs JT (1995). Implication of cell kinetic changes during the progression of human prostatic cancer. Clin. Cancer Res. 1(5):473-480.
Bruckheimer EM, Kyprianou N (2000). Apoptosis in prostate carcinogenesis: a growth regulator and a therapeutic target: a review. Cell Tissue Res. 301:153-162
Chang HY, Yang X (2000). Proteases for cell suicide: functions and regulations of caspases. Microbiol. Mol. Biol. Rev. 64(4):821-846.
Christodoulou MI, Kontos CK, Halabalaki M, Skaltsounis AL, Scorilas A (2014). Nature promises new anticancer agents: interplay with apotosis-related BCL2 gene family. Anticancer Agents Med. Chem. 14:375-399.
Deng XK, Yin W, Li WD, Yin FZ, Lu ZY, Zhang XC, Hua ZC (2006). The anti-tumor effects of alkaloids from the seeds of *Strychnos nux-vomica* on hepG2 cells and its possible mechanism. J. Ethnopharmacol. 106(2):179-186.
Fabian A, Germishuizen G (1997). Wild flowers of northern South Africa. Fernwood Press, Vlaeburg.
Fil'chenkov OO, Zavelevych MP, Khranovs'ka NM, Zaika LA, Potopal's'kyi AL (2006). Modified alkaloids from *Chelidonium majus* L. induce G2/M arrest, caspase-3 activation, and apoptosis in human acute lymphoblastic leukemia MT-4 cells. *Ukr Biokhim Zh*. 78(5):81-7.
Ganguly T, Khar A (2002). Induction of apoptosis in a human erythroleukemic cell line K562 by tylophora alkaloids involves release of cytochrome C and activation of caspase-3. Phytomedicine. 9(4):288-295.
Griffin C, Sharda N, Sood D, Nair J, McNulty J, Pandey S (2007). Selective cytotoxicity of pancratistatin-related natural Amaryllidaceae alkaloids: evaluation of the activity of two new compounds. Cancer Cell Int. 7:10.
Griffin C, McNulty J, Pandey S (2011). Pancratistatin induces apoptosis and autophagy in metastatic prostrate cancer cells. Int. J. Oncol. 38:1549-1556.
Ito C, Itoigawa M, Nakao K, Murata T, Tsubo M, Kaneda N, Furukawa(2006). Induction of apoptosis by carbazole alkaloids

isolated from *Murraya koenigii.* Phytomedicine. 13(5):359-365.
Jackson R, McNeil B, Taylor C, Holl G, Ruff D, Gwebu E T (2002). Effect of aged garlic extract on caspase-3 activity, in vitro. Nutr. Neurosci. 5(4):287-290.
Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ (2004). Cancer statistics. C A Cancer J.Clin. 54:8-29.
Juma BF, Majinda RRT (2004). Erythrinaline alkaloids from the flowers and pods of *Erythrina lysistemon* and their DPPH radical scavenging properties. Phytochemistry 65(10):1397-1404.
Kumar S, Pathania AS, Saxena AK, Vishwakarma RA, Ali A, Bhushan S (2013). Anticancer potential of flavonoids isolated from the stem bark of *Erythrina suberosa* through induction of apoptosis and inhibition of STAT signal pathway in human leukemia HL-60 cells. Chem. Biol. Interact. 205:128-137.
Kyprianou N, Isaacs JT (1988). Activation of programmed cell death in the rat ventral prostate after castration. Endocrinology 122(2):552-562.
Kyprianou N, Isaacs JT (1989). "Thymineless" death in androgen-independent prostatic cancer cells. Boichem. Biophys. Res. Commun. 165(1):73-81.
Liew SY, Looi CY, Paydar M, Cheah FK, Leong KH, Wong WF, Mustafa MR, Litaudon M, Awang K (2014). Subtidine, a new monoterpenoid indole alkaloid form bark of *Nauclea subdita* (Korth.)Steud. induces apoptosis in human prostrate cancer cells. Plos One 9:e87286.
Marcelli M, Cunningham GR, Walkup M, He Z, Sturgis L, Kagan C, Mannucci R, Nicoletti I, Teng B, Denner L (1999). Signaling pathway activated during apoptosis of the prostate cancer Cell Line LNCaP: Overexpression of caspase-7 as a new gene therapy strategy for prostate cancer. Cancer Res. 59(2):382-390.
Mohammed MMD, Ibrahim NA, Awad NE, Matloub AA, Mohammed-Ali AG, Barakat EE, Mohamed AE, Colla PL (2012). Anti-HIV-1 and cytotoxicity of the alkaloids of *Erythrina abyssinica* Lam. growing in Sudan. Nat. Prod. Res. 26(17):1565-1575.
NAPRALERT (Natural Products Alerts) Internet Database, June 25 (2016). Ethnopharmacology, biological activity and phytochemical information on genus *Erythrina*.
Nicholson DW, Ali A, Thornberry NA, Vaillancourt JP, Ding CK, Gallant M, Gereau Y, Griffin PR, Labelle M, Lazebnik YA, Munday NA, Raju AM, Samulson ME, Yamin TT, Yu VI, Miller DK (1995). Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature. 376(6535):37-43.
Parrish AB, Freel CD, Kornbluth S (2013). Cellular mechanisms controlling caspace activation and function. Cold Spring Harb. Perspect. Biol. 5:a008672.
Siegel RL, Miller KD, Jemal A (2016). Cancer statistics, 2017. CA Cancer J. Clin. 66:7-30.
Tu H, Borkowsski A, Jacobs SC, Kyprianou N (1996). Incidence of apoptosis and cell proliferation in prostate cancer: relationships with TGF-β and bcl-2 expression. Int. J. Cancer. 69:357-363.

Related Journals:





www.academicjournals.org