Indian Journal of Traditional Knowledge
Vol. 16(1), January 2017, pp. 51-58

Apoptosis-inducing effects of *Tulbaghia violacea* Harv methanolic extracts on human Jurkat leukemia T cells

Jared Mackenzie[1], Kogi Moodley[2], Irene Mackraj[2*], Anil Chuturgoon[1], Alisa Phulukdaree[1], Metse Serumula[1] & Rene Myburg[1]

Discipline of Medical Biochemistry[1] and Physiology[2], School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of Kwa Zulu-Natal, Private Bag X7, Congella, 4013, Durban, South Africa; [*]Deparment of Physiology University of Kwa Zulu-Natal Durban, South Africa
E-mail: mackraji@ukzn.ac.za

Received 21 June 2016, revised 04 July 2016

The aim of the present study was to investigate the effects of methonolic extracts of *Tulbaghia violacea* (TVL), on Jurkat cells (human T lymphocyte). Jurkat cells were treated with IC_{50} concentrations of TVL (leaf, rhizome and stalk) extracts as determined by MTT assay and glutathione (GSH) concentration was measured using the GSH-Glo assay. The comet and DNA fragmentation assays were used to determine DNA damage induced by the TVL extracts, and mitochondrial membrane potential activity performed by JC-1assay. The results showed that all TVL extracts except leaf increased apoptosis. TVL leaf instead showed increased necrosis when compared to other TVL components. In addition, TVL extracts induced caspase 3/7, 8 and 9 activity and induced PARP cleavage. These findings suggest that TVL exerts anti-cancer activity primarily through the mechanism of apoptosis. The results suggest that TVL rhizome and stalk induce apoptosis through the intrinsic pathway due to increased activation of caspase 9 and 3/7, with TVL rhizome having the greatest effect on human T lymphocyte cells. Thus, this study suggest that TVL extracts have the potential to induce cell death in Jurkat cells by mitochondria mediated pathway through the involvement of caspase-3/7 in particularly in TVL rhizome.

Keywords: Jurkat cell, Caspase 3/7, Apoptosis, *Tulbaghia violacea*

IPC Int. Cl.[8]: A61K 36/00, A01D 4/04, A01D 4/16, A01D 9/00, G06M 11/02, G01N 33/48

Leukemia, an indiscriminate disease, characterized by an abnormal increase of malignant leucocytes, was diagnosed in over 54000 people in the USA in 2015[1]. These diagnoses encompassed many subtypes of the disease, and resulted in a mortality rate of 45%[1]. About 90 % of all leukemia cases are diagnosed in adults[2]. In almost all forms of cancer treatments, the preferred intervention is through altering cell proliferation or inducing cytotoxicity leading to apoptosis. The intracellular redox environment has been suggested to modulate several cellular processes such as cell proliferation, apoptosis and survival[3]. Oxidative stress induces activation of caspases, a family of cysteine proteases that are involved in induction of apoptosis[4].

The use of natural substances is favoured when considering new treatment methods for leukemia and other diseases, as many have been shown to present with minimal side effects compared to conventional treatment methods[5-7]. Many natural products preferentially inhibit the growth of tumor cells by targeting one or more signaling cascades leading to induction of apoptosis, thus providing a promising therapeutic strategy against cancer[8,9]. *Tulbaghia violacea* (TVL) or “wild garlic” is a plant that is indigenous to the Eastern Cape of South Africa[10]. It is believed to have similar properties to garlic due to the presence of cysteine-derived sulphur compounds found in both plants that produce garlic like odour when the plants leaves and bulbs are crushed or bruised[11]. Traditionally, the leaves are consumed as vegetables[10,12-14]. However, the leaves and bulbs of TVL have also been used by traditional healers to treat various ailments. These include fever, tuberculosis, stomach and cardiovascular problems, as well as oesophageal cancer[10,11,13,14]. A previous study showed that methanolic extracts of TVL leaves and bulbs had inhibitory effects in MCF7, WHCO3, HT29 and HeLa cancer cells[15]. In addition, recent reports also confirmed the pro-apoptotic activities of TVL

*Corresponding author

extracts associated with caspase-3 cleavage and the activation of DNA fragmentation in cancer cell lines[16]. Mechanisms of apoptosis are often investigated through the use of various leukemia cell lines, since anti-cancer drugs or cytotoxic reagents are more effective in leukemia than in other cancer diseases[17]. To the best of our knowledge, there is no report available on the effects of TVL extracts on human T lymphocyte cancer cells, thus leading to the aim of our study. Hence, in this study, TVL extracts were evaluated for its mechanism of apoptotic induction in Jurkat cell lines.

Methodology

Sample collection, authentication with preparation and extraction

TVL was harvested and authenticated by Botanist, Professor H Baijnath (Department of Botany, UKZN). The rhizome, leaf and stalk sections were washed thoroughly with water and left to dry for 48 hrs at room temperature. A voucher specimen was deposited in the ward herbarium of UKZN. The dried plant components (501.62 gm) were blended in methanol using a commercial blender and the active components extracted in methanol for 48 hrs. After 48 hrs, each mixture was filtered twice. The filtered mixtures were evaporated overnight and a rotary evaporator (Genevac LTD, Ipswich, England) was used to remove remaining methanol from the extracts, resulting in a percentage yield of 0.8% at 79 °C and 85 °C. For applications in cell culture, the dried plant extract was weighed and dissolved in cell culture medium.

Cell culture

Human leukemia cell lines (Jurkat cells) were used in this study. The cells were maintained in 10% complete culture medium (CCM: RPMI 1640, supplemented with 10% foetal calf serum, 1% penstrep fungizone and 1% L-glutamine) in a 37 °C humidified incubator with 5% CO_2 saturation. The cell number was determined using the trypan blue method. Treatments were performed by dissolving the respective TVL extracts in media in order to obtain IC_{50} concentrations (TVL leaf= 256 µg/ml, TVL, rhizome= 225 µg/ml, TVL stalk= 216 µg/ml). Jurkat cells were then cultured using this media for 24 hrs and then used for further experimental assays.

MTT cell proliferation assay

Cell proliferation was determined using the 3-[4,5-dim- ethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay as described by Freimoser *et al.*, 1999[18]. The cells were plated at a cell density of 4x104. After 24 hrs the spent medium was replaced with fresh medium containing increasing concentration (0 – 500 µg/ml) of the TVL extracts. A negative control of untreated cells was included. All treatments, including the negative control were done in triplicate.

Single Cell Gel Electrophoresis (SCGE) Assay

A SCGE (or comet) assay was performed in order to detect DNA fragmentation. This assay was performed following the protocol used by Xu *et al.*, 2013[19] with minor modifications. In brief, slides containing cell suspension were embedded between 2 layers of Low Melting Point Agarose (LMPA). The coverslips were removed and the slides submerged in cold lysing solution (500 ml; 2.5M NaCl, 100mM EDTA, 1% Triton X-100, 10mM Tris (*p*H 10) and 10% DMSO) for 1 hr at 4 ºC. The lysing solution was removed, and the slides were placed into an electrophoresis tank to equilibrate for 20 min in alkaline electrophoresis buffer (300 mM NaOH and 1mM Na_2EDTA). After equilibration, the tank was sealed, and a current of 300 mA (25V) was applied for 35 min. The buffer and slides were carefully removed and the slides were washed three times (5 min per wash) with neutralisation buffer (0.4M Tris; *p*H 7.4). All slides were then stained with ethidium bromide (20 µg/ml) and covered with a coverslip. The slides were viewed using a fluorescent microscope (Olympus IX5I inverted microscope with 510-560 nm excitation and 590nm emission filters) (analysis Image Processing Software, Novell). Approximately 50 comets per treatment were captured and analyzed by measuring tail length (µm), and by assigning a visual score to each comet based on its appearance according to Collins, 2004[20].

Annexin V-Fluos Apoptotic Assay

The Annexin V-Fluos assay was performed to detect externalized phosphatidylserine, an early marker of apoptosis according to the manufacturer's instructions. The samples ($1x10^5$ cells/ treatment) were incubated (10–15 min) in triplicate in 100µl binding buffer containing Annexin-V-FLUOS, followed by the addition of 0.5 ml incubation buffer. The data was acquired using a FACS Calibur flow cytometer (BD Biosciences, South Africa) using the 488 nm excitation and a 515 nm band pass filter for fluoresce in detection and analyzed using Cell Quest PRO v4.02.

Caspase determination

Activities of initiator caspases 8, 9 and effector caspase 3/7 were evaluated using luminescence-based assay kits (Promega Caspase-Glo®, South Africa), according to manufacturer's instructions. For each measurement, 1x105 treated cells were aliquoted in triplicate on a luminometer plate. Briefly, for each assay, 100µl of caspase 3/7, 8 or 9 reagent was added to treated cells and mixed using a plate shaker. The plates were then incubated at room temperature for 30 min and samples analyzed using Modulus TM microplate luminometer (Turner Biosystems, Sunnyvale, USA).

DNA fragmentation

In order to confirm the results found in the SCGE assay, a DNA fragmentation assay was performed. Treated cells ($1X10^6$ cells/treatment) were centrifuged (Eppendorf centrifuge 5804R, Hamberg, Germany) at 400xg for 10 min at 24 ºC. Cell lysis of the pellet was performed for 15min. Potassium acetate was added to the lysed cells, vortexed and mixed by inversion for 8 min. The cells were centrifuged (Biofuge Pico, Germany) at 13 000 rpm for 5 min, and the remaining supernatant was transferred into a clean Eppendorf tube. The sample was washed with isopropanol followed by ethanol, and thereafter DNA hydration solution [10mM EDTA (*p*H8), 100mM Tris-Cl (*p*H 7.4) and dH_20] was added to the pellet. The tubes were then vortexed and placed into a water bath for 15 min and DNA concentration of each sample was measured using a Nanodrop (Nanodrop 2000). DNA was electrophoresed and imaged using the UV Tech Alliance 2.7 System (Quantity 1 analysis software).

Glutathione (GSH) determination

The GSH assay was performed according to the manufacturer's instructions. Briefly the cells (1X104Cells/treatment) were suspended in PBS and transferred to a luminometry plate. The assay was performed in triplicate. The 2X GSH-Glo Reagent as added to each sample and standard and mixed gently. Following an incubation period at room temperature for 30 min in the dark, Luciferin Detection Reagent was added to each sample and mixed. The plates were incubated in the dark for 15 min and read on a Modulus TM microplate luminometer (Turner Biosystems, Sunnyvale, USA). The concentration of GSH for the treated samples was determined by extrapolation from a standard curve.

Mitochondrial membrane potential (JC–1) Assay

Flow Cytometry was used to assess the polarity of the mitochondrial membrane by determining the state of JC–1(5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimi dazol-carbocyanineiodide), a lipophilic fluorochrome in the cell. Once JC-1 enters the cytoplasm, it forms monomers and can be taken up by the mitochondria, where it forms aggregates. Red aggregates are measured in the red (FL-2) channel of the flow cytometer, whereas green monomers are measured in the green (FL-1) channel. Following the manufacturer's instructions, treated cell samples ($1x10^6$ cells/sample) were prepared in triplicate and 0.5 ml of JC-1 working solution was added to each sample pellet. These were then incubated for 15 min at 37 °C in a CO2 incubator. Each sample was washed and then re-suspended in sheath fluid, and data was acquired using a FACSC alibur bench top cytometer (BD Biosciences, South Africa) using the 488 nm excitation and a 515 nm band pass filter for fluoresce in. The data analysis was performed by Cell Quest PRO v4.02.

Statistical analysis

All data were expressed as mean ± standard deviation. All statistical analyses were performed on Graph Pad Prism, version 5 (Graph Pad Software, San Diego, California USA). Statistical analyses of the various tests were performed using one–way analysis of variance (ANOVA), with a Bonferroni multiple comparisons test. Values of $p < 0.05$ were considered statistically significant.

Results and discussion

Anti-proliferative effect of TVL extract

The anti-proliferative effects of TVL extracts were representative as IC_{50} value calculated from linear plot between the concentration and % cell viability. The results revealed that TVL leaves extract had a greater inhibitory effect on the growth of the cells compared to the rest of TVL extract (Table 1). Extensively, the natural phytochemical compounds from medicinal plants have been screened extensively for their anti-cancer activity[21]. Medicinal plants indigenous to the Southern African region are

Table 1—IC_{50} values obtained for each extract following an MTT assay

Extracts	IC50 (µg/ml)
Tulbaghia violacea leaf	256
*Tulbagh*ia *violacea* rhizome	225
*Tulbagh*ia *violacea* stalk	216

currently under intense investigation because of their therapeutic potential in the treatment of diseases (including cancer) and because of the expansive natural heritage of the region. Previous studies have reported that TVL has shown pro-apoptotic activity in MCF7, WHCO3, HT29, CHO, MCF7, and HeLa cancer cells[15-16]. This was associated with caspase-3 cleavage and the activation of DNA fragmentation. However, the precise mechanism by which TVL extract induces cell death in leukemia is unclear. Hence, in this study, TVL extracts were evaluated for its mechanism of apoptotic induction in Jurkat cell lines. This cell type provides an ideal *in vitro* model system for studying the cellular and molecular events involved in the proliferation and differentiation of leukemic cells[22]. Furthermore, Jurkat cells that are human T lymphocytes elucidating T cell leukemia[23]. Indeed, the balance between the pro-apoptotic and anti- apoptotic proteins, dysfunction of mitochondria and activation of caspases are the important factors deciding apoptotic cell death of tumor cells[24]. In this study, we aimed to identify the apoptotic effects of TVL extract on human T leukaemic cell line (Jurkat). The study showed that TVL leaves extract had a greater inhibitory effect on the growth of the cells compared to other components. The methanolic TVL extracts showed anti proliferative effects in the leukaemic cell line in keeping with previous different cancer cell line studies[15-16].

The effect of TVL extract on DNA damage and fragmentation using SCGE Assay

Representative images from each treatment are shown in Fig. 1 in Jurkat cells. Fig. 1A represents the control group and shows an intact core in the nucleus. In the treatment groups (1B, C and D), the DNA is shown to be migrating out of the nucleus, resulting in comet tail formation. The length of the comet tail (Fig. 2) gives an indication of the DNA damage. A significant ($p<0.05$) increase in comet tail length was seen for all 4 extracts compared to the control, with TVL rhizome (21.10 ± 22.49 5µm) exhibiting the greatest increase in comet tail length (3-fold increase) (Fig. 2). Table 2 represents the visual score of the "comets" following treatment. A significant increase in visual score was seen following all treatments with greatest increase attributed to the rhizome. The effect of TVL extract on DNA fragmentation in Jurkat cells. The single strand DNA breaks following treatment with all 4 extracts, with more single strand breaks occurring in TVL rhizome when compared to the other TVL extracts and double strand DNA breaks are occurring in the control. The DNA laddering pattern that results due to DNA fragmentation following treatment with the extracts. Laddering is more pronounced in the TVL treatments, even though no double strand breaks were observed (data not shown). In the present study, TVL extracts increased DNA damage as shown by the SCGE and DNA fragmentation assay. The tail lengths and visual

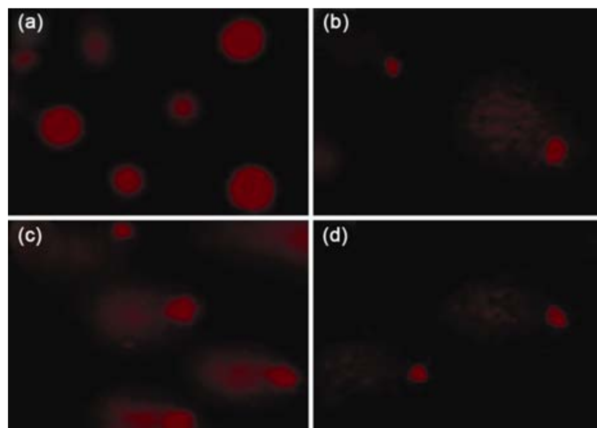

Fig. 1—Fluorescent microscope images of Jurkat cells (following treatment with TVL extracts) showing DNA fragmentation, indicated by the "comet" like appearance of the cells. A) Control, B) TVL Leaf, C) TVL Rhizome, and D) TVL Stalk (magnification= 400X).

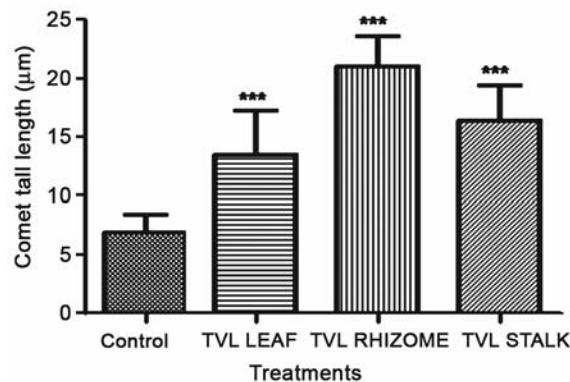

Fig. 2—The comet tail length in Jurkat cells, following treatment with TVL extracts. All extracts resulted in an increase in comet tail length, with the greatest increase seen following treatment with TVL rhizome. ***$p<0.0001$ by comparison with the control group.

Table 2—Visual score of comets following treatment with extracts.***$p<0.0001$ by comparison with the control group

Cell numbers	Visual Scores			
	Control	TVL Leaf	TVL Rhizome	TVL Stalk
1 – 25	5	57	80	66
26 - 50	6	66	77	71
Total (/200)	11	123***	157***	137***

scores recorded were the greatest in the rhizome. Although the TVL leaf and stalk extracts both increased migration of DNA from the nucleus, this was not as significant as that seen in the rhizome extract. The presence of comet tails indicates the presence of fragmented DNA that migrates out of the nucleus. Studies have shown that initial DNA damage is due to cleavage of the supercoiled DNA, resulting in an "open circular form"[25]. This represents single strand breaks. Further cleavage results in double strand breaks or "linear double stranded DNA molecules"[25].The DNA fragmentation assay showed high levels of single strand breaks in the TVL rhizome treated cells. These results are of significance, as this study is to the best of our knowledge, one of the first to show the presence of DNA damage in Jurkat cells following treatment with TVL extracts. The results are in keeping with others, regarding GSH production following treatment, as increased ROS and RNS have been known to result in both single and double strand DNA breaks[26]. Reactive oxygen species have been known to cause DNA damage through inducing damage at the apurinic/apyrimidinic sites, which can lead to mutations or cell death[24]. Previous studies have shown that TVL extracts are able to induce apoptosis in MCF-7, WHCO3, HT29 and HeLa cancer cell lines[15-16]. This is in keeping with the results observed in the SCGE and DNA fragmentation assays.

Effect of TVL extract on apoptosis using Annexin V-Fluos

The Fig. 3 A&B shows the effect of TVL extract on apoptosis and necrosis. The control group showed a low percentage (5.040 ± 0.04%) of apoptotic cells, and only 3.330 ± 0.12% of necrotic cells, suggesting that 91.63% of cells were viable. Treatment with TVL rhizome (6.650 ± 0.02%) and TVL stalk (8.210 ± 0.02%) resulted in a significant ($p<0.0001$) increase in percentage of cells undergoing apoptosis when compared to the control (5.040 ± 0.04%). TVL stalk extract had the most profound effect, resulting in a 1.6 fold increase ($p<0.0001$). However, it must be noted that treatment with TVL leaf (4.890 ± 0.02%) resulted in no significant change in percentage of apoptotic cells, instead a slight but non-significant ($p>0.05$) decrease was observed. Treatment with TVL leaf (4.020 ± 0.02%) resulted in a significant increase ($p<0.0001$) in percentage cells undergoing necrosis when compared to the control (3.330 ± 0.12%), whereas TVLrhizome (2.640 ± 0.1%) resulted a significant decrease ($p<0.0001$). The Annexin V-Fluos assay, which detects externalised phosphatidylserine as a marker of apoptosis, showed that rhizome and stalk extracts all induced a significant increase in the percentage of cells undergoing apoptosis, with the greatest increase observed for stalk. In order to determine which apoptotic pathway these extracts were inducing, caspase activation was determined. The activation of caspase 3/7 ($p<0.05$ for TVL leaf, rhizome and stalk) and 9 ($p<0.05$ for TV leaf and rhizome) by all TVL extracts suggests that these are acting through the intrinsic apoptotic pathway. These are known as effector and initiator caspases, respectively, and are responsible for the cleavage of PARP and other downstream events involved in induction of apoptosis[30]. During the intrinsic pathway, cytochrome C is released by the mitochondria, resulting in apoptosis in some formation, leading to the activation of caspase 9 and then caspase 3/7, which initiate

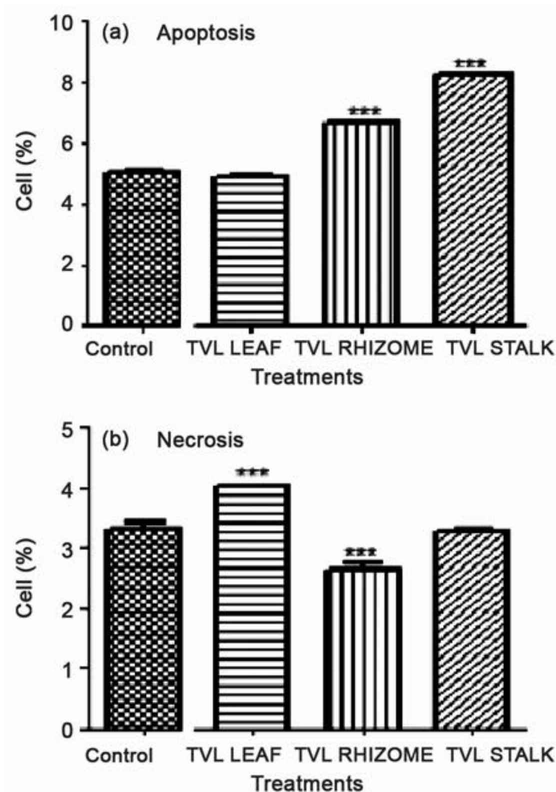

Fig. 3—The percentage of apoptotic and necrotic Jurkat cells following treatment with TVL extracts. A) % Cells undergoing apoptosis. All extracts, except TV leaf, resulted in a significant increase in % cells undergoing apoptosis. B) % Cells undergoing necrosis. TV leaf induced a significant increase in % cells undergoing necrosis, whereas TVL rhizome resulted in a significant decrease. ***$p<0.0001$ by comparison with the control group.

apoptosis[31]. TVL stalk shows a significant increase in mitochondrial depolarisation, which correlates with the induction of apoptosis and caspase 3/7 activation. This also confirms the hypothesis that suggests that this extract may act through the intrinsic apoptotic pathway. Studies have shown that mitochondrial dysfunction appears to occur prior to cell shrinkage and nuclear fragmentation when apoptosis is taking place[32].

Effect of TVL extract on Caspase activity

The effect of TVL extract on caspase activation in jurkat cells showed in Fig. 4 A-C. A significant ($p<0.05$) increase in the activity of all caspases was observed for TVL leaf (caspase 3/7 = 660000 ± 13370RLU, caspase 8 = 1872000 ± 25120RLU, caspase 9 = 3750000 ± 41700RLU). However, treatment with TVL rhizome extract only resulted in increased caspase 3/7 (58700 ± 62070RLU) and caspase 9 (3818000 ± 22150RLU) activity, whereas TVL stalk (518700 ± 12830RLU) resulted in a significant increase only in caspase 3/7 activity. TVL leaf was the only extract that did not show a significant change ($p>0.05$) in percentage cells undergoing apoptosis. However, it was the only extract that resulted in a significant increase in percentage cells undergoing necrosis. These results are specific to treatment with the TVL leaf extract,

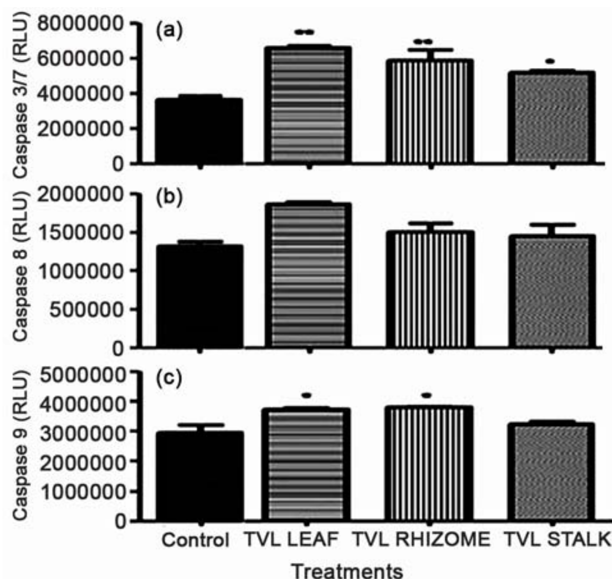

Fig. 4—The caspase concentration A) Caspase 3/7, B) Caspase 8, and C) Caspase 9 in Jurkat cells following treatment with TVL extracts. TV extracts increased caspase 3/7 expression and TV leaf and bulb significantly increased caspase 9 expression. * $p<0.05$ and ** $p< 0.001$ by comparison with the control group.

and are unusual, as caspase 3/7 and 9 expression increased significantly following treatment with all extracts, including TVL leaf. A possible explanation for this occurance is the development of secondary necrosis within the Jurkat cells following treatment. Secondary necrosis is defined as "an autolytic process of cell disintegration with release of cell components that occurs when there is no intervention of scavengers and the full apoptotic programme is completed"[33]. This process is different to primary necrosis, as it occurs after apoptosis and results in the release of activated caspase 3[33]. Activation of caspase 3/7 results in the cleavage of Poly (ADP-ribose) polymerase-1 [PARP], which can be used as a marker of apoptosis[16]. Fragments of PARP produced during cleavage have been shown to contain a molecular weight of 89 kDa and 24 kDa[15]. The present study revealed that treatment with TVL extracts results in PARP cleavage, confirming that apoptosis is taking place. Another possible explanation for induction of necrosis by TVL leaf may be the hyper activation of PARP. In some cases, PARP can become hyper activated resulting in necrosis[34].

Effect of reduced Glutathione on activity of TVL extract determination of GSH

There was a significant ($p< 0.05$) increase in GSH concentrations for TVL leaf (22.93 ± 0.05136 μM) and stalk (22.06 ± 1.944 μM) compared to the control (15.03 ± 0.3982 μM). However, there was a non-significant ($p> 0.05$) increase for TVL rhizome (18.17 ± 0.5145 μM). All extract induced GSH Production. A significant increase in GSH was seen following treatment with TVL leaf and stalk (data not shown). It is accepted that an increase in oxidative stress requires increased action by antioxidants to prevent deleterious outcomes[27]. As a result, constant exposure to increased oxidative stress can ultimately lead to a corresponding depletion of cellular antioxidants. In this study, GSH concentration significantly increased in Jurkat cells treated with TVL leaf and stalk extracts, with slight increases ($p>0.05$) following treatment with rhizome. A study by Kubec *et al.*, 2002[11] revealed varying content of S-(methylthiomethyl)-, S-methyl-, and S-ethylcysteine derivatives in the rhizomes, leaves, and stems of TVL. Cysteine is an essential component of GSH, thus the increased cysteine could contribute to increased concentration of GSH. Glutathione acts as a cofactor for glutathione peroxidase, scavenges ROS directly, buffers NO through formation of a S-nitrosoglutathione

adduct and is involved in the regulation of various other important antioxidants, such as vitamins C and E[28]. Glutathione peroxidase acts as a potent antioxidant enzyme, catalysing the reduction of H_2O_2 to H_2O and is also required for the detoxification of precursors of lipid peroxidation (lipid hydroperoxides), resulting in the formation of alcohol[29]. Another possible explanation for the GSH effect is the presence of more sulphur containing compounds in TVL stalk and leaf extracts, which may spare GSH following treatment[14].

Effect of TVL extract on mitochondrial membrane potential (MMP) using JC-1 Assay

Treatment with TV stalk (49.2 ± 0.2%) resulted in a significant ($p< 0.05$) increase in % depolarisation when compared to the control (44.10 ± 0.2%). However, treatment with all other extracts resulted in no significant change ($p>0.05$) in MMP (data not shown). Our study suggests that TVL extracts have the potential to induce cell death in a leukaemic cell line (Jurkat cells) by mitochondria mediated pathway through the involvement of caspase-3/7 in particularly in TVL rhizome. The data of the current work appear useful for further research aiming to chemically identify the specific compounds responsible for the anticancer activities of TVL extracts.

Acknowledgement

We would like to acknowledge Dr S Murugesan for review of the manuscript and the financial assistance of the National Research Foundation (NRF). The authors would also like to acknowledge all technical assistance received.

References

1 Rebecca LS, Kimberly DM & Ahmedin J, Cancer Statistics, 2015, CA Cancer, *J Clin,* 65 (2015) 5–29.
2 Mathers D, Boschi-Pinto C, Lopez AD & Murray C, "Cancer incidence, mortality and survival by site for 14 regions of the world", Global programme on Evidence for Health Policy Discussion Paper No.13 (World Health Organization), 2001.
3 Gong-Liang Z, Ying L, Jun-YZ, Qiong J, Wei-Q G, Li-Ming S & Hong-Man H, Oxidative stress-mediated anti proliferative effects of furan-containing sulfur flavors in human leukemia Jurkat cells, *Food Chem*,180 (2015)1–8.
4 Polverino AJ & Patterson SD, Selective activation of caspases during apoptotic induction in HL-60 cells, Effects of a tetrapeptide inhibitor, *J Biol Chem,* 272 (1997) 7013–7021.
5 Silva IT, Costa GM, Stoco PH, Schenkel EP, Reginatto FH & Simões CM, *In vitro* antiherpes effects of a C-glycosyl flavonoid-enriched fraction of Cecropia glaziovii Sneth, *Lett Appl Microbiol*, 51 (2010)143–148.
6 Lai PK & Roy J, Antimicrobial and chemopreventive properties of herbs and spices, *Curr Med Chem*, 11 (2004) 1451–1460.
7 Milner JA, Recent advances on the nutritional effects associated with the use of garlic as a supplement, *J Nutr*, 131 (2001) 1027S–1031S.
8 Khan N, Afaq F & Mukhtar H, Apoptosis by dietary factors: the suicide solution for delaying cancer growth, *Carcinogenesis*, 28 (2007) 233–239.
9 Pan MH & Ho CT, Chemopreventive effects of natural dietary compounds on cancer development, *Chem Soc Rev,* 37 (2008) 2558–2574.
10 Hutchings A, Scott AH, Lewis G, Cunningham A, Zulu Medicinal Plants- An Inventory, (University of Natal Press, Scottsville, South Africa), 1996.
11 Kubec R, Velısek J & Musah RA, The amino acid precursors and odor formation in society garlic (Tulbaghia violacea Harv), *Phytochemistry,* 60 (2002) 21–25.
12 Van Wyk BE & Gericke N, Peoples plants: a guide to Useful Plants of Southern Africa, 1st edn, (Brizza Publications), 2000.
13 Van Wyk B E, Van Oudtshoorn B & Gericke N, Medicinal plants of South Africa. Brizza Publications 1st Edition, 1997.
14 Moodley K, Mackraj I, Naidoo Y, Cardiovascular effects of Tulbaghia violacea Harv. (Alliaceae) root methanolic extract in Dahl salt-sensitive (DSS) rats, *J Ethnopharm,* 146 (2013) 225–231.
15 Bungu L, Frost, CL, Brauns SC & van de Venter M, *Tulbaghia violacea* inhibits growth and induces apoptosis in cancer cells *in vitro*, *Afr J Biotech,*5 (2006) 1936–1943.
16 Lyantagaye L, Methyl-α-D-glucopyranoside from *Tulbaghia violacea* extract induces apoptosis in vitro in cancer cells, *Bang J Pharm,* 8 (2013) 93–101.
17 Park HS, Hwang JH, Kim GY, Cha HJ, Kim WJ, Kim ND, Yoo YH & Choi YH, Induction of Apoptosis by fucoidan in Human Leukemia U937 cells through activation of p38 MAPK and modulation of Bcl-2 family, *Mat Drugs*, 11 (2013) 2347-2364.
18 Freimoser FM, Jakob CA, Aebi M & Tuor U, The MTT [3-(4, 5-dimethylthia- zol-2-yl)-2, 5-diphenyltetrazolium bromide] assay is a fast and reliable method for colorimetric determination of fungal cell densities, *Appl Environ-mental Micro,* 65 (1999) 3727–3729.
19 Xu XR, Zhu JQ, Ye T, Wang CL, Zhu YF, Dahms HU, Jin F & Yang Wx, Improvement of single-cell gel electrophoresis (SCGE) alkaline comet assay, *Aquat Biol*, 18 (2013) 293–295.
20 Collins, AR, The comet assay for DNA damage and repair: principles, applications, and limitations, *Mol Biotechnol, 26* (2004) 249–261.
21 Christopher L, Regina A, Felix A, Isaac T, Takuhiro U, Gideon A, Offeibea A, Doris N K & Samuel EI, Anti-proliferative effect of *Ficus pumila* L. on human leukemic cell lines, *Int J Basic Clin Pharmacol,* 4 (2015) 330–336.
22 Salaro E, Rambaldi A, Falzoni S, Amoroso FS, Franceschini A, Sarti AC, Bonora M, Cavazzini F, Rigolin GM, Ciccone M, Audrito V, Deaglio S, Pelegrin P, Pinton P, Cuneo A & Di Virgilio F, Involvement of the P2X7-NLRP3 axis in leukemic cell proliferation and death, *Sci Rep*, 25 (2016) 26280.
23 Abraham RT & Weiss A, Jurkat T cells and development of the T-cell receptor signaling paradigm, *Nat Rev Immunol*, 4 (2004) 301–308.

24 Halder B, Bhattacharya U, Mukhopadhyay S & Giri AK, Molecular mechanism of black tea polyphenols induced apoptosis in human skin cancer cells: involvement of Bax translocation and mitochondria mediated death cascade, *Carcinogenesis,* 29 (2008) 129–138.
25 Burrows CJ & Muller JG, Oxidative Nucleobase Modifications Leading to Strand Scission, *Chem Rev,* 98 (1998) 1109–1151.
26 Martin LJ, DNA Damage and Repair: Relevance to Mechanisms of Neurodegeneration, *J Neuropathol Exp Neurol,* 67 (2008) 377–387.
27 Mittler R, Oxidative stress, antioxidants and stress tolerance, *Trends in Plant Sci*, 7 (2002) 405–410.
28 Valko M, Free radicals and antioxidants in normal physiological functions and human disease, *Int J Biochem Cell Bio*, 39 (2007) 44–84.
29 Sies H, Glutathione and its role in Cellular Functions, *Free Rad Biol and Med*, 27 (1999) 916–992.
30 Tang W, Liu Q, Wang X, Wang P, Cao B, Mi N & Zhang J, Involvement of Caspase 8 in Apoptosis Induced by Ultrasound-Activated Hematoporphyrin in Sarcoma 180 Cells *in vitro*, *J Ultras Med,* 27 (2008) 645–656.
31 Jiang X & Wang X, Cytochrome C - Mediated Apoptosis, *Annual Rev Biochem*, 73 (2004) 87–106.
32 Kroemer G, Galluzzi L & Brenner C, Mitochondrial Membrane Permeabilization in Cell Death, *Physiol Rev*, 87 (2007) 99–163.
33 Silva MT, Secondary necrosis: The natural outcome of the complete apoptotic program, *Fed Euro Biochem Soc Lett*, 823 (2010) 4150–4180.
34 Zong W & Thompson CB, Necrotic death as a cell fate, *Genes Dev*, 20 (2006) 1–15.