مجلة جامعة سبها للعلوم البحتة والتطبيقية
Sebha University Journal of Pure & Applied Sciences
Journal homepage: www.sebhau.edu.ly/journal/index.php/jopas

Effect of Typha capensis crude rhizome extract on LNCaP prostate cancer cell line

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Keywords:
Typha capensis
LNCaP cells
DNA fragmentation
MTT assay
TUNEL assay

ABSTRACT

Typha capensis, commonly referred to as one of indigenous medicinal plants that are traditionally used to treat male fertility problems and various other ailments. Previous studies revealed that T. capensis has indeed a beneficial effect on male reproductive functions and aging male symptoms. LNCaP cells incubated with different concentrations of crude aqueous extract of T. capensis rhizomes (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) and control (without extract) for 24 and 96 hours, after incubation. The following parameters were evaluated: cell morphology and viability (determined by means of MTT assay). LNCaP cells showed a decline in cell viability at the incubation period 96 hours (-82.4%) more than 24 hours (-64.7%) indicating more cell death. cell early apoptosis (determined by means of Annexin V-Cy3 binding), DNA fragmentation (determined by means of the TUNEL assay). After 96 hours of exposure, all concentrations caused a dose-dependent increase in early apoptosis in the cells. At higher concentrations (10 and 100 µg/ml), a significant increase of 38.8% (P=0.009) and 52.3%(P<0.001), respectively, in the percentage of cells with signs of early apoptosis was significant. a significant increase of TUNEL-positive cells was found between the control and 1, 10, 100 µg/ml.

تأثير مستخلص جذور نبات تيفا كابينسس الخام على الخلايا سرطان البروستاتا

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الكلمات المفتاحية:
تيفا كابينسس
خلايا البروستات المسرطنه
التجزئة للحمض النووي
اختبارالحيويه للخلايا
اختبار التجزئة للحمض النووي

الملخص

تيفا كابينسس يشار إليه عادةً على أنه أحد النباتات الطبية الأصلية التي تُستخدم تقليديًا لعلاج مشاكل خصوبة الرجال وأمراض أخرى مختلفة. كشفت الدراسات السابقة أن *تيفا كابينسس* له بالفعل تأثير مفيد على الوظائف التناسلية للذكور وأعراض الشيخوخة عند الذكور. تم تحضين خلايا البروستات المسرطنه بتركيزات مختلفة من المستخلص المائي الخام لجذور *تيفا كابينسس* 0.01 ، 0.02 ، 0.1 ، 1 ، 10 و 100 ميكروغرام / مل والتحكم بدون مستخلص لمدة 24 و 96 ساعة ، بعد الحضانة. تم تقييم المعلمات التالية: مورفولوجيا الخلية وصلاحيتها تم تحديدها عن طريق مقايسة اختبار الحيويه للخلايا أظهرت خلايا البروستات المسرطنه انخفاضًا في قابلية الخلية للحياة في فترة الحضانة 96 ساعة (82.4-٪) أكثر من 24 ساعة (64.7-٪) مما يشير إلى زيادة موت الخلايا. موت الخلايا المبرمج المبكر للخلايا يتم تحديده عن طريق ربط Annexin V-Cy3 ، تجزئة الحمض النووي يتم تحديده عن طريق اختبار التجزئة للحمض النووي. بعد 96 ساعة من التعرض، تسببت جميع التركيزات في زيادة تعتمد على الجرعة في موت الخلايا المبرمج المبكر في الخلايا. في التركيزات الأعلى (10 و 100 ميكروغرام / مل) ، كانت هناك زيادة معنوية قدرها 38.8٪ (P = 0.009) و 52.3٪ (P <0.001) ، على التوالي ، في النسبة المئوية للخلايا التي ظهرت عليها علامات موت الخلايا المبرمج المبكر. تم العثور على زيادة كبيرة في الخلايا الإيجابية اختبار التجزئة للحمض النووي بين مجموعة التحكم و 1 ، 10 ، 100 ميكروغرام / مل.

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Article History : Received 01 December 2021 - Received in revised form 21 January 2021 - Accepted 26 January 2021

Introduction

1. Prostate cancer

In comparison to other types of cancer, prostate cancer progresses surprisingly slowly and has been reported to take as long as 30 years before a tumor of the prostate becomes large enough to cause detectable symptoms [1]. Men over the age of 50 are more likely to be diagnosed with prostate cancer, although it has been known to occur in younger men. Typically, cancer is detected in the prostate in most men by the age of 80 [2]. However, despite prostate cancer being quite a common occurrence in men over the age of 50, most of these men typically do not die as a result of the prostate cancer [3]. Nevertheless, African-American men generally more likely to be diagnosed with prostate cancer at younger ages, along with faster rates of prostate cancer progression, in comparison to men of other racial groups [4].

Classified as an adenocarcinoma, prostate cancer is a glandular cancer that typically starts when otherwise normal cells of the prostate begin to mutate and change into cancerous cells [5]. The most common region in which cancer of the prostate develops is the peripheral zone. Initially, small clusters of cancerous cells in the prostate stay around otherwise normal glands of the prostate. Hereafter, cancers of the prostate often tend to invade the surrounding lymph nodes, bone and other distant body sites in a process called metastasis [6].

Typical diagnosis of prostate cancer is done with a blood test, as prostate cancer has been shown to be linked with elevated prostate-specific antigen (PSA) levels. The use of PSA as a diagnostic tool since the 1980s has drastically improved screening for prostate cancer and significantly reduced the occurrence of metastatic prostate [7]. As a result, prostate cancer is now more typically localized when diagnosed, with no sign of metastasis beyond the region of the pelvis. Many different types of treatment options for localized prostate cancer exists [8]. These treatment options include active surveillance, radical prostatectomy, external beam radiotherapy and brachytherapy. Once prostate cancer reaches a state of metastasis, the deprivation of androgens, known as hormone therapy, is normally the first method of treating the cancer [9].

2. Herbal medicine

Traditional medicines, in developing countries, are commonly used due to strong cultural beliefs or as due to an inability to afford conventional medications used in Western medicine [10]. A continual effort to investigate and categorize the many different medicinal plants used all across Africa has gone largely unrecognized and hasn′t been as well documented as Indian and Chinese traditional medicines. It is projected that over 5000 plants, in Africa, have been identified as being used in some form of traditional medicine. Despite this, however, a small percentage of these identified plants have been investigated [11]. Extracts made from plants are commonly used in studies that investigate their effects on different biological systems, with regard to the bioactive compound responsible for the observed effect, do provide adequate and accurate analyses. However, it is important that the way these plants are prepared and subsequently the manner in which they are used in traditional medicine is taken into account when active compounds have been any identified from these many extracts [11]. To achieve this, the method in which the plants is normally prepared needs to undergo investigation so that the link between the observed effects and the dosage of plant preparation may be established. It must be mentioned that although the study of plants have the potential to make a significant contribution to modern medicine, often by yielding new drugs with an array of applications, the way these plants are intended to be used in traditional medince mat be entirely different [11].

3. *Typha capensis*

The *T. capensis* rhizomes are used in traditional medicine during pregnancy to ensure easy delivery, for venereal diseases, dysmenorrhea, diarrhoea, dysentery, and to enhance the male potency and libido. Typha genus contain flavones and other phenolic compounds, which exhibit anti-oxidative capacity. *Typha capensis*, commonly referred to as the bulrush, belongs to the family Typhaceae. It is usually found in wet or seasonally wet places [12]. The plant is described as a robust, reed like plant of up to three metres in height. The rhizomes are thick, fleshy and spongy and creep horizontally Medicinal uses of the rhizomes of *Typha capensis* include prescription during pregnancy to ensure easy delivery, dysmennorhea, venereal diseases, dysentery, diarrhoea, and to enhance the male potency and libido [13]. Other uses include treatment of genital problems, promotion
of fertility in women and to improve blood circulation. Its use in childbirth stems from the claim that it strengthens uterine contractions and promotes expulsion of the placenta [14,15,16,17]. The patient is prescribed one or two cups of a decoction of the roots in boiling water, which is taken daily for a week [18,19].

The phytochemistry of several species of *Typha capensis* have noted several flavones and other phenolic compounds [20], long chain hydrocarbons as well as various triterpenoids with a steroidal skeleton typhasterol have been identified [21,22]. However, the hexane extract has revealed two new phenolic compounds, namely, typharin and typhaphthalide. The acetone extract has also produced several known flavan-3-ols, which were isolated as mixtures in their free phenolic form. These include afzelechin, epiafzelechin, catechin and epicatechin. All the above compounds have previously been isolated from other plant species.

The crude methanolic extract of *T. capensis* showed inhibition of growth of some of the bacteria, which are believed to be pathogenically responsible for the conditions, which this plant is used to treat [13].

Typha capensis has anti-inflammatory,diuretic, immunosuppressive activity and is a well-known traditional medicine. Chemical properties are due to presence of sterol [19] typhaphthalide, typharin [13], flavonolglycoside [22], essential fatty acids and phenolic acids [23]. Even though *Typha capensis* has good nutritive properties, its antioxidative property remains untouched. Synthetic antioxidants have untoward side effects, hence, it is essential that a superior drug is isolated from plant sources. Also, an in vitro study, investigating the effect of *Typha capensis* on various sperm parameters showed that an aqueous extract of the rhizomes had significant detrimental effects on sperm motility, viability, sperm ROS production as well as on sperm $\Delta\psi m$ [24].

4. Aim of the study

The aim of the study was to investigate the effect of *Typha capensis* as one of South Africa′s indigenous medicinal plants and it′s phytochemicals on male reproductive function.

Material and methods:

Collection and processing of *Typha capensis* rhizomes

After collections the rhizomes were washed, chopped into 2-3 cm pieces and allowed to dry at 25ºC in a dried oven. Thereafter, the dried rhizomes were milled into a fine powder for the extraction process. For preparation of the aqueous rhizome extract the powdered rhizomes were infused in hot (75-80 °C) distilled water (50g dried rhizome extract /1L distilled water). The resulting mixture was allowed to cool down at room temperature for 2 hour, after which it was filtered (Qualitative filter paper 32.0 cm). The filtrate was then frozen at -20ºC and finally freeze-dried using a Vertis-freeze drier to yield the water-soluble extract.

According to traditional healers‟ suggestion, a handful of dried rhizome is used for the treatment of patients per patient is take in per day. Three handfuls of the rhizomes were weighed out and an average of 70 g was obtained. The aqueous extraction yielded an average of 7.65 g of the extract per 50 g dried rhizome on presumption that an average male weighs 75kg; the extract concentration was calculated. A stock solution of 10000 µg/ml of *T.capensis* was prepared in Dulbecco‟s Modified Eagle Medium / Nutrient Mixture F12 Ham

(DMEM/F-12, 1:1 mixture) or Roswell Park Memorial Institute (RPMI) 1640 Medium or Keratinocyte-SFM (1X) serum Free Medium for cell culture-related assays (depending on the experimental design and the cells used). These stock solutions underwent serial dilutions to yield different concentrations. The Medium used depended on the cells used the cell culture.

LNCaP prostate cancer cell line

The LNCaP prostate cancer cell line derived from a needle aspirate biopsy in 1977 from a 50-year old male, diagnosed with stage D prostate cancer. This cell line was purchased from ATCC. LNCaP cells are an ideal model for investigating early prostate cancer. Cells were cultured in 25 and 75 cm2 sterile tissue culture flasks and were passaged at regular intervals when cells reached approximately 80-90% confluency. The culture medium was aspirated from the flasks and cells were washed with 2-4 ml sterile PBS. Thereafter, 0.5 - 1 ml 1X 0.25% trypsin/EDTA were added to the flask, gently shaken and under occasional visual control incubated at 37°C until cells began to detach. This process took approximately 4-7 minutes. In order to inactivate the trypsin, 2 ml of RPMI 1640 were added and cells were re-suspended by careful aspiration using a pipette. Finally, the cell suspension was transferred from the flask to a 15 ml test tube and centrifuged at 125 xg for 5 minutes. The supernatant was discarded and the cell pellet re-suspended in 5 ml of RPMI 1640. Following this, 1 ml of the cell suspension was transferred into a new tissue culture flask with fresh RPMI 1640 growth medium and passage recorded. Afterwards, LNCaP cells, which appeared to be growing at a slow rate, were left for up to 48 hours to re-attach. Fresh growth medium was then added to the first flask and incubated for further growth. The cells were then either passaged into a new 25 cm2 and 75 cm2 flasks, respectively, with fresh medium or seeded into 6-, 24- or 96-well plates. A dilution of cells was made to produce the final cell number required for experiments. Cell morphology was observed and compared with cell viability.

LNCaP cell viability

LNCaP cells were grown to 80% confluence and then trypsinated with 2 ml 0.25% trypsin/EDTA. To inactivate, 2 ml of RPMI 1640 growth medium were added. Subsequently, the cells were seeded at 8×103 cells/well in 200 µl of complete culture medium for a 24-hours exposure and 3×103 cells/well in 200 µl of complete culture medium for a 96-hours exposure in a sterile 96-well plate. After exposing cells to various concentrations of the *T. capensis* rhizome extract for 24 and 96 hours, respectively. Thereafter, 20 µl of MTT (5 mg/ml in PBS) were added to each well. The plates were incubated 37ºC for an additional 4 hours. Then, the medium was removed from the wells and the formazan crystals formed by the reduction of MTT in living cells were solubilized in 100 µl of dimethylsulfoxide (DMSO). Subsequently, the optical density (OD) of the samples was measured with an ELISA reader (GloMax Multi Detection System). The absorbance was read at 560 nm.

Determination of apoptosis

Determination of early apoptotic events by means of Annexin V-Cy3 binding

Annexin V binds to phosphatidyl-serine, which translocates from the inner to the outer leaflet of the plasma membrane as an early sign of apoptosis. Annexin is a family of calcium binding proteins that bind phospholipids in the presence of calcium [25].

Cell surface phosphatidyl-serine was detected by Annexin V conjugated with the fluorophore Cy3 using the commercially available Annexin V-Cy3.18 conjugate (AnnCy3) apoptosis detection kit. This kit also comprises 6-carboxyfluorescein diacetate (6-CFDA) to detect live cells.

The binding of Annexin V-Cy3 to phosphatidyl-serine is observed as red fluorescence. On the other hand, 6-carboxyfluorescein is used for the detection of living cells. When 6-CFDA enters living cells, cellular esterases hydrolyze it producing a fluorescent compound, 6-carboxyfluorescein (6-CFDA), which is observed as green fluorescence. Therefore, when cells are incubated with both AnnCy3 and 6-CFDA three staining patterns are detected with this double-staining procedure: (1) live cells stain only with 6-CFDA (green), (2) necrotic cells stain only with AnnCy3 (red), and (3) cells in the early stages of apoptosis stain with 6-CFDA (green) and AnnCy3 (red).

Determination of DNA fragmentation by means of the TUNEL assay

To determine DNA fragmentation, which indicates an endpoint of apoptosis, the Dead End Colorimetric TUNEL System kit was used. This is a non-radioactive assay which allows for accurate and rapid detection of DNA fragmentation.

For cells better sticking to the slide, poly-L-lysine was diluted 1:10 in distilled water and slides were coated by spreading 200 µl of the solution in water onto the surface of a clean glass slide. Once dry, slides were rinsed in distilled water and allowed to air dry for approximately 30 minutes and then stored at 4°C.

Statistical Analysis

All statistical calculations were performed using the MedCalc statistical software (Version 12.3; Mariakerke, Belgium). After testing for normal distribution by means of the Kolmogorov-Smirnov test, appropriate statistical tests, either parametric (Pearson correlation, repeated measures one-way ANOVA, paired samples (t-test) or non-parametric (Spearman Rank correlation, Wilcoxon test) were performed. Data were expressed as mean ± SEM. A P-value of $P<0.05$ was considered significant.

Results

Effect of *T. capensis* crude rhizome extract on LNCaP cell viability

The viability of LNCaP cells as well as cell morphology after being exposed to different concentrations of *T. capensis* crude rhizome extract (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) was observed and recorded. The morphology of LNCaP cells was found to have changed after exposure, there was a clear increase in detachment and clumping of cells and apoptotic bodies could also be seen indicate to cell death.

The viability of LNCaP cells was tested after exposure to different concentrations of *T. capensis* crude rhizome extract (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) by means of the MTT test. After 24 hours of exposure values at all concentrations decreased. As from 0.01 µg/ml values declined (-5%) and reached a lowest value (-64%) at 100 µg/ml when compared to the control. One-way ANOVA exhibited a significant trend (ANOVA trend analysis: $P<0.0001$). (Figure 1).

The viability of LNCaP cells was tested after exposure to different concentrations of *T. capensis* crude rhizome extract (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) by means of the MTT test. After 96 hours of exposure viability for all concentrations decreased. As from 0.01 µg/ml, values declined (-7%) and reached a lowest value (-82%) at 100 µg/ml ($P < 0.0001$) when compared to the control. One-way ANOVA exhibited a significant trend (ANOVA trend analysis: $P<0.0001$). (Figure 2).

Results reveal that the viability of LNCaP cells at the highest concentrations (10, 100 µg/ml) showed declines in cell viability at the incubation period 96 hours (-72 and -82.4%) more than 24 hours (-57.8 and -64.7%) indicate to more cell death.

Effect of *T. capensis* crude rhizome extract on Annexin V-Cy3 binding in LNCaP cells

The effect of *T. capensis* rhizome extract F1 fraction of the summer season on Annexin V-Cy3 binding to LNCaP cells as an indicator of apoptosis was analysed after exposure to different concentrations (0.01, 0.02, 0.1, 1, 10 and 100µg/ml). After being exposed for 24 hours at low concentrations (0.01, 0.02, 0.1 and 1 µg/ml), an increase of Annexin V-Cy3 binding indicated early signs of apoptosis when compared to the control. At higher concentrations (10 and 100µg/ml), increases of +28% ($P=0.00199$) and +33.8% ($P=0.00163$), respectively in the percentage of cells with signs of early apoptosis were significant. One-way ANOVA exhibited a significant positive trend (ANOVA trend analysis: $P<0.0001$) (Figure 3).

After 96 hours of exposure, all concentrations caused a dose-dependent increase in early apoptosis in the cells. At higher concentrations (10 and 100 µg/ml), a significant increase of 38.8% ($P=0.009$) and 52.3%($P<0.001$), respectively, in the percentage of cells with signs of early apoptosis was significant. One-way ANOVA exhibited a significant position trend after exposure for 96 hours (ANOVA trend analysis: $P<0.0001$) (Figure 4).

DNA fragmentation in LNCaP cells

LNCaP cells were incubated with increasing concentrations of *T. capensis* rhizome extract F1 fraction of the summer season (0.01,

0.02, 0.1, 1, 10 and 100 µg/ml) and analysed for DNA fragmentation. After exposure of LNCaP cells for 24 hours at higher concentrations (1, 10, 100 µg/ml) the percentage of TUNEL-positive cells increased by +14%, +21.6% and +29%, respectively, in a dose-dependent manner. No significant change between the control and 0.1 µg/ml (P=0.066) was found. However, a significant dose-dependent increase in TUNEL-positive cells was found between the control and 1, 10 and 100 µg/ml (P=0.0047, P=0.003 and P=0.001, respectively). One-way ANOVA exhibited a significant trend (ANOVA trend analysis: P<0.0001) (Figure 5).

After exposure for 96 hours, LNCaP cells exhibited an increasing percentage of TUNEL-positive cells at higher concentrations (1, 10, 100 µg/ml) of +16.67, +25.6 and +34%, respectively. No significant effect between the control and 0.1 µg/ml (P=0. 0624). However, a significant increase of TUNEL-positive cells was found between the control and 1, 10, 100 µg/ml (P=0.0028, P=0.001 and P<0.0001, respectively). One-way ANOVA exhibited a significant trend (ANOVA trend analysis: P<0.0001) (Figure 6).

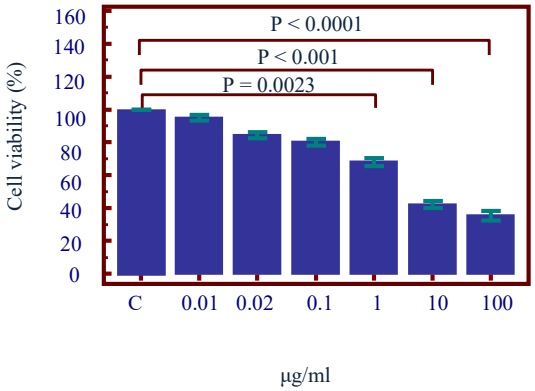

Figure 1: LNCaP cells viability (determined by means of MTT assay).

Incubation with different concentrations of *T. capensis* crude rhizome extract (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) for 24 hours, viability for all concentrations decreased as from 0.01 µg/ml values declined (-5%) reached a lower value (-57%) at 10 µg/ml (P<0.001) when compared to the control. However, at the higher concentrations (100 µg/ml) a significant decrease of -64% (P<0.0001) was observed. One-way ANOVA exhibited a significant trend (ANOVA trend analysis: P<0.0001).

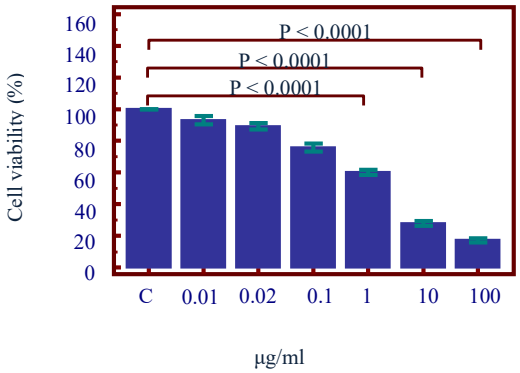

Figure 2: LNCaP cells viability (determined by means of MTT assay).

Incubation with different concentrations of *T. capensis* crude rhizome extract (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml), for 96 hours, viability for all concentrations decreased as from 0.01 µg/ml values declined (-7%) reached a lower value (-72%) at 10 µg/ml (P < 0.0001) when compared to the control. However, at higher concentration (100 µg/ml) a significant decrease of -82.4% (P<0.0001) was observed. One-way ANOVA exhibited a significant trend (ANOVA trend analysis: P<0.0001).

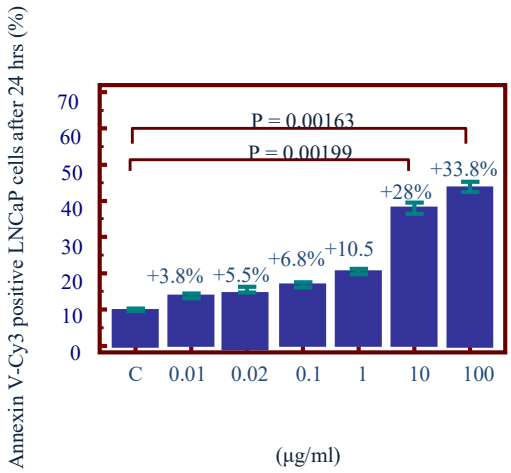

Figure 3: Effect of *T. capensis* rhizome extract F1 fraction of the summer season (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) on Annexin V-Cy3 binding indicating cell apoptosis in LNCaP cells. After exposure for 24 hours at low concentrations (0.01, 0.02, 0.1 and 1 µg/ml), LNCaP cells showed signs of early apoptosis. At higher concentrations (10 and 100 µg/ml), the increase of +28% (P=0.00199) and +33.8% (P=0.00163), respectively, in the percentage of cells with signs of early apoptosis is sinificant. One-way ANOVA exhibited a significant positive trend (ANOVA trend analysis: P<0.0001).

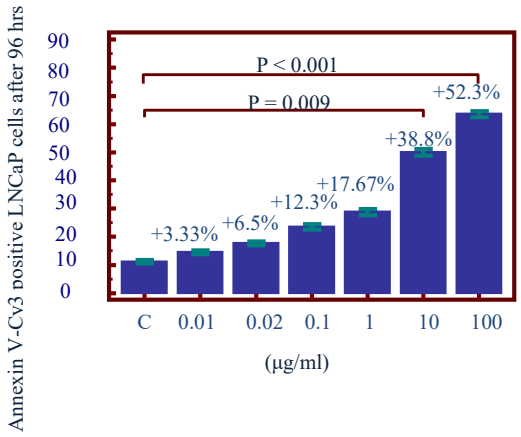

Figure 4: Effect of *T. capensis* rhizome extract F1 fraction of the summer season (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) on Annexin V-Cy3 binding indicating cell apoptosis in LNCaP cells. After 96 hours of exposure, cells showed a dose-dependent increase in early apoptosis when compared to the control. At higher concentrations (10 and 100 µg/ml) a significant increase of +38.8% (P=0.009) and +52.3% (P<0.0001), respectively in the percentage of cells with signs of early apoptosis is evident. One-way ANOVA exhibited a significant positive trend after exposure for 96 hours (ANOVA trend is: P<0.0001).

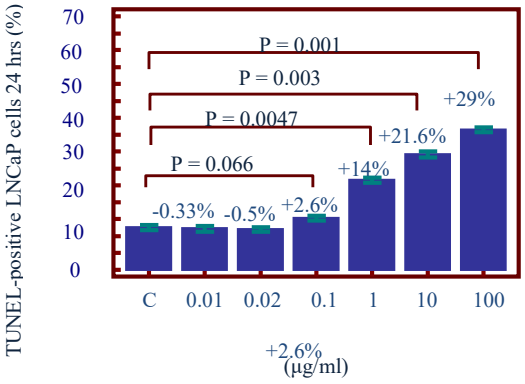

Figure 5: Effect of *T. capensis* rhizome extract F1 fraction of the summer season (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) on DNA fragmentation in LNCaP cells. After exposure of LNCaP cells for 24

hours at higher concentrations (1, 10, 100 µg/ml), a dose-dependent increase in the percentage of TUNEL-positive cells by 14%, 21.6% and 29%, respectively, is seen. This is no differance between the control and the extract concentration of 0.1 µg/ml (P=0.066). However, a significant dose-dependent increase of TUNEL-positive cells was found between the control and 1, 10 and 100 µg/ml (P=0.0047, P=0.003 and P=0.001, respectively). One-way ANOVA exhibited a significant trend (ANOVA trend analysis: P<0.0001).

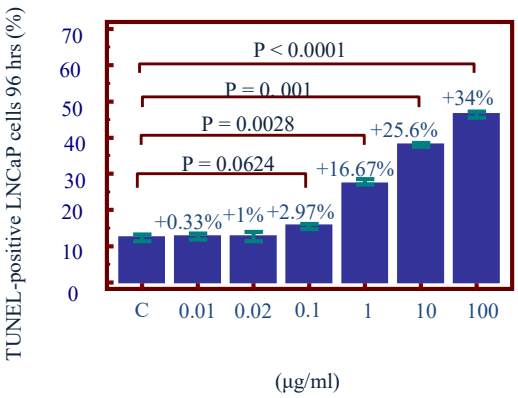

Figure 6: Effect of *T. capensis* rhizome extract F1 fraction of the summer season (0.01, 0.02, 0.1, 1, 10 and 100 µg/ml) on DNA fragmentation in LNCaP cells. After exposure for 96 hours, LNCaP cells exhibited an increasing percentage of TUNEL-positive cells at higher concentrations (1, 10, 100 µg/ml) of 16.67, 25.6 and 34%, respectively. No significant effect between the control and 0.1 µg/ml (P=0.0624) is evident. However, a significant increase of TUNEL-positive cells was found between the control and 1, 10, 100 µg/ml (P=0.0028, P=0.001and P<0.0001, respectively). One-way ANOVA exhibited a significant trend (ANOVA trend analysis: P<0.0001).

Discussion

Cytotoxic effects of *T. capensis* aqueous rhizome extract on LNCaP cells,

Nevertheless, many plants investigated for suggested anti-cancer abilities often prove true. A study conducted by [26], for example, investigated anticancer claims of eurycomanone which is a cytotoxic compound found in *Eurycoma longifolia* Jack. These authors demonstrated the cytotoxicity of eurycomanone, killing 50% of HeLa cells, reducing viability and proliferation significantly, thus making it a potent anti-proliferative agent. Moreover, [27] demonstrated the cytotoxicity of eurycomanone against human hepato carcinoma cells, suggesting that eurycomanone is cytotoxic on cancerous liver cells, HepG2 and less toxic on normal cells Chang's liver and WLR-68. Similarly, [28]reported that eurycomanone was cytotoxic on cancerous cells (CaOv-3, HeLa, HepG2, HM3KO, MCF-7) and less toxic on normal cells (MDBK, Vero), demonstrating the potential of plants to act as effective anticancer agents [29].

This possibility of anticancer effects is true for many plants as determined [30], showing the effects of crude aqueous extracts of a panel of medicinal plants on the growth and invasion of cancer cells, showing that extracts of *Larrea tridentata* (Creosote Bush) and *Juniperus communis* (Juniper Berry) significantly decreased the growth of MCF-7/AZ breast cancer cells [31].

In the current study, the MTT assay was used to determine the cytotoxicity of *T. capensis.* when the LNCaP cells were exposed to the aqueous *T. capensis* rhizome extract over 24 and 96 hours respectively, a significant decrease in cell viability was noted at all concentrations of the extract, indicating a reduced mitochondrial dehydrogenase activity. Moreover, at both the 24-hour and 96-hour exposure, LNCaP cells revealed a remarkable dose-dependent decrease in viability, cellular death is clearly evident at 10 and 100 µg/ml. The results present that the cancer cells, in contrast to the non-cancerous TM3 cells, are stressed and dying under the cytotoxic effects of *T. capensis* at all concentrations. This confirms the idea that this extract has anticancer abilities and warrants its use in this manner.

Effects of *T. capensis* aqueous rhizome extract on cell early apoptosis in LNCaP,

When LNCaP cells were exposed to *T. capensis* rhizome extract F1 fraction of the summer season for 24 and 96 hours, LNCaP cells shown to increase in a dose-dependent manner with significant increase at the higher concentration. All concentrations yielded an increase in percentages of early signs of apoptosis. At higher concentrations (10 and 100 µg/ml), a significant increase between of +28% to +52% in the percentage of early signs of apoptosis was significant (P<0.001). This confirms that *T. capensis* rhizome extract F1 fraction of the summer season induces early signs of apoptosis in LNCaP cells.

Effects of *T. capensis* aqueous rhizome extract on DNA fragmentation in LNCaP

Apoptosis occurs normally during development, aging, and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defence mechanism such as in immune reactions or when cells are damaged by disease or noxious agents [32]. Apoptosis is a programmed cell death characterized by morphological features and extensive DNA fragmentation [33].

However, after exposing LNCaP cells were exposed for 24 and 96 hours the *T. capensis* rhizome extract F1 fraction caused enough DNA damage characterized by stained cell nuclei at high concentrations (1, 10 and 100 µg/ml) to drive the cell toward decreasing capacity of cell repair mechanisms, fragmentation and cell death. This confirms that treatment with *T. capensis* rhizome extract F1 fraction induced DNA fragmentation in the cancer cell line LNCaP in a concentration-dependant manner.

[34] concluded that LNCaP cells treated with extracts from *Epilobium parviflorum* and *Epilobium hirsutum*. Incubation of LNCaP cells with aqueous Epilobium extracts (20, 50, 70 mg/ml). Cell apoptosis was determined by staining with propidium iodide (PI) and Annexin V–fluorescein isothiocyanate. This resulted in a reduction of proliferation in a concentration-dependent manner. The percentage of late apoptotic cells was significantly increased, in a dose-dependent manner, and apoptotic cells in the early phase of apoptosis (Annexin V+/PI–). Cells treated with extracts, especially in lower concentration of 20 mg/ml, were necrotic the percentage of necrotic cells was statistically significant.

In a study conducted by [35], the cytotoxic and apoptotic activities of *Ficus pseudopalma* (FP) Blanco leaf extracts were investigated against the human prostate PRST2 cancer cell line. A significant decrease in the viability of PRST2-treated cells (P <0.001) in a concentration-dependent manner was observed. Using APO-BrdU TUNEL assay, it was observed that the apoptotic activities of the extracts increased in a concentration-dependent manner.

Conclusion

The objective of this study was to investigate the effect aqueous rhizome extract toward cell viability and DNA fragmentation in the cancer cell line LNCaP in a concentration-dependant manner. Results obtained clearly demonstrated that LNCaP cell viability were significantly higher after exposure to aqueous rhizome extract.

Moreover, this study, for the first time investigated the effects of bioactive aqueous rhizome extract of *Typha capensis* rhizomes toward male reproductive functions, health and towards the treatment of prostate cancer. Furthermore, high dosages of the active compounds were shown to have negative effect on the percentage of DNA fragmentation in LNCaP cells when compared to the effect of the low dose. This may indicate that although seemingly effective toward the treatment of prostate cancer, further investigation into the ideal dosage, along with the safety of the dose may be required.

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