European Journal of Therapeutics
pISSN: 2564-7784
eISSN: 2564-7040

Eur J Ther. 2023;29(3):298-306.
https://doi.org/10.58600/eurjther1625

Original Research

Evaluation of in Vitro Cytotoxic and Apoptotic Effect of Tarantula Cubensis Alcoholic Extract on Human Prostate Cancer Cells

Deniz Gul[1], Haci Ibrahim Cimen[2], Asuman Deveci Ozkan[3], Gamze Guney Eskiler[3], Huseyin Cakıroğlu[4], Yavuz Tarik Atik[1], Anil Erdik[5], Osman Kose[2], Hasan Salih Saglam[2], Ahmet Gokce[2]

[1]Department of Urology, Sakarya Training and Research Hospital, Sakarya, Turkey
[2]Department of Urology, Faculty of Medicine, Sakarya University, Sakarya, Turkey
[3]Department of Medical Biology, Faculty of Medicine, Sakarya University, Sakarya, Turkey
[4]Experimental Medicine Research and Application Centre, Sakarya University, Sakarya, Turkey
[5]Department of Urology, Sakarya Toyotasa Emergency Hospital, Sakarya, Turkey

Received: 2023-05-31 / **Accepted**: 2023-06-20 / **Published Online:** 2023-06-26

Correspondence
Deniz Gul, MD
Sakarya Training and Research Hospital, Department of Urology,
Address: Adnan Menderes Cd. Saglik Sk. No:195 Adapazari, Sakarya, Turkey
E-mail: dr.guldeniz@gmail.com

ABSTRACT

Objective: Prostate cancer (PCa) is the second most common cancer in men worldwide and few studies have been reported investigating the effects of homeopathic therapy on PCa. Tarantula cubensis alcoholic extract (TCAE), is used in veterinary medicine as homeopathic medicine and there are various studies about the therapeutic efficacy of TCAE in treating different diseases. However, studies about the efficacy of TCAE in cancer treatment are limited.

It aimed to investigate the therapeutic efficacy of TCAE, which is used as homeopathic medicine in PCa.

Methods: DU-145 and LNCaP cells were used as PCa cell lines, and HUVEC cells were used as control cell lines. The effect of TCAE (25, 50, 100 and 250 µM) on cell viability was evaluated by Water Soluble Tetrazolium Salts-1 (WST-1) analysis, and its apoptotic effects were assessed by Annexin V analysis and acridine orange staining.

Results: TCAE decreased the viability rates in DU-145 and LNCaP cells in a time-dependent manner ($p<0.01$). The lowest viability rates for DU-145 and LNCaP cells were determined as 62.76±4.21% and 55.68±1.84% at 250 and 25 µM doses, respectively, for 48 h ($p<0.01$). Moreover, TCAE did not induce any cytotoxic effect on HUVEC cells ($p<0.01$). Apoptotic cell rates were found as 30.45±0.78% and 45.02±1.32% in DU-145 and LNCaP cells at 250 and 25 µM TCAE, respectively ($p<0.01$). Furthermore, impaired cell/cytoplasm ratio, chromatin condensation, membrane blebbing, and vacuolar damage were observed in DU-145 and LNCaP cells.

Conclusion: TCAE exerts cytotoxic and apoptotic effects on PCa cells. Additionally, due to androgen receptor status, LNCaP cells were more sensitive than DU-145 cells. However, further molecular studies are needed to determine the potential of TCAE as a new chemotherapeutic agent in PCa.

Keywords: Apoptosis; DU-145; LNCaP; Prostate cancer; Tarantula cubensis alcoholic extract

INTRODUCTION

Prostate cancer (PCa) is the second most common cancer in men worldwide [1]. Age, genetic, metabolic, hormonal and infectious-related factors and diet are considered to be risk factors associated with PCa, but the underlying causes of its onset and progression have not been fully elucidated [2]. There are different treatment options with or without combinations for localized disease and metastatic disease. All these treatment options have significant and severe side effects, and consequently, to avoid these side effects, different treatment investigations have become the focus of attention of researchers.

Homeopathic treatment is included in complementary and

alternative medicine practices. In this treatment, the principle is that ‘’a substance that can cause symptoms in a healthy person may promote self-healing in a person with a disease with similar symptoms’’ [3]. Few studies have been reported investigating the effects of homeopathic therapy in PCa. The treatment response to the different homeopathic agents used in most of these studies is controversial [4-6]. However, it has been reported that the homeopathic medicine has positive effects on PCa cells due to reducing cell proliferation [7].

Tarantula cubensis alcoholic extract (TCAE), used in veterinary medicine as homeopathic medicine, is obtained by processing the entire ‘Tarantula cubensis’ spider in 60% alcohol [8]. Many therapeutic effects have been described for TCAE, such as; anti-inflammatory, demarcating, antiphlogistic, necrotizing, and resorptive influences, and it is effective in wound healing [9] respectively, and 29% of those cured by the tarantula poison (Theranekron. Despite the underlying mechanisms of the TCAE effect is not clear, it is thought to activate the defense mechanism against inflammation and proliferation [10]. The remedy can separate healthy tissues at the cellular level with its demarcating effect, and rapid healing and epithelialization are provided with its regenerative effect [11]. With this regard, there are various studies in the literature about the therapeutic efficacy of TCAE in treating different diseases. It is effective in peripheral nerve [12], tendon [13] and open wound [14] healing. Clinical benefits have been reported in oral, skin and teat papillomatosis in animals [15-17]. On the other hand, studies about the efficacy of TCAE in cancer treatment are limited. The positive results of some in vivo or in vitro studies on breast and colon cancer increase the importance of TCAE in cancer treatment [18-21]. However, there is no study in the literature about the effect of TCAE on PCa. In this present study, we aimed to investigate the therapeutic efficacy of TCAE, which is a homeopathic medicine, in PCa.

Main Points;

- Prostate cancer is one of the most common cancers in men, and alternative methods with fewer side effects are worth investigating in its treatment.
- Tarantula cubensis alcoholic extract (TCAE) is a homeopathic drug used in veterinary medicine, has anti-inflammatory and antioxidant effects, and has shown antitumoral effects on various cancers.
- This study has shown that TCAE has significant anti-tumoral effectiveness on prostate cancer cells.
- The anti-tumoral activity of TCAE in prostate cancer cells has been demonstrated as having both cytotoxic and apoptotic effects on two different types of prostate cancer cells, suggesting that TCAE may be beneficial as an alternative option in the treatment of prostate cancer.

MATERIALS AND METHODS

Cell culture

In this study, DU-145 and LNCaP cells were used as PCa cells in all analyses, and human umbilical vein endothelial cells (HUVEC) were used as a control cell only in the viability assay. All cells were purchased from the American Type Culture Collection (ATCC). TCAE (Theranekron®) was commercially purchased from Richter Pharma AG. The DU-145 and LNCaP cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640, Gibco) medium, and HUVEC cells were grown in Dulbecco’s modified eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS Thermo Fisher Scientific) and 1% penicillin/streptomycin (Gibco). All cells were incubated in a humidified incubator with 5% CO_2 at 37 °C.

Cell Viability Assay

To determine the cytotoxic effects of TCAE, a WST-1 cell viability assay was performed. For this purpose, an equal number of DU-145, LNCaP and HUVEC cells ($2x10^4$ cells/well) were seeded on a 96-well plate. After 24 h, the cells were treated with different concentrations of TCAE (25, 50, 100 and 250 µM) for 24 and 48 h. Following administration of TCAE, 10 µl of WST-1 reagent (Biovision) was added to each well with 100 µl medium and incubated at 37 °C for 30 min. After incubation time the absorbance values from the wells were obtained from the microplate reader (Allsheng) at 450nm. We selected the most effective exposure time and concentration of TCAE for further analysis. Each experiment was performed in triplicate.

Annexin V Assay

The apoptotic effects of TCAE on PCa cells were determined with Muse Annexin V & Dead Cell Assay. For this purpose, an equal number of DU-145 and LNCaP cells ($1x10^5$ cells/well) were seeded on a 6-well plate. After 24 h, the cells were treated with different concentrations of TCAE (25, 50, 100 and 250 µM) for 48 h. Following administration of TCAE, the cells were centrifuged at 1200 rpm and the cell pellet was washed with 1 ml of PBS twice and stained with MuseTM Annexin V & Dead Cell Assay Kit (Millipore) at room temperature for 30 min. After 30

min incubation, the stained cells were analyzed with the Muse Cell Analyzer (Millipore) according to the instructions. Each experiment was performed in triplicate.

Acridine Orange (AO) Staining

To further analyze the apoptotic effects of TCAE on PCa cells, the cell morphology was observed with AO staining. For this purpose, an equal number of DU-145 and LNCaP cells ($1x10^5$ cells/well) were seeded on a 6-well plate. After 24 h, the cells were treated with different concentrations of TCAE (25, 50, 100 and 250 µM) for 48 h. Following administration of TCAE, the cells were fixed with 1 ml of 4% paraformaldehyde (PFA) and stained with 1 ml of AO (100 mg/ml) dye at room temperature and dark for 30 minutes. Stained cells were visualized with EVOS FL Cell Imaging System (Thermo Fisher Scientific) according to the instructions.

Statistical Analysis

SPSS 22.0 (IBM, USA) was used for statistical analysis, and the results were expressed as the mean ± standard deviation of three independent experiments. One-way analysis of variance (ANOVA) followed by Tukey's test was used for multiple comparisons. The relationship between drug dose and viability rates was evaluated with the Pearson correlation test, and p-values less than 0.05 were considered statistically significant.

RESULTS

Evaluation of the Cytotoxic Effects of TCAE on PCa Cells

WST-1 was performed in DU-145 and LNCaP also HUVEC cells to determine the cytotoxic effect of TCAE on PCa cells. Our results showed that TCAE had a cytotoxic effect on two different types of PCa cells. According to our results, TCAE decreases the viability of cells time-dependently ($p<0.01$, Figure 1). After 48 h incubation with 25, 50, 100, and 250 µM TCAE, the viability of DU-145 cells significantly reduced to 68.13±1.93%, 68.15±1.72%, 66.14±4.76% and 62.76±4.21%, respectively ($p<0.01$, Figure 1A). Additionally, the viability of LNCaP cells reduced to 55.68±1,84%, 57.11±2.06%, 59.44±0.66%, and 62.54±0.78%, respectively for 48 h ($p<0.01$, Figure 1B). Furthermore, after incubation with TCAE (25, 50, 100, 250 µM) for 48 h, the proliferation of HUVEC cells was detected as 88.63±0.09%, 113.94±0.41%, 111.74±1,30% and 99.04±1.02%, respectively ($p<0.01$, Figure 1C). Therefore, TCAE treatment for 48 hours was more effective than 24-hour treatment in both DU-145 and LNCaP cells and did not cytotoxicity in HUVEC cells ($p<0.01$). In addition, the most effective dose of TCAE was 250 and 25 µM in DU-145 cells and hormone-sensitive LNCaP cells, respectively. These findings showed that LNCaP cells were more sensitive to TCAE than DU-145 cells.

Evaluation of the Apoptotic Effects of TCAE on PCa Cells

Annexin V analysis was performed in DU-145 and LNCaP cell lines to determine the effect of TCAE on apoptotic cell death. The obtained results demonstrated that TCAE increased the percentage of apoptotic cells in different types of PCa cells and showed a significant increase, especially in the percentage of early apoptotic cells ($p<0.01$, Figure 2). After 48 h of incubation with 25, 50, 100, and 250 µM TCAE, and the percentage of total apoptotic cells amounted to 22.54±1.00%, 23.53±1.12%, 25.86±0.44%, and 29.80±0.39% in DU-145 cells, respectively ($p<0.01$, Figure 2A, 2C). Additionally; 25, 50, 100, 250 µM TCAE concentrations increased the percentage of total apoptotic cells (44.06±1.58%, 36.65±0.84%, 36.99±0.73%, and 28.43±2.37% respectively) in LNcaP cells for 48 h ($p<0.01$, Figure 2B, 2C).

Evaluation of the Morphological Effects of TCAE on PCa Cells

AO staining was performed to demonstrate the morphological changes caused by TCAE in PCa cells associated with apoptotic cell death. Our results revealed that TCAE caused morphological changes associated with apoptotic cell death in two different types of PCa cells (Figure 3). At the end of 48 hours, impaired cell/cytoplasm ratio, chromatin condensation, membrane blebbing and vacuolar damage were observed in PCa cells due to the increasing concentration of TCAE compared to the control group (Figure 3). However, TCAE caused more apoptotic changes in the cell morphology of LNCaP cells than DU-145 cells.

DISCUSSION

The anti-cancer effects of TCAE have been shown in clinical veterinary medicine. However, it has not yet been fully elucidated the underlying molecular mechanisms of TCAE in human cancers. Although there are various investigations on its effects on breast and colon cancer, there has been no study on its impact on PCa. In this study, for the first time, the cytotoxic and apoptotic effects of TCAE on two different types of PCa cells were determined in vitro. The findings showed that TCAE decreased viability rates and increased apoptotic cell rates in PCa cells. Thus, with the present study, data supporting that TCAE may be a potential anti-cancer therapeutic agent in the treatment of PCa were obtained.

Clinical observations and research on the homeopathic effects of TCAE have primarily been on wound healing. It has been shown that TCAE stimulates and accelerates epithelization [11,14] and has anti-inflammatory effects on animals [22]. Makav et al. [23] reported that TCAE has therapeutic efficacy in an experimentally generated gastric ulcer model in rats, exhibits lower gastric erosion and better efficacy than ranitidine, and causes a decrease in the levels of pro-inflammatory cytokines IL-1β and IL-6.

Few studies are evaluating the effects of TCAE on the genitals. It has been shown that TCAE accelerates postpartum uterine regeneration in cows [24]. Kozlu et al. [25] suggest that TCAE may have remedial effects in an experimentally generated ischemia-reperfusion injury model in the rat ovary. It has also been reported that TCAE causes atrophy in endometriosis foci in an experimentally established endometriosis model in rats [26] and the results of this study may suggest that TCAE may also have hormonal effects. However, its mechanism of action is unclear.

Studies about cancer treatment have shown that TCAE is effective in treating some types of cancer (breast and colon) [19,20,27]. However, the first study that pioneered and guided these studies was reported by Koch and Stein in 1980. In this study, TCAE inhibits tumor growth by forming a demarcation line in canine mammary tumors [28]. More recently, Gultiken and Vural [18] investigated the efficacy of TCAE in canine mammary tumors and stated that TCAE administration is beneficial in terms of tumor regression, recurrence and metastasis. In addition, Er et al. [21], in their study investigating the effects of TCAE in an experimentally generated colon cancer model in rats, is reported that TCAE may have an anti-cancer effect and increased TNF-α level.

In the literature, studies evaluating the effects of homeopathic medicines on PCa are limited, and the first study was reported by Jonas et al. in 2006. In this study, five different homeopathic drugs widely used have no effect on cell viability, gene expression, and apoptosis in DU-145, LNCaP and MAT-LyLu PCa cells. However, in the same study, in rats injected with MAT-LyLu PCa cells, a reduction in tumor incidence and volume and an increase in apoptotic cell death are reported after treatment with homeopathic drugs [4]clinical and laboratory research has been equivocal, and no rigorous research has been done on cancer. In 1999, the US National Cancer Institute

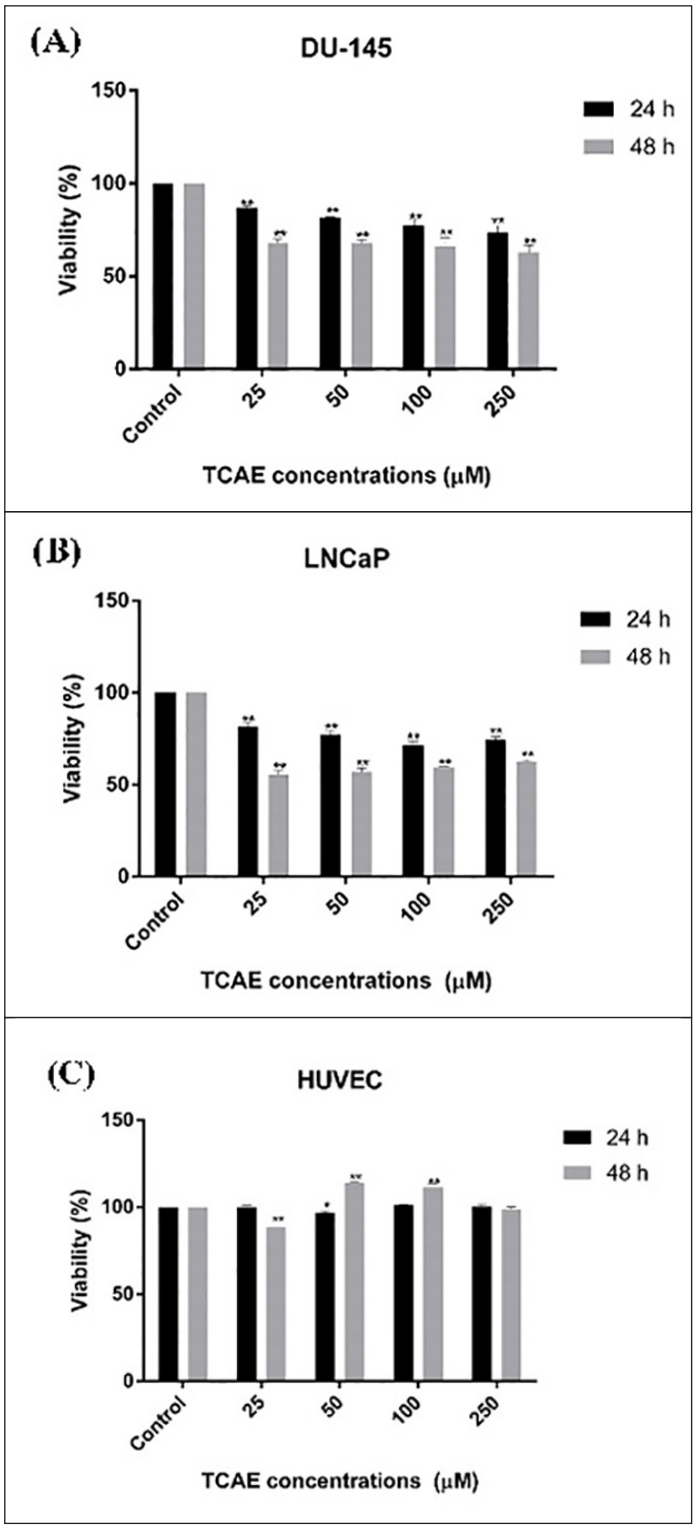

Figure 1. The cytotoxic effect of TCAE on DU-145, LNCaP and HUVEC cells. Following administration of TCAE (25, 50, 100 and 250 μM) for 24 and 48 hours, the viability rates of (A) DU-145, (B) LNCaP and (C) HUVEC cells were determined with WST-1 viability assay. (TCAE: Tarantula cubensis alcoholic extract, WST-1: Water Soluble Tetrazolium Salts-1, μM: micromolar *:$p<0.05$; **:$p<0.01$).

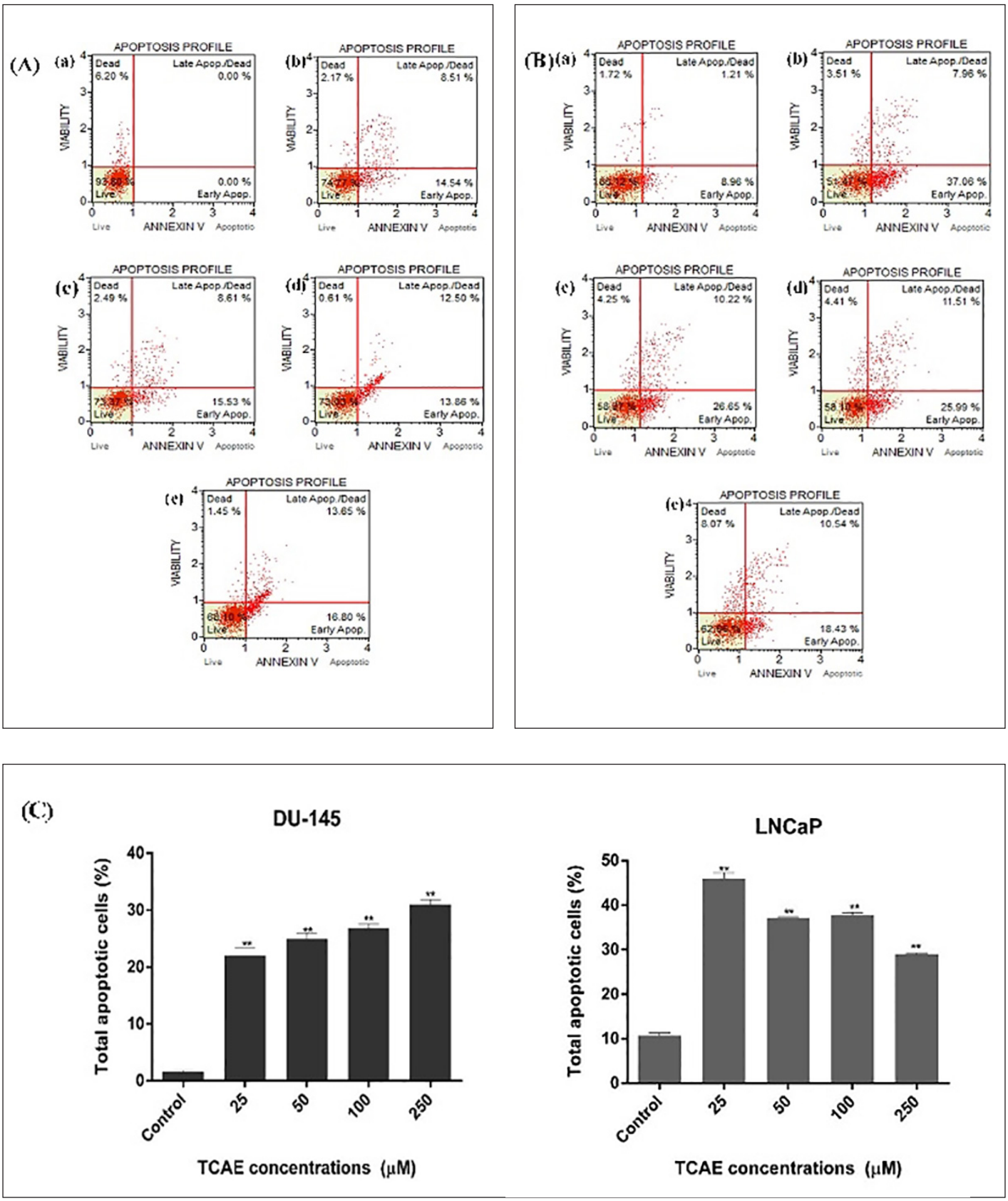

Figure 2. The results of Annexin V analysis. (A) DU-145 and (B) cells treated with (a) control, (b) 25 μM, (c) 50 μM, (d) 100 μM ve (e) 250 μM TCAE for 48h. (C) Statistical comparisons of the percentage of TCAE induced total apoptotic cell death in DU-145 and LNCaP cells. (TCAE: Tarantula cubensis alcoholic extract, μM: micromolar, *:p<0.05; **:p<0.01).

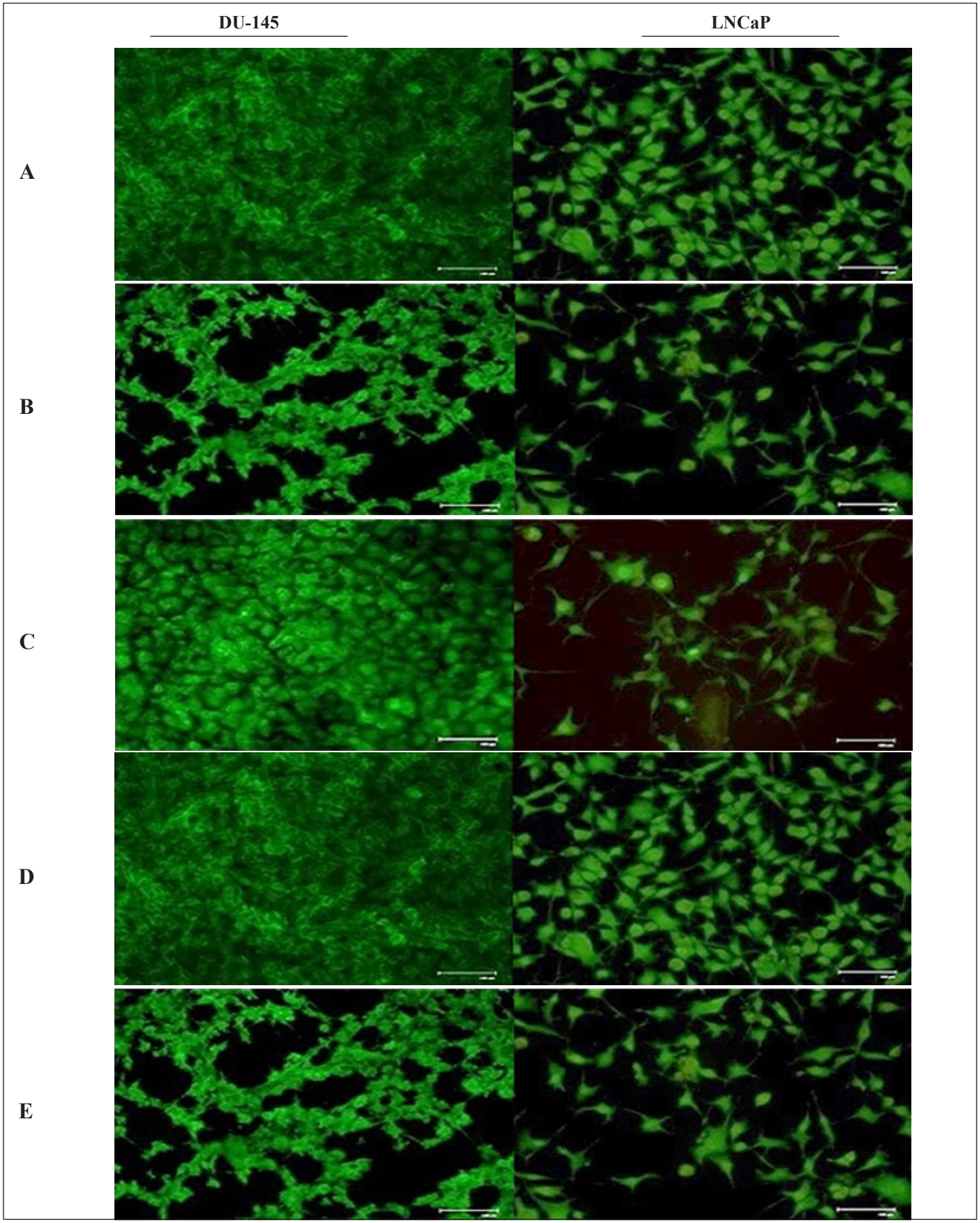

Figure 3. The effects of TCAE on cell morphology of DU-145 and LNCaP cells. The cells were treated with (A) control, (B) 25 μM, (C) 50 μM, (D) 100 μM ve (E) 250 μM TCAE for 48h. (TCAE: Tarantula cubensis alcoholic extract, μM: micromolar)

evaluated the effects of homeopathic treatment of cancer from a clinic in India and has released a request for protocols to conduct further research into this treatment. Therefore, the authors conducted a series of carefully controlled laboratory studies evaluating the effects of commonly used homeopathic remedies in cell and animal models of prostate cancer. STUDY DESIGN: One hundred male Copenhagen rats were randomly assigned to either treatment or control groups after inoculation with prostate tumor cells. METHODS: Prostate tumor cells DU-145, LNCaP, and MAT-LyLu were exposed to 5 homeopathic remedies. Male Copenhagen rats were injected with MAT-LyLu cells and exposed to the same homeopathic remedies for 5 weeks. In vitro outcomes included tumor cell viability and apoptosis gene expression. In vivo outcomes included tumor incidence, volume, weight, total mortality, proliferating cell nuclear antigen (PCNA. In subsequent studies, Thangapazham et al. [5] do not detect significant changes in apoptotic genes and cytokine levels after homeopathic treatment in an animal model of PCa. Also, homeopathic drug administration to PCa cells (DU-145, LNCaP, MAT-LyLu) does not affect cell growth and viability and does not cause changes in apoptotic gene expression [6]. On the other hand, MacLaughlin et al. [7] stated that 'sabal serrulata' used in homeopathic treatment causes a reduction in PC-3 and DU-145 PCa cells with the rate of 33% and 23%, respectively.

In our study, TCAE reduced the viability of both PCa cell lines in a time-dependent manner, and the viability rates of LNCaP cells were lower than DU-145 cells for 48 hours. The most effective dose was found to be 25 μM in LNCaP cells and 250 μM in DU-145 cells. Furthermore, TCAE did not have any cytotoxic effects on HUVEC cells. The increase in the viability of HUVEC cells at increasing doses of TCAE suggests that it may cause a proliferative effect on non-carcinogenic cells. Er et al. [19] reported that TCAE has an inhibitory effect on the proliferation of the MCF-7 breast cancer cell line, and this effect increases in a concentration and time-dependent manner. In another in vitro study, Ghasemi-Dizgah et al. [20] investigated the impact of TCAE on different cell types (MCF-7 and HN-5 cancer cell lines and HEK293 control cells). TCAE decreases cell proliferation in a dose-dependent manner. In addition, its cytotoxic effect on MCF-7 and HN-5 is higher than HEK293. These studies support our findings, and TCAE did not show any cytotoxic effect on healthy cells.

Furthermore, the highest apoptotic cell rates were obtained in DU-145 and LNCaP cells, at 250 and 25 μM TCAE concentrations, respectively. These findings were consistent with the results of the viability assay. In addition, the total apoptotic cell rates were higher in LNCaP cells than in DU-145 cells. Therefore, hormone-sensitive PCa may be more sensitive to apoptosis-targeted therapies. Several studies have shown the apoptotic effect of TCAE on cancer cells. Er et al. [19] reported that TCAE administration for 6 hours increases apoptosis in MCF-7 cells. In canine mammary tumors, the rate of apoptotic cells has increased following TCAE administration [27]. Ghasemi-Dizgah et al. [20] stated that TCA treatment induces DNA fragmentation and caspase-3 activity in the cells.

Limitations

This study has a few limitations. Protein and gene expression analyses were not performed and therefore it could not be determined which apoptotic pathways play a role. In addition, the lack of testing on animal models is another limitation.

CONCLUSION

In conclusion, TCAE showed an anti-cancer effect on PCa cells by inducing apoptosis in our study. However, further investigations of the underlying molecular mechanisms of TCAE-mediated apoptosis in PCa cells are required. Furthermore, TCAE alone cannot achieve sufficient efficacy in the treatment of PCa. Therefore, combination treatment studies are needed to evaluate the possible synergistic effects of TCAE with chemotherapy drugs. Thus, TCAE treatment may reduce the side effects of chemotherapy drugs in PCa treatment.

Conflict of Interest: The authors declare that they have no conflict of interest in the publication.

Informed Consent: Informed consent was not obtained.

Funding: This work was supported by the Sakarya University Scientific Research Projects Unit [SAU-BAPK] under grant [Number 2020-7-25-16].

Author Contributions: Conception: DG, HİÇ; Design: DG, HİÇ; Supervision: DG, HİÇ; Materials: DG, HİÇ, ADÖ, GGE, HÇ; Data Collection and/or Processing: DG, ADÖ, GGE, HÇ; Analysis and/or Interpretation: DG, ADÖ, GGE, HÇ, YTA; Literature Review: DG, HÇ, YTA, AE, OK, HSS, AG; Writing: DG, HİÇ, ADÖ, GGE, YTA, AE, OK, HSS, AG; Critical Review: DG, YTA, AE, OK, HSS, AG.

Ethical Approval: Informed consent was not obtained.

REFERENCES

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 71(3):209–249. https://doi.org/10.3322/caac.21660

[2] Mottet N, Cornford P, van den Bergh RCN, Briers E, De Santis M, Fanti S, Gillessen S, Grummet J, Henry AM, Lam TB, et al. (2020) EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer. Eur Assoc Urol. 1–182.

[3] Pingel S. (1992) Homeopathy. Basic aspects and principles of use in dermatology. Hautarzt. 43(8):475–482.

[4] Jonas WB, Gaddipati JP, Rajeshkumar N V, Sharma A, Thangapazham RL, Warren J, Singh AK, Ives JA, Olsen C, Mog SR, Maheshwari RK. (2006) Can homeopathic treatment slow prostate cancer growth? Integr Cancer Ther. 5(4):343–349. https://doi.org/10.1177/1534735406294225

[5] Thangapazham RL, Rajeshkumar N V, Sharma A, Warren J, Singh AK, Ives JA, Gaddipati JP, Maheshwari RK, Jonas WB. (2006) Effect of homeopathic treatment on gene expression in Copenhagen rat tumor tissues. Integr Cancer Ther. 5(4):350–355. https://doi.org/10.1177/1534735406294226

[6] Thangapazham RL, Gaddipati JP, Rajeshkumar N V, Sharma A, Singh AK, Ives JA, Maheshwari RK, Jonas WB. (2006) Homeopathic medicines do not alter growth and gene expression in prostate and breast cancer cells in vitro. Integr Cancer Ther. 5(4):356–361. https://doi.org/10.1177/1534735406294224

[7] MacLaughlin BW, Gutsmuths B, Pretner E, Jonas WB, Ives J, Kulawardane DV, Amri H. (2006) Effects of homeopathic preparations on human prostate cancer growth in cellular and animal models. Integr Cancer Ther. 5(4):362–372. https://doi.org/10.1177/1534735406295350

[8] Richardson-Boedler C. 2002. The brown spider Loxosceles laeta: source of the remedy Tarentula cubensis? Homeopathy. 91(3):166–170. https://doi.org/10.1054/homp.2002.0029

[9] Stampa S. (1986) A field trial comparing the efficacy of sulphamonomethoxine, penicillin, and tarantula poison in the treatment of pododermatitis circumspecta of cattle. J S Afr Vet Assoc. 57(2):91–93.

[10] Day C. (1998) Veterinary homeopathy: principles and practice. In: Schoen AM, editor. Complement Altern Vet Med. St. Louis: Mosby; p. 485–513.

[11] Sardari K, Kakhki EG, Mohri M. (2007) Evaluation of wound contraction and epithelialization after subcutaneous administration of Theranekron® in cows. Comp Clin Path. 16(3):197–200. https://doi.org/10.1007/s00580-006-0657-8

[12] Kizilay Z, Aktas S, Kahraman Cetin N, Kilic MA, Ozturk H. (2019) Effect of Tarantula cubensis extract (Theranekron) on peripheral nerve healing in an experimental sciatic nerve injury model in rats. Turk Neurosurg. 29(5):743–749. https://doi.org/10.5137/10195149.JTN.26162-19.2

[13] Oryan A, Moshiri A, Raayat AR. (2012) Novel application of Theranekron® enhanced the structural and functional performance of the tenotomized tendon in rabbits. Cells Tissues Organs. 196(5):442–455. https://doi.org/10.1159/000337860

[14] Gul Satar NY, Cangul IT, Topal A, Kurt H, Ipek V, Onel GI. (2017) The effects of Tarantula cubensis venom on open wound healing in rats. J Wound Care. 26(2):66–71. https://doi.org/10.12968/jowc.2017.26.2.66

[15] Cam Y, Kibar M, Atasever A, Atalay O, Beyaz L. (2007) Efficacy of levamisole and Tarantula cubensis venom for the treatment of bovine cutaneous papillomatosis. Vet Rec. 160(14):486–488. https://doi.org/10.1136/vr.160.14.486

[16] Icen H, Sekin S, Simsek A, Kochan A, Tunik S. (2011) The efficacy of Tarantula cubensis extract (Theranekron) in treatment of canine oral papillomatosis. Asian J Anim Vet Adv. 6(7):744–749. https://doi.org/10.3923/ajava.2011.744.749

[17] Paksoy Z, Gülesci N, Kandemir FM, Dinçel GÇ. (2015) Effectiveness of levamisole and tarantula cubensis extract in the treatment of teat Papillomatosis of cows. Indian J Anim Res. 49(5):704–708.

[18] Gültiken N, Vural MR. (2007) The effect of Tarantula cubensis extract applied in pre and postoperative period of canine mammary tumours. J Istanbul Vet Sci. 2:13–23.

[19] Er A, Corum O, Corum D, Hitit M, Donmez H, Guzeloglu A. (2017) Alcoholic extract of Tarantula cubensis induces apoptosis in MCF-7 cell line. Biomed Res. 28(8):3660–3665.

[20] Ghasemi-Dizgah A, Nami B, Amirmozafari N. (2017) Tarantula cubensis venom (Theranekron®) selectively destroys human cancer cells via activating caspase-3-mediated apoptosis. Acta Medica Int. 4(1):74. https://doi.org/10.5530/ami.2017.4.14

[21] Er A, Ozdemir O, Coskun D, Dik B, Bahcivan E, Faki HE, Yazar E. (2019) Effects of Tarantula cubensis alcoholic extract and Nerium oleander distillate on experimentally induced colon cancer. Rev Med Vet (Toulouse). 1–3:15–21.

[22] Gürbulak K, Akçay A, Gümüşsoy KS, Sist B, Steiner S, Abay M, Canoğlu E, Bekyürek T. (2014) Investigation of the efficacy of Tarantula cubensis extract (Theranekron D6) in the treatment of subclinical and clinical mastitis in dairy cows. Turkish J Vet Anim Sci. 38(6):712–718. https://doi.org/10.3906/vet-1405-101

[23] Makav M, Gelen V, Gedikli S, Atila Uslu G, Uslu H, Eroglu HA. (2020) Therapeutic effect of Tarantula cubensis extract on indomethacin induced gastric ulcers in rats. Thai J Vet Med. 50(4):559–566.

[24] Kaçar C, Zonturlu AK, Oral H, Yildiz S, Ari UC. (2007) The effects of Theranekron (R) application on uterus involution and vaginal efluence in cows early puerperal period. Kafkas Univ Veterinerlik Fakültesi Derg. 13(1):11–15.

[25] Kozlu T, Güler F, Peker Akalın P, Kazak F, Ergün Y, Aslan E. (2021) The influence of Theranekron® on glucose-6-phosphate dehydrogenase activity in rat ovaries, intact and with ischemia-reperfusion injury. Vet Arh. 91(1):81–88. https://doi.org/10.24099/vet.arhiv.0828

[26] Dolapcioglu K, Dogruer G, Ozsoy S, Ergun Y, Ciftci S, Soylu Karapinar O, Aslan E. (2013) Theranekron for treatment of endometriosis in a rat model compared with medroxyprogesterone acetate and leuprolide acetate. Eur J Obstet Gynecol Reprod Biol. 170(1):206–210. https://doi.org/10.1016/j.ejogrb.2013.05.026

[27] Gultiken N, Guvenc T, Kaya D, Agaoglu AR, Ay SS, Kucukaslan I, Emre B, Findik M, Schäfer-Somi S, Aslan S. (2015) Tarantula cubensis extract alters the degree of apoptosis and mitosis in canine mammary adenocarcinomas. J Vet Sci. 16(2):213–219. https://doi.org/10.4142/jvs.2015.16.2.213

[28] Koch H, Stein M. (1980) Konservative behandlung von neoplasmen der milchdrüse des hundes mit theranekron. Prakt Tierarzt. 61:424–430.

How to Cite;

Gul D, Cimen HI, Ozkan AD, Eskiler GG, Cakıroğlu H, Atik YT, Erdik A, Kose O, Saglam HS, Gokce A (2023) Evaluation of in Vitro Cytotoxic and Apoptotic Effect of Tarantula Cubensis Alcoholic Extract on Human Prostate Cancer Cells. Eur J Ther. 29(3):298-306. https://doi.org/10.58600/eurjther1625