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Research Article

Evaluation of the Antiproliferative Effect of Extract from Equisetum arvense L. on Hepatocellular Carcinoma SNU-449 Cells

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ABSTRACT: Equisetum arvense L. (Horsetail) extract (HTE) has been used traditionally in the treatment of various ailments. However, its potential as an anticancer agent, particularly in hepatocellular carcinoma (HCC), is still not well understood. The objective of this study is to investigate the anticancer potential of HTE in the SNU449 HCC cells. To assess the antiproliferative and migratory properties of HTE on HCC, a cell viability was analyzed at 24 and 48nd hours using seven different concentrations of HTE (ranging from 7.81 to 500 ppm). The lowest concentration that effectively affected cell viability was determined, and subsequent experiments were carried out using this dose at the 24-hour mark. The MTT, colony formation, wound healing, and Western blotting assays to measure CASP-3 and Cleaved CASP-3 expressions were also included in the analysis. The MTT assay identified 326 ppm as the minimum effective dose at the 24-hour time point. Colony formation assays showed a notable difference between treated and untreated cells, with a surviving fraction of 46.9% in HTE-treated cells. The wound healing assay indicated that HTE-treated cells exhibited a 43.4% wound closure rate after 24 hours. Western blot analysis revealed the normalized volume ratios for Caspase-3 were 53222328 in the treated cells, and 7948593 in the control, while for Cleaved Caspase-3, the ratios were 707454 in treated cells and 596409 in control cells. The results suggest that HTE has antiproliferative and migratory properties on SNU-449 HCC cells. Further investigations are required to understand the underlying mechanisms of these effects.

Keywords: *Equisetum arvense L., Horsetail, Antiproliferative Effects, SNU-449 Cell Line*.

1 INTRODUCTION

Primary liver carcinoma, particularly hepatocellular carcinoma (HCC), is an epithelial malignancy originating in the liver and accounts for over 80% of all liver cancer cases. In 2020, approximately 9.06 million new cases and 8.3 million deaths were reported globally [1]. HCC, the most common form of primary liver cancer, originates from hepatocytes and typically has a doubling time of 4-5 months [2]. It is a highly invasive tumor that rapidly grows, infiltrates blood vessels, and spreads to distant organs via the bloodstream. HCC often develops in individuals with chronic liver conditions such as hepatitis B and C infections, non-alcoholic fatty liver disease, and cirrhosis [3]. Other common risk factors include exposure to aflatoxins, obesity, diabetes mellitus, and alpha-1 antitrypsin deficiency [4]. Despite advances in treatment, the prognosis for HCC

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remains poor, as current therapeutic strategies are limited by inadequate molecular understanding, lack of early detection biomarkers, and resistance to chemotherapy [5].

The global incidence of HCC continues to rise, with estimates projecting over 1 million cases by 2025. Recent advances in phytomedicine and chemotherapy highlight the anticancer potential of phytochemicals, which possess a wide range of biological activities [6]. Medicinal plants and their extracts offer promising candidates for the development of new drugs and therapies due to their diverse pharmacological properties. Among these, *Equisetum arvense* L. has attracted significant attention due to its favorable chemical composition and therapeutic benefits [7,8].

Equisetum arvense L., a widely distributed medicinal plant [9], is found in regions across America, North Africa, and Asia [10]. Known commonly as horsetail or diarrhea herb, this plant has traditionally been used for various ailments, including as a diuretic, anti-inflammatory, anti-edema, and for treating fractures, wounds, and other conditions [11]. Previous phytochemical studies have declared that It has alkaloids, organic biomolecules, phytosterols, ascorbic acid, silicic acid, phenols, tannins, flavonoids, saponins, triterpenoids, tartaric acid, caffeic acids, apigenin, and kaempferol in its composition [12,13]. The peduncle contained silicic acid and silicates (5-8%), calcium (1.3%), potassium (1.8%), and various minerals [14]. Additionally, compounds such as nicotine, palustrine, and palustrinene have been isolated from the plant [15]. Many experimental and clinical studies report that *E. arvense* L. is a medicinal plant with hopeful therapeutic potential in managing various medical disorders [16,17]. Given its established medicinal uses, *Equisetum arvense* L. was selected for this study to assess its potential antiproliferative and migratory effects against hepatocellular carcinoma cells, specifically the SNU-449 cell line.

2 MATERIAL AND METHOD

2.1 Preparation of SNU449 Cell Culture

The SNU449 human hepatocellular carcinoma cell line (ATCC, CRL 2234) was cultured in RPMI-1640 Medium (Sigma), supplemented with 10% heat-inactivated FBS, (Sigma) and 1% Penicillin-Streptomycin-Neomycin (Sigma), and maintained at 37°C in a 5% CO_2 humidified incubator.

2.2 Cell Viability Assay

The Cell viability was measured using a colorimetric MTT assay as described by van Meerloo et al [18]. SNU449 cells were resuspended in RPMI-1640 medium and plated at a density of 10,000 cells per well in a 96-well plate, after which the plate was incubated

overnight. HTE (Concentrations ranged from 7.81 to 500 ppm) (Solgar, Turkey) was then exposed. After 24 hours MTT solution (5 mg/mL in PBS) was then added, following which the plate was incubated for 2–4 hours. Subsequently, 100 µL of DMSO (Merck) was added, and the absorbances were measured at 570 nm (Biotek, Synergy H1m). The median inhibitory concentration was then determined.

2.3 Colony Formation Assay

Colony formation assay was determined using the method described by Franken et al [19]. SNU449 cells were seeded into 6-well plates at a density of 1000 cells per well and treated with 326 ppm of HTE for 24 hours. Treatment was followed by the replacement of the medium with fresh RPMI-1640, which was refreshed every 2 to 3 days over 14 days. Colonies were then fixed with a solution of methanol: acetic acid (3:1) for 5 minutes. They were subsequently stained with 0.5% crystal violet for 15 minutes, and then counted using a microscope (Leica, DMi8). Plate efficiency (PE) and surviving fraction (SF) were then calculated using the following formulae:

- **PE** = (number of colonies formed / number of cells seeded) * 100
- **SF** = (PE of treated cells / PE of control cells) * 100

2.4 Wound Healing Assay

SNU449 cells were seeded in 60 mm culture dishes and grown to 80% confluence. It was created a scratch with a sterile 100 µL pipette tip in the cell monolayer. Cells were then treated with 326 ppm HTE for 24 hours, and images of the wound area were taken at specific time points using a cell imager (Leica, Paula). The imager's software assessed confluence, gap closure, migration rate, and half-gap time [20].

2.5 Western Blotting Assessment of Caspase 3 and Cleaved Caspase 3

Protein expressions of Caspase-3 (Cell Signaling, no. 14220) and Cleaved Caspase-3 (Cell Signaling, no. 9664) were assessed by Western blotting. Bands were visualized using Clarity Max Western ECL Substrate (Bio-Rad, no. 1705062) and analyzed using the Bio-Rad ChemiDoc system. Band quantification was performed using Bio-Rad Image Lab software (Version 6.1.0 build 7).

2.6 Statistical Analysis

The Kolmogorov-Smirnov test was applied to evaluate the normality. Data are presented as median (IQR). Protein expression levels were normalized and expressed as fold changes relative to the untreated control. Group comparisons were performed using the Kruskal-Wallis test for multiple groups. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS 27 (IBM).

3 RESULT

3.1 Cytotoxicity of Horsetail Extract (HTE) in the SNU449 Cell Line

MTT assay was performed to assess the effect of HTE on SNU449 cell viability. The results indicated that the minimum effective dose was 326 ppm at 24 hours. The absorbance values for the HTE-treated and untreated groups were 0.316 (range: 0.234-0.388) and 0.615 (range: 0.481-0.759), respectively (Figure 1).

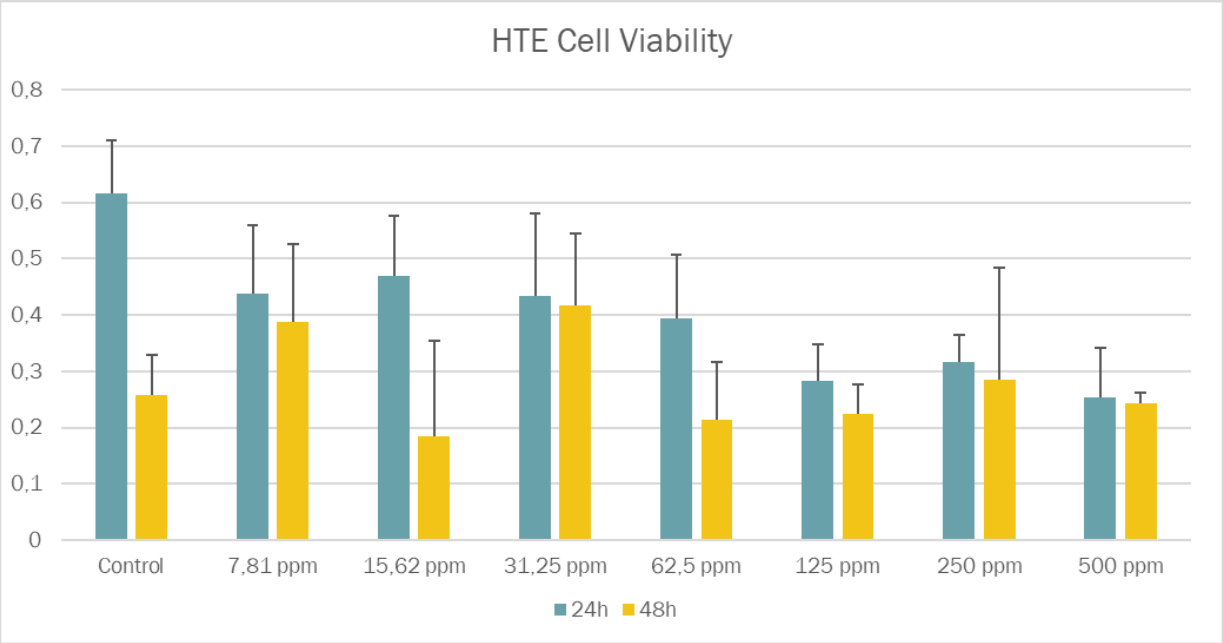

Figure 1. Effects of HTE on Cell viability of SNU449 cells.

3.2 Effects of HTE on the Migratory Characteristics of SNU449 Cells

The migratory characteristics of SNU449 cells were determined via a wound healing assay after 24 hours of HTE treatment. The results are summarized in Figure 2, where the wound closure rate was 43.4% in HTE-treated cells after 24 hours.

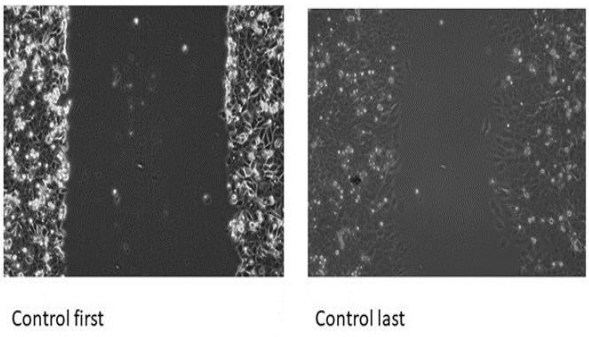

Figure 2a. Control wound healing.

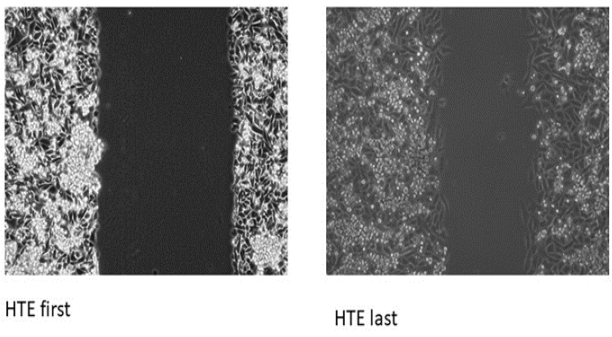

Figure 2b. HTE wound healing.

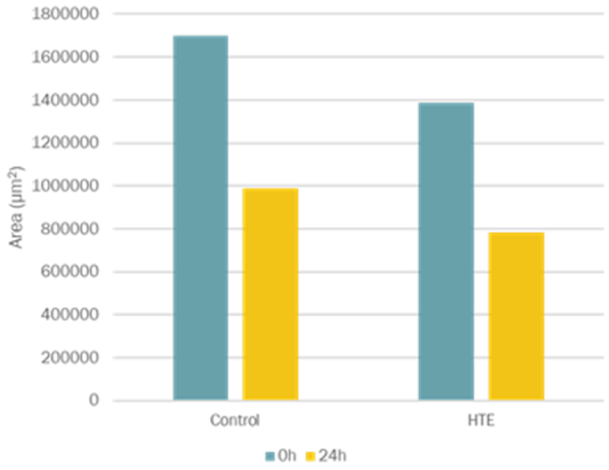

Figure 2c. Wound area.

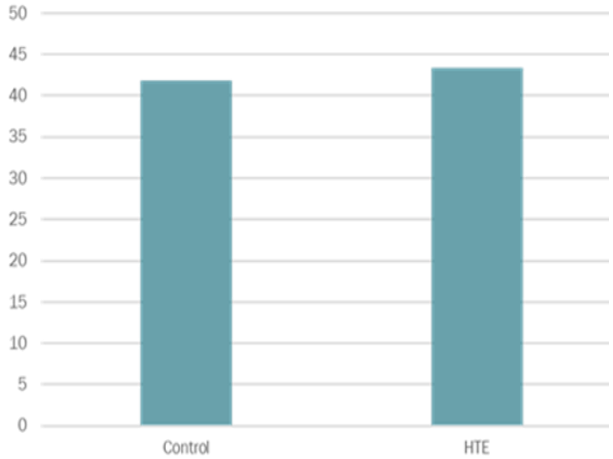

Figure 2d. Wound healing rate.

Figure 2. Migratory characteristics of SNU449 cells following 24-hour treatment with control or HTE.

3.3 Effects of HTE on the Proliferative Potency of SNU449 Cells

Colony formation assays revealed a significant difference in cell proliferation between HTE-treated and untreated cells, with a surviving fraction of 46.9% relative to the control (Figure 3).

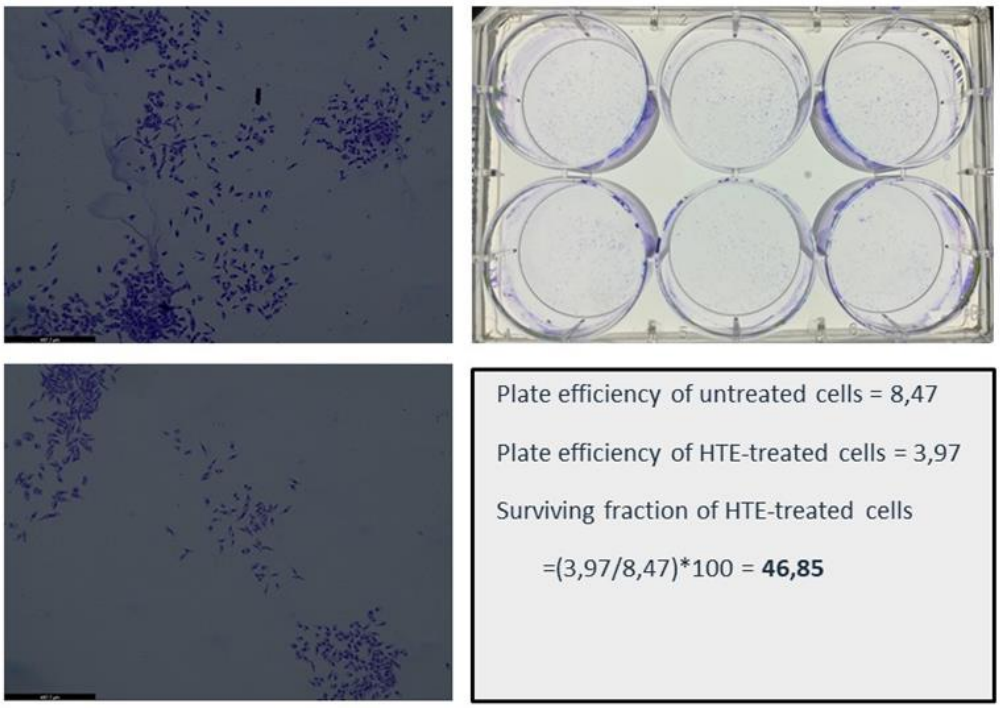

Figure 3. Proliferative potency of SNU449 cells.

3.4 Effects of HTE on Protein Expressions of Caspase 3 and Cleaved Caspase 3

Normalized volume ratios for Caspase-3 were 53222328 and 7948593 in HTE-treated and control cells, respectively. For Cleaved Caspase-3, normalized volume ratios were 320607 and 707454 in HTE-treated and control cells, respectively (Figure 4).

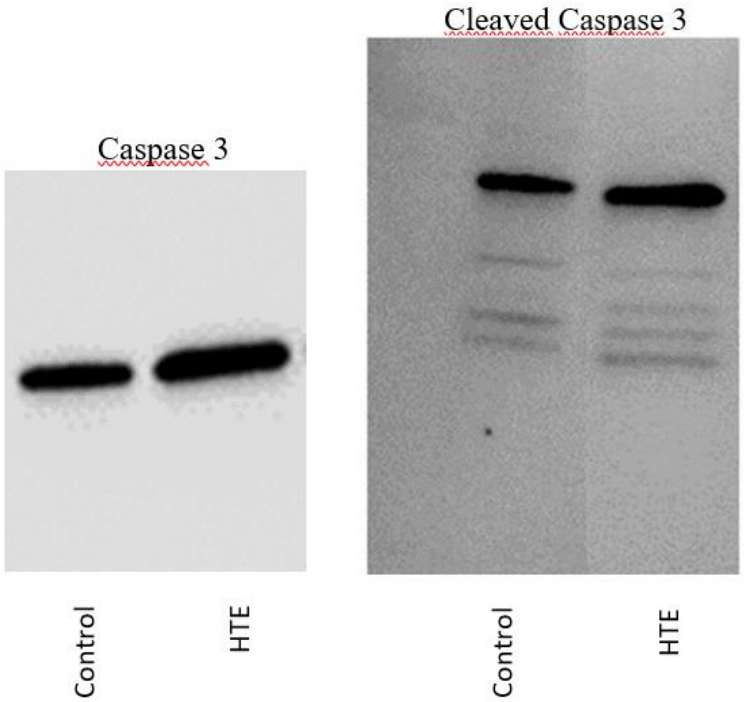

Figure 4. Changes in Caspase 3 and Cleaved Caspase 3 levels of control and HTE treatment groups

4 DISCUSSION

Several studies have reported the various biological activities of hydroalcoholic extracts of *Equisetum arvense* L., including analgesic, sedative, anti-inflammatory, antioxidant, antiseptic, antidepressant, hypoglycemic, and diuretic effects [11]. Additionally, *Equisetum arvense* L. has shown positive effects in treating liver conditions [12]. Notably, the methanolic extract of *Equisetum arvense* L. has exhibited hepatoprotective effects on tacrine-induced cytotoxicity in HepG2 cells [21]. Studies have also demonstrated that *Equisetum arvense* L. possesses anticancer properties [22].

The antiproliferative properties of *Equisetum arvense* L. extracts were evaluated on HeLa, HT-29, and MCF7 human cancer cells using the sulforhodamine B assay. The inhibition of cell growth was found to be contingent upon the type of cell line, extract type, and extract concentration [15]. Yamamoto et al. also observed cytotoxic effects of *Equisetum arvense* L. through apoptosis in the human leukemia U937 cell line. Furthermore, protein extracts from

Equisetum arvense L. have been shown to inhibit proliferation in cancer cell cultures [23].

Additionally, *Equisetum arvense* extract exhibited significant antiproliferative effects against melanoma B16 cells [24]. In general, the extract has demonstrated inhibitory effects on tumor cells, which depend on factors such as concentration, extract type, and cancer cell sensitivity. The inhibition rate increases with higher extract concentrations, likely due to the presence of compounds in the extract that alter the physiological state of the cells, arrest the cell cycle at specific stages, prevent proliferation, or induce apoptosis in cancer cells [25]. The hepatoprotective properties of phenolic compounds and flavonoids isolated from *Equisetum arvense* L. also support the use of this plant in traditional medicine for treating liver diseases [21].

In this study, the antiproliferative and migratory properties of *Equisetum arvense* L. extract were evaluated against the human hepatocellular carcinoma SNU449 cell line. Initially, we investigated whether HTE influenced the proliferative capacity of SNU449 cells. We found that low doses of HTE stimulated cell proliferation, while higher doses exhibited cytotoxic effects. The MTT assay revealed that 326 ppm was the effective minimum dose at 24 hours. The colony formation assay further confirmed a significant difference between the extract of *Equisetum arvense* L. exposed and control cells, suggesting that HTE affects the proliferative capacity of SNU449 cells. However, HTE did not alter the levels of caspase-3 and cleaved caspase-3 proteins. This suggests that HTE may exert its effects through alternative mechanisms, which warrant further investigation in future studies.

5 CONCLUSION

HTE has a moderate antiproliferative effect independent of the apoptotic pathway. In future studies, it may be considered to investigate the effect of the combination of the HTE with other chemotherapeutic agents and its effect on different signaling pathway mechanisms in tumor cells.

6 CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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