

Effect of the Wnt/ β -catenin pathway inhibitors on cell proliferation and migration of HEC-1A endometrial adenocarcinoma: experimental cell culture model

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ABSTRACT

Objectives: The most diagnosed tumor among infiltrating tumors of the female genital tract is endometrial carcinoma. The Wnt/ β -catenin signaling pathway has an important role in organogenesis, self-renewal of tissues, and adult stem cell maintenance. However aberrant activation of it causes many types of tumors and also related to the prognosis of patients. Therefore, we aimed to investigate whether Wnt/ β -catenin pathway inhibitors have any effect on the proliferation and migration of tumor cells.

Methods: As cancer cell line, HEC-1A endometrial adenocarcinoma was used. The Wnt/ β -catenin pathway inhibitors effects on proliferation and migration were demonstrated by real-time cell analysis device and wound healing model respectively.

Results: Wnt/ β -catenin pathway inhibitors FH535 (25 μ M at 36th hour, $p < 0.05$; 50 μ M at 48th hour $p < 0.001$) and niclosamide inhibited cell proliferation (10, 25 and 50 μ M at 60th hours; $p < 0.01$, $p < 0.05$ and $p < 0.001$, respectively) whereas ICRT14 and IWP-2 did not. However only niclosamide which is also an antihelmintic drug inhibited migration of the cells in all concentrations tested (10, 25 and 50 μ M, $p < 0.05$).

Conclusions: The present study shows that the Wnt/ β -catenin pathway has an substantial role in both proliferation and migration of endometrial adenocarcinoma. We suggest that the antihelmintic drug niclosamide could be further investigated for its potential therapeutic effect in endometrial adenocarcinoma.

Keywords: Wnt/ β -catenin, endometrial adenocarcinoma, niclosamide, migration

In the female genital tract most frequent infiltrating tumor is endometrial carcinoma which mostly occurs in the postmenopausal age [1, 2]. Endometrial cancers are mainly diagnosed at early stages, and 5-year of survival is between 20% to 91% depending on the stage. Hormone therapy, older age, exercise, diet and, diabetes are risk factors for developing endometrial cancer [3].

The Wnt/ β -catenin signaling pathway has important roles in organogenesis, development, adult stem

cell functions, and renewal of tissues. However abnormal activation of it causes many types of tumors [4, 5]. It has been reported that mutations of the β -catenin gene result in endometrial tumorigenesis [6-8]. Besides malignant transformation and tumor progression also proposed to require activation of the pathway [9]. Estrogens could induce Wnt/ β -catenin signaling and may cause endometrial hyperplasia even cancer. In contrast, progesterone was suggested as an inhibitor of this signaling [9]. This pathway also have coopera-

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tive with other pathways such as cyclooxygenase, EGFR signaling and Notch signaling [10].

In endometriosis patients inhibition of Wnt/ β -catenin signaling reported to prevent migration, invasion and proliferation, of epithelial and stromal cells of [10].

Although Wnt/ β -catenin signaling inhibitors have not yet been in clinical practice for endometrial cancer treatment, experimental data have promising consequences [9]. Wnt/ β -catenin signaling pathway has a substantial role in cell proliferation, migration and cell fate specification, [10]. HEC-1A cells are experimental models for postmenopausal endometrial cancer and it enables investigations for regulatory and signaling mechanisms [11, 12]. Therefore, we aimed to investigate whether Wnt/ β -catenin signaling inhibitors have any effect on the proliferation and migration of HEC-1A endometrial adenocarcinoma cells.

METHODS

Cell Line

HEC-1A which is a human originated endometrial carcinoma and often used as an experimental model for type 2 postmenopausal endometrial cancers was used as a cell culture (provided from ATCC[®] HTB-112TM). 10% fetal bovine serum (Biological Industries USA) 1% Penicillin/streptomycin (Biological Industries USA), L-glutamine (PAN-Biotech, Germany) were added to McCoy's 5A (Lonza, US) and served as medium for cells. The incubator was adjusted to 5% CO₂ at 37°C. Cells were getting confluent approximately in 10-14 days and had a 95-99% viability rate. Cells that were contaminated or had phenotypic changes were excluded from the study.

Preparation of Drugs

Wnt/ β -catenin pathway inhibitors FH535, ICRT14, IWP-2, and niclosamide were purchased from Sigma-Aldrich, USA. FH535, ICRT14, IWP-2, and niclosamide (10, 25, and 50 μ M each) were dissolved in the 0.5% dimethyl sulfoxide (DMSO). The data recorded from the migrations and proliferation with Wnt/ β -catenin pathway inhibitors were compared against control group (DMSO 0.5%).

Proliferation Studies

The effect of the Wnt/ β -catenin pathway inhibitors

on proliferation was recorded by real-time cell analysis (xCELLigence) device. The xCELLigence is used for real-time monitoring of cell proliferation. It enables recording of electronic impedance of E-Plates. Each well of the E-plate were seeded with 10000 cells and incubated for 24 hours. Thereafter Wnt/ β -catenin pathway inhibitors FH535, ICRT14, IWP-2, and niclosamide (10, 25, and 50 μ M each) were added to the wells. E-plates were put into the incubator which also contains the device.

Migration Model

The wound healing model used for migration studies. Each well of 24 well plate were seeded with 30000 cells. When cells were reach to confluency after approximately 10 days they were incubated with serum-free medium for 24 hours. Thereafter, by using a 20 μ l pipette the wound lines were opened on cells that grown on plates. After the administration of 1% FBS medium to wells, FH535, ICRT14, IWP-2, and niclosamide (10, 25, and 50 μ M each) were applied. After the inhibitor agent administration ($t = 0$) and subsequently at 24th and 48th hours, the wound images were measured. Relative cell motility was calculated by the formula (wound width at $t = 0$ hours) - (wound width at $t = 24$ or 48 hours).

Statistical Analysis

Cell proliferation obtained from xCELLigence system was expressed as cell index "CI" and $CI > 0.2$ was considered as positive. Data were presented as mean \pm SEM. One-way analysis of variance and post hoc Bonferroni test was used for statistical evaluation or t-test were used when appropriate. A P value smaller than 0.05 was considered significant.

RESULTS

The Effect of DMSO on Cell Proliferation and Migration

Following the incubation cells were gradually proliferated in both saline and DMSO groups (Fig. 1a). The concentrations of DMSO in both E-plate wells and migration wells were 0.5% and did change neither cell proliferation (Fig. 1a, $p > 0.05$) nor cell migration (Fig. 1b, $p > 0.05$).

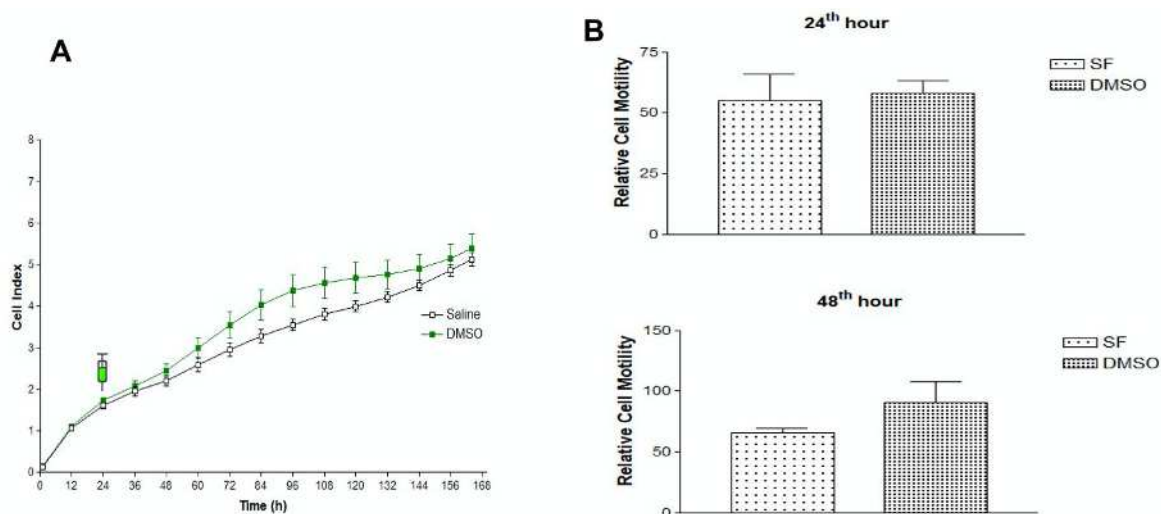


Fig. 1. Effect of DMSO (vehicle) on the proliferation (a) and migration (b) of HEC1A cells by real-time cell analysis and wound healing model respectively. (a) DMSO was added at the 24th hour (̸). DMSO (0.5%) did not altered cell proliferation (n = 4) (p > 0.05). (b) Treatments were made at the 0th hour for migration. The concentration of DMSO was 0.5 % in wells and did not change cell migration (p > 0.05).

The Effect of FH535 on Cell Proliferation and Migration

10 µM of FH535 did not change cell proliferation (Fig. 2a, p > 0.05). 25 µM of FH535 starts to inhibit cell proliferation 12 (36th hour on the graph) hours after the application (Fig. 2a, p < 0.05). 25 µM of FH535 decreased proliferation between the 36th and 84th hours and after the 84th hours started to cause cell death (Fig. 2a, p < 0.001). The higher 50 µM concentrations of FH535 start to cause cell death at earlier times between the 48th and 168th hours (Fig. 2a, p < 0.001). However FH535 did not change the cell migration of HEC-1A cells (Fig. 2b, p > 0.05).

The Effect of ICRT14 on Cell Proliferation and Migration

10, 25, and 50µM of ICRT14 did not change cell proliferation of endometrial adenocarcinoma HEC-1A cells (Fig. 3a). ICRT14 did not change the cell migration of HEC-1A cells (Fig. 3b, p > 0.05).

The Effect of IWP-2 on Cell Proliferation and Migration

10, 25, and 50µM of IWP-2 did not change cell proliferation of endometrial adenocarcinoma HEC-1A cells (Fig. 4a). IWP-2 did not change the cell migration of HEC-1A cells (Fig. 4b, p > 0.05).

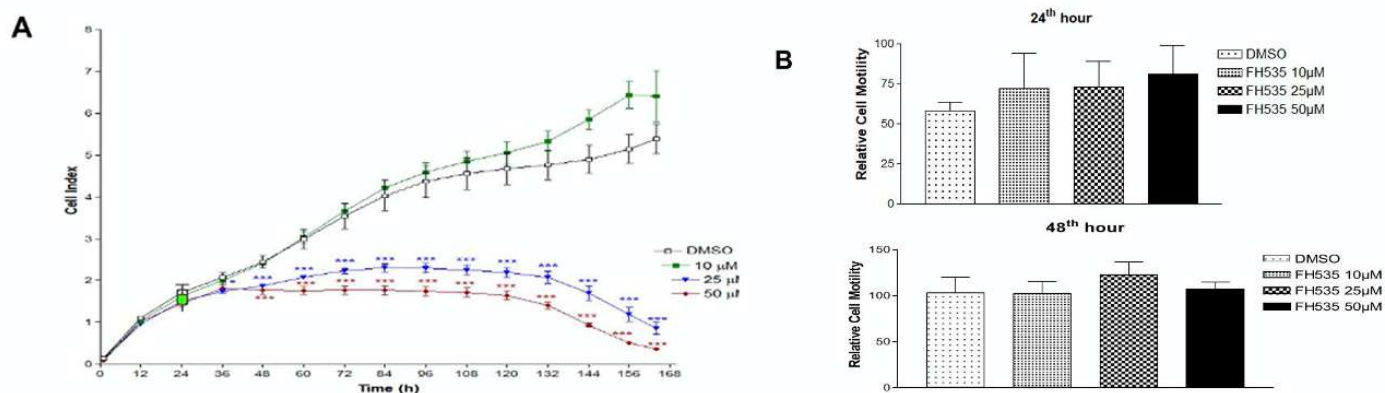


Fig. 2. The effect of FH535 on cell proliferation (a) and migration (b). (a) Treatments were made at the 24th hour (̸) for proliferation. 10 µM of FH535 did not change cell proliferation. 25 µM of FH535 started to inhibit cell proliferation at 36th hour. 25 µM of FH535 decreased proliferation between the 36th and 84th hours and after the 84th hours started to cause cell death. 50 µM concentrations of FH535 start to cause cell death at earlier times between the 48th and 168th hours (*p < 0.05, *** p < 0.001). (b) Treatments were made at the 0th hour for migration. FH535 did not change the cell migration of HEC-1A cells (p > 0.05).

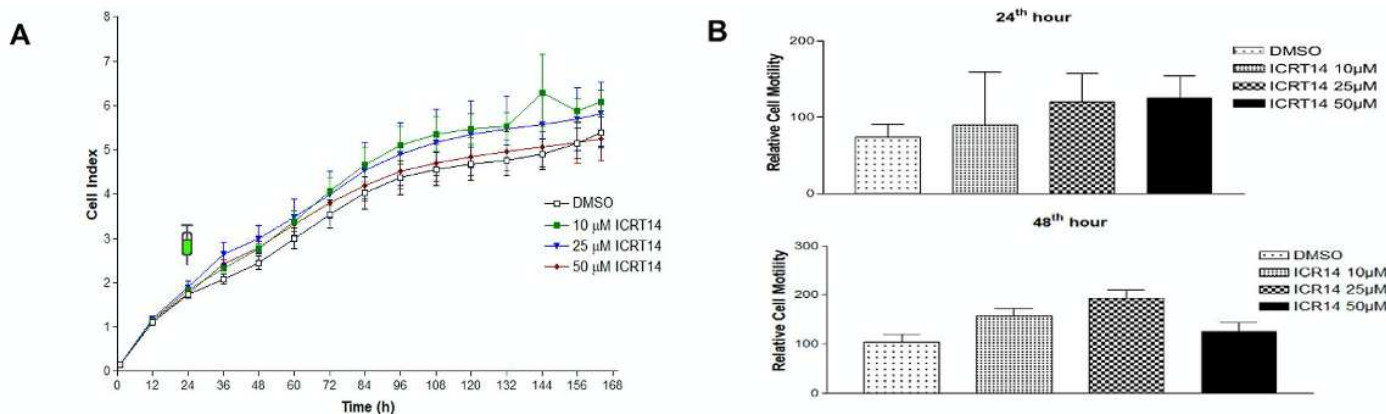


Fig. 3. The effect of ICRT14 on cell proliferation (a) and migration (b). (a) Treatments were made at the 24th hour (☉) for proliferation. 10, 25, and 50μM of ICRT14 did not change cell proliferation of endometrial adenocarcinoma HEC-1A cells. (b) Treatments were made at the 0th hour for migration. ICRT14 did not change the cell migration of HEC-1A cells ($p > 0.05$).

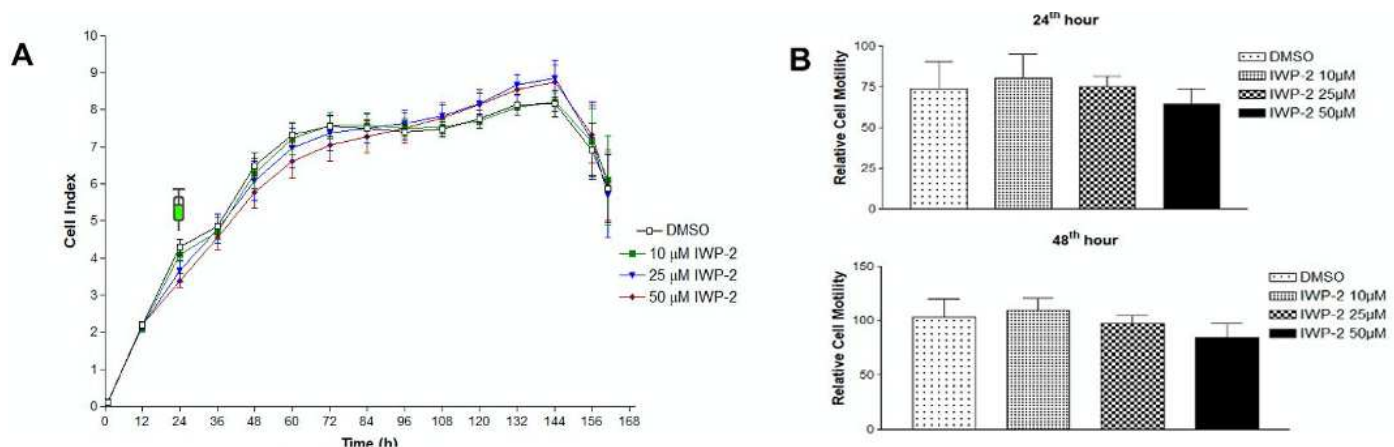


Fig. 4. The effect of IWP-2 on cell proliferation (a) and migration (b). (a) Treatments were made at the 24th hour (☉) for proliferation. 10, 25, and 50μM of IWP-2 did not change cell proliferation of endometrial adenocarcinoma HEC-1A cells. (b) Treatments were made at the 0th hour for migration. IWP-2 did not change the cell migration of HEC-1A cells ($p > 0.05$).

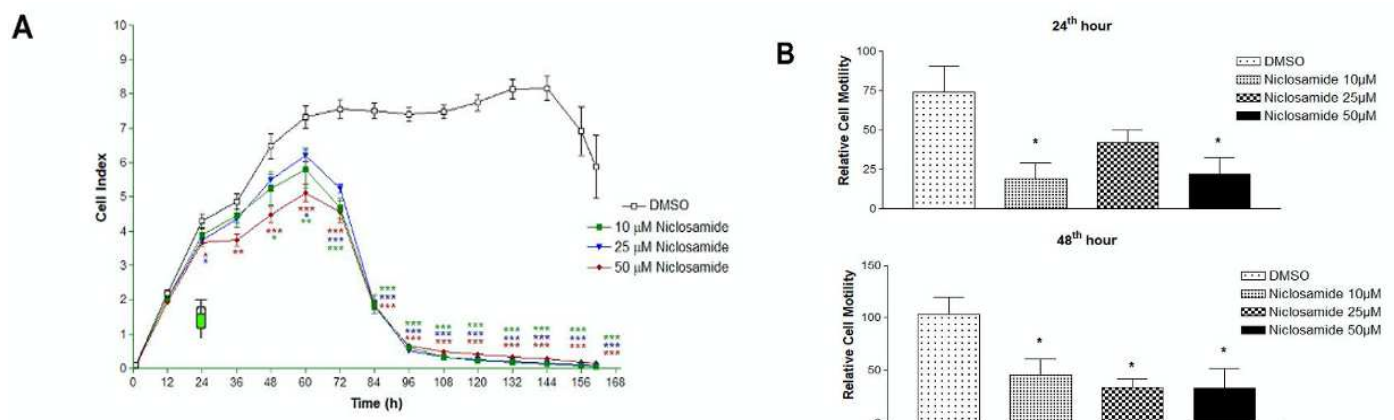


Fig. 5. The effect of niclosamide on cell proliferation (a) and migration (b). (a) Treatments were made at the 24th hour (☉) for proliferation. 10, 25, and 50μM of niclosamide inhibited cell proliferation. While the effect of 25 and 50μM of niclosamide started immediately after the application, the effect of 10μM observed at 48th hour. After the 60th hour there was a fast decrease in cell index with all concentrations used ($*p < 0.05$, $***p < 0.001$). (b) Treatments were made at the 0th hour for migration. Niclosamide significantly inhibited cell migration of HEC-1A cells at all concentrations tested ($p < 0.05$).

The Effect of Niclosamide on Cell Proliferation and Migration

10, 25, and 50 μ M of niclosamide inhibited cell proliferation. While the effect of 25 and 50 μ M of niclosamide started immediately after the application, the effect of 10 μ M observed at 48th hour. After the 60th hour, there was a fast decrease in cell index with all concentrations used (Fig. 5a). Niclosamide significantly inhibited cell migration of HEC-1A cells at all concentrations tested (Fig. 5b, $p < 0.05$).

DISCUSSION

The effects of four Wnt/ β -catenin pathway inhibitors on cell proliferation and migration that are important for cancer prognosis were tested in HEC-1A cells which are being used as an experimental model for type 2 postmenopausal endometrial cancers [12]. Wnt/ β -catenin pathway inhibitors, FH535, and niclosamide inhibited cell proliferation whereas ICRT14 and IWP-2 did not. However, only niclosamide which is also an antihelmintic drug inhibited migration of the cells.

FH535 is a cell-permeable sulfonamide compound that inhibits β -catenin-Tcf/LEF interaction in the Wnt/ β -catenin pathway but also suppresses the Peroxisome Proliferator-Activated Receptor (PPAR γ/δ) signal. PPARs belong to the nuclear receptor protein group and act as transcription factors that regulate the gene expression [13].

In hepatocellular carcinoma cells, FH535 suppressed the proliferation, and 50 μ M significantly reduced the distance between the two lines in the wound healing test [14]. It has been reported that FH535 inhibited cell proliferation (10 μ M, 20 μ M, and 40 μ M) and migration at 24 hours in wound healing experiment (20 μ M and 40 μ M) in colon cancer cell lines [15]. In another study, the effect of FH535 on pancreatic cell lines were examined (PANC-1 and BXPC-3), and 20 μ M FH535 concentration did not change the expression of β -catenin in BXPC-3 cells but decreased the expression of β -catenin in PANC-1 cells. In the wound healing experiment, the 20 μ M concentration of FH535 significantly reduced migration at 8 and 12 hours [16]. Also, it has been reported that FH535 inhibits proliferation at concentrations of 0.1 μ M, 1 μ M, and 10 μ M in osteosarcoma cells [17]. The antiprolif-

erative effect observed in hepatocellular carcinoma cells with the addition of FH535 at 50 μ M concentration is similar to our study, but the antiproliferative effect observed in colorectal cancer cells with the addition of FH535 at 10 μ M concentration was not noticed in HEC1A cells. Besides, inhibition of migration in the wound healing experiment in hepatocellular carcinoma, colorectal cancer, pancreas, and osteosarcoma cells was not observed in HEC1A cells. Different effects observed in the same concentration in the cell lines suggested that the inhibitory action of FH535 may change depending on the cell type.

In our study, the 25 μ M concentration of FH535 reduced proliferation starting from the 24th hour, as well as reducing the number of cells after the 84th hour. 50 μ M concentrations of FH535 tend to decrease proliferation after 12 hours and started to decrease the number of cells after 24 hours. Niclosamide administration reduced proliferation from the first moment of administration and additionally decreased the number of cells after 36th hour at all concentrations. In this study, cell death mechanisms have not been studied, but we suggest that the decrease in the number of cells at the specified hours may be related to the activation of cell death mechanisms. Because the cells in the control group at the same time were still growing at their normal rate.

Niclosamide inhibits the co-receptor LRP6 via increasing the Wnt receptor Fzd1 internalization, inhibits Dvl2 expression via inhibiting the β -catenin/TCF complex formation, and thereby inhibits the Wnt/ β -catenin signaling at multiple levels. In addition, niclosamide also inhibits Notch, STAT, 3NF- κ B, and mTORC1 signaling pathways. At the same time it may induce growth inhibition, apoptosis and cell cycle arrest via targeting the mitochondria of cancer cells. [18].

Gyamfi *et al.* [19] have shown that niclosamide inhibits both proliferation and migration in breast cancer cell lines. Arend *et al.* [20] showed antiproliferative effects of it in ovarian cancer cells (0.1-4 μ M). Zhao *et al.* [21] have demonstrated that it inhibits proliferation in renal cell carcinoma lines. Wang *et al.* [22] showed that it inhibits proliferation in hepatocellular cell lines, induces apoptosis, and increases the cisplatin sensitivity of cancer cells. Liu *et al.* [23] reported that 0.1 μ M, 0.25 μ M, and 0.5 μ M niclosamide suppress cell migration in prostate cancer cells. Li *et*

al. [24] have demonstrated that different concentrations of niclosamide between 0.1 μ M-10 μ M in human osteosarcoma cell lines inhibit cell proliferation by stopping the cell cycle. Likewise in our study niclosamide prevented both proliferation and migration in endometrium adenocarcinoma HEC1A cells. Among other inhibitors tested in this study, only niclosamide inhibited migration. Based on this finding, it could be suggested that this effect by niclosamide is due to a nonspecific effect of it rather than the Wnt/ β -catenin pathway inhibition. However, further and detailed molecular studies are needed to confirm this suggestion. One of the limitations of this study is that expression and activation studies have not been performed for the Wnt / β -catenin pathway.

ICRT14 belongs to the thiazolidinedione class of β -catenin responsive transcription inhibitors, and also prevents TCF binding to DNA [13]. In one study, ICRT14 has been shown to induce significant G0/G1 cell cycle arrest in colon cancer cell lines, and in another study, increased sensitivity to chemotherapy in pediatric acute lymphocytic leukemia patients by inhibiting Wnt [25, 26]. IWP-2 blocks Wnt production by directly inhibiting the porcupine active site, it can also affect CK1 δ/ϵ related pathways [27]. Kleszcz *et al.* [28] have demonstrated that IWP-2 inhibits porcupine, reducing the Wnt signal, thereby inducing apoptosis in neck and head carcinoma cells. Tong *et al.* [29] showed that artemisinin derivatives can significantly inhibit tumor metastasis and lung tumorigenesis via Wnt/ β -catenin signaling. In our study, ICRT14 and IWP2 did not change proliferation and migration at the concentration and time intervals tested.

CONCLUSION

In this study, we examined the possible effects of Wnt/ β -catenin pathway inhibitors on proliferation and migration in endometrial adenocarcinoma HEC1A cells, which are important parameters for cancer. We have shown that FH535 inhibits proliferation, and niclosamide both proliferation and migration. We suggest that antihelminthic drug niclosamide which is a cheap antiparasitic drug could be regarded as a potential therapeutic agent in endometrium adenocarcinoma. Further studies on the effects of niclosamide and its derivatives on proliferation and migration and

other parameters of cancer are needed to elucidate the pathogenesis of endometrial cancers, prevent progression of the disease and develop new treatment strategies.

Authors' Contribution

Study Conception: YÇ, İÜ; Study Design: YÇ, RNT, İÜ; Supervision: İÜ; Funding: İÜ; Materials: İÜ, RNT; Data Collection and/or Processing: YÇ, RNT, İÜ; Statistical Analysis and/or Data Interpretation: YÇ, RNT, İÜ; Literature Review: YÇ, İÜ; Manuscript Preparation: İÜ, RNT and Critical Review: İÜ, RNT.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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