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Research Article

Histological analysis of TGFβ1 and VEGFR expression in cervical carcinoma treated with *Rhodomyrtus tomentosa*

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Abstract

Cervical carcinoma is one of the most common malignant carcinomas around the world, including Indonesia. *Rhodomyrtus tomentosa* is an herbal medicine that is often used in Asia as a therapeutic agent to stop cancer metastases. The process of neoangiogenesis in cervical cancer depends on VEGFR activity. Increased TGFβ1 production is also linked to cervical cancer, suggesting that gene inactivation contributes to the emergence of cervical carcinoma.

Group C- was the control group, Group C+ was the cancer model group, CER100 was the group of rats with cancer + 100 mg/kg body weight (BW) of *R. tomentosa*, CER200 was the group of rats with cancer + 200 mg/kg BW of *R. tomentosa*, and CER400 was the group of rats with cancer + 400 mg/kg BW *R. tomentosa*. Rats were dissected after administration of *R. tomentosa* for 30 days. Immunohistochemical staining of the cervical tissue was performed with TGFβ1 and VEGFR antibodies. VEGFR expression was significantly different from TGFβ1 expression ($p < 0.01$). The highest expression was observed at the lowest dose of *R. tomentosa* (100 mg/kg BW), and the lowest expression was observed at 200 and 400 mg/kg BW. The administration of *R. tomentosa* can repair tissue damage and decrease the expression of TGFβ1 and VEGFR via histopathological parameters, indicating the importance of the activity of these proteins in the development of neoangiogenesis in cervical cancer.

Keywords

cervical cancer, immunohistochemistry, molecular therapy, *Rhodomyrtus tomentosa*, TGFβ1, VEGFR

Introduction

Cervical carcinoma of the uterus is one of the most prevalent malignant carcinomas that endangers the lives of women (Conesa-Zamora 2013). Cervical carcinoma accounts for one-half of all malignant tumours that develop in the female reproductive system (Conesa-Zamora 2013). Molecular therapy can be used to cure cancer while using natural herbs (Yin et al. 2013). In cancer treatments, including chemotherapy, certain herbs help to minimise side effects (Yin et al. 2013). Herbs are typically boiled in water to create plant extracts. *Rhodomyrtus tomentosa* is one of the herbal remedies that is frequently employed by the Asian population (Yin et al. 2013). The ornamen-

tal plant *R. tomentosa* (family Myrtaceae) is indigenous to South and Southeast Asia (Djati and Christina 2019). The rhodomyrtone content of this plant has been demonstrated to inhibit cell migration, adhesion, and A431 cell invasion, and it has potential as a novel therapeutic agent to stop cancer metastasis (Tayeh et al. 2017). In stomach carcinoma, this plant has been shown to induce cell cycle arrest and encourage apoptosis (Tayeh et al. 2017; Zhang et al. 2020). These herbs can heal diabetic wounds, the histology of the placenta, and increase HSP-70 expression while lowering lipid peroxidation (Situmorang and Ilyas 2018; Ilyas et al. 2019; Irianti et al. 2020; Ilyas and Situmorang 2021; Manurung et al. 2021). *R. tomentosa* has a potent antioxidant activity and the potential to be a source of nutrients that enhance health due to its high phenolic content (Zhang et al. 2018).

For tumour invasion and metastasis to occur in a mutually beneficial manner, vascular endothelial cell growth factor (VEGF) is required. Furthermore, there is a direct connection between angiogenesis and the development of the lymphatic system (Lv et al. 2018). The development of new blood and lymphatic vessels in cervical squamous cell carcinoma is influenced by the Ang family and VEGF during angiogenesis, namely in cervical cancer (Lv et al. 2018). VEGF is crucial for the development of new blood vessels. In precancer, activated VEGFR causes concurrent activation of the PLC-Raf kinase-MEK-MAP kinase, PI3K-AKT, and MAP kinase pathways to promote cell proliferation and endothelial cell survival (Dang et al. 2017). Angiogens, like VEGF, a novel angiopoietin target, can be induced by gene expression regulation and factor signal transduction pathways, and they represent a promising novel approach for the clinical treatment of tumours (Dang et al. 2017). An increase in VEGF expression in precancerous changes and cervical cancer is indicative of the role of this proangiogenic factor in the mechanism of neoangiogenesis (Tomao et al. 2014). The discovery of increased VEGF expression in a subset of patients with suboptimal histological characteristics highlights the significance of VEGF activity in the neoangiogenesis and progression of cervical cancer (Tomao et al. 2014; Dang et al. 2017).

Transforming growth factor β1 (TGFβ1) can boost normal cervical remodelling and inhibit cervical cell growth induced by human papillomavirus (HPV) (Wang et al. 2021). HPV infection affects TGFβ signalling (Wang et al. 2021). The presence of TGFβ1 in human cervical cancer suggests that gene inactivation contributes to the emergence of cervical carcinoma. Increased TGFβ1 production or inhibition, mutation of the TGF-transmembrane receptor, or lack of expression and/or mutation of Smads are all linked to cervical cancer (Taylor et al. 2011; Principe et al. 2014). Immune cells of varying types and numbers can be detected in nests of tumour cells that are surrounded by varying densities of intratumoral stroma in cervical cancer (Principe et al. 2014). Transforming growth factor-1 (TGF-1) regulates epithelial cell proliferation and the development of the stroma and extracellular matrix (ECM) and suppresses the immune system (Taylor et al. 2011). Increased TGFβ1 synthesis or changes in intracellular and post-receptor signalling pathways have been linked to several cancers (Taylor et al. 2011). Therapeutic strategies should be designed to prevent the invasive phenotype induced by TGFβ1 while preserving its growth-inhibiting effects and inducing its apoptosis (Taylor et al. 2011; Principe et al. 2014).

The findings of a study on VEGFR1 and TGFβ1 expression highlight the potential of *R. tomentosa* as a molecular therapy for cancer and provide strong support for its therapeutic use in modern medicine. The effect of *R. tomentosa* on VEGFR1 and TGFβ1 expression in rat cervical histopathology should be investigated before employing human cells. To increase cell penetration and bioavailability, *R. tomentosa* was formulated into a micro-colloidal form. It is intended to use this plant to produce drugs for human molecular cancer therapy.

Materials and methods

Materials

R. tomentosa leaves were discovered in the Lintong Nihuta, North Sumatera, Indonesia. The plants were found in the Lintong Nihuta sub-district of Humbahas Regency at elevations ranging from 1,000 to 1,500 m above sea level and located at 02°4'20"–2°16'15"N and 98°52'40"–98°56'20"E. Lintong Nihuta District has 479 ha of peatland, accounting for 16.03% of the total peatland area in Humbang Hasundutan Regency (Hutagaol et al. 2021).

Preparation of *Rhodomyrtus tomentosa*

Preparation: The leaves and twigs of *R. tomentosa* were separated. The leaves were cleansed of any soil or dust that adhered to them, and they were dried for 7 days at room temperature and smoothed.

Extraction: 500 grams of *R. tomentosa* dry powder were macerated in 96% technical ethanol for 24 hours at room temperature. Maceration with a 96% technical ethanol solvent yielded the ethanol extract of *R. tomentosa*. The maceration products were filtered using a Buchner funnel and a vacuum pump. Using the same method, the filtered residue was macerated twice more. A rotary evaporator was used to concentrate the ethanol extract, which was then dried for 8 hours to produce a solid ethanol extract.

Production of micro-colloidal *R. tomentosa* (CER): An ethanol extract of the leaves was prepared by sonication as follows: 0.5 mg of *R. tomentosa* extract was added to a Tween 20 solution. Capryol 90 was added, and the solution was homogenised. PEG-400 was added, and the solution was sonicated. The prepared substance was dissolved in distilled water (1:100) and sonicated with an ultrasonic device (Sonicator Ultrasonic Homogenizers and Emulsifiers), and the micro-colloidal *R. tomentosa* was ready for use in animals experiments.

Experimental animals

This study was conducted at the University of Sumatera Utara's (**USU**) Biology, Pathology and Anatomy Laboratory of the Faculty of Medicine from January 2022 to August 2022. The study was conducted using a completely randomised design. This type of research is known as an experimental study. There were five groups: Group C- was the control group, Group C+ was the cancer model group, Group CER100 was the group of rats with cancer + 100 mg/kg body weight (BW) of *R. tomentosa*, Group CER200 was the group of rats with cancer + 200 mg/kg BW of *R. tomentosa*, and CER400 was the group of rats with cancer + 400 mg/kg BW *R. tomentosa*. *R. tomentosa* leaf ethanol extract was administered for 30 days orally. The animals were euthanized with administering an anaesthetic combination of 300 mg/kg BW of ketamine and 15–30 mg/kg BW of xylazine was administered then rats were dissected for taken the cervix and cervical tissues were stained with VEGFR1 and TGFβ1 antibodies using immunohistochemical techniques.

Rat model of cervical cancer

Thirty female *Rattus norvegius* were used in this study. The rats were aged 10–15 weeks and weighed 180–200 g. Before being kept in cages at constant room temperature (25.0 ± 3.0 °C) and a humidity level of 35–60%, male rats were introduced to the laboratory environment for 2 weeks. The cages were lit for 12 hours and darkened for 12 hours. Female rats were given unrestricted access to water and free access to corn and pellets. Rats were placed in a plastic container measuring 40 cm × 30 cm. The rats were injected vaginally with 50 mg of benzopyrene diluted with corn oil. The tumour was identified when a lump was found due to administration of benzopyrene for three months, and samples were sent to the Anatomical Pathology Laboratory of the USU to ensure that the tissue was tumorous. The rats were then administered *R. tomentosa* for 1 month. The dose was in accordance with the acute toxicity test and previous studies (Situmorang et al. 2021, 2022a, b). The USU Faculty of Mathematics and Natural Sciences Ethics Committee for Handling Experimental Animals approved this study (Ethical Clearance No. 042/KEPH-FMIPA/2022).

Measurement of superoxide dismutase

Superoxide dismutase (SOD) analysis was performed using the blood of the rats with cervical cancer. The Superoxide Dismutase Activity Kit was used to measure SOD activity. After dilution with a uniquely coloured sample diluent, the sample is loaded into wells. Xanthine oxidase reagent was added after the substrate, and the mixture was allowed to sit at room temperature for 20 minutes. In the presence of oxygen, xanthine oxidase generates superoxide, which converts the colourless substrate in the detection reagent to a yellow product that is detectable at 450 nm.

Measurement of malondialdehyde

Blood plasma samples from the rats were assessed with traditional thiobarbituric reactive species spectrophotometry (TBARS). The Malondialdehyde (MDA) Assay Kit (competitive enzyme-linked immunosorbent assay) (ab238537) was used for rapid detection and quantification of the protein MDA. This kit enables the quantification of MDA addition in a determined protein sample by comparing its absorbance with a known MDA-BSA standard curve. Then, the MDA-TBA2 condensation product can be measured via UV-VIS spectrophotometry.

Making paraffin blocks

Cervical organs were fixed in formalin and immersed in xylol for 15 minutes. After 5 minutes of alternating immersion in 96% and 70% pure alcohol, the tissues were washed with distilled water. After exposure to haematoxylin dye for 5 minutes, the tissues were washed in distilled water for 3 minutes, and the eosin stain was applied for 1 minute. Prior to immersion in xylol, the slides were dried in 70%, 96%, and 100% alcohol. Light microscopy analysis was then performed (Economou et al. 2014).

Immunohistochemistry

The histological changes in TGFβ1 and VEGFR expression in cervical carcinoma were investigated using immunohistochemistry after *R. tomentosa* leaf extract administration. The paraffin-fixed cervix samples were deparaffinised and treated for 30 minutes with 1% H_2O_2 in methanol to decrease endogenous peroxidase activity. The slides were then washed with 0.01 M Tris-buffered saline (pH 7.4). The tissue slices were treated with TGF-1 monoclonal antibody (catalogue #MA1-169 [B11-4C3]), VEGFR1 (soluble) polyclonal antibody (catalogue #36-1100), and Antigen Affinity-Purified Polyclonal Antibody (eBioscience Inc, San Diego, USA). The VECTASTAIN Elite ABC Kit (Vector Laboratories, USA) was used to detect immunoreactivity, which Mayer's haematoxylin neutralised (McCluggage 2007).

Data analysis

The Kruskal–Wallis and Mann–Whitney tests were performed on categorical (ordinal) or numerical data that were not normally distributed after data collection.

Results

Analysis of superoxide dismutase expression in rats with cervical carcinoma treated with *Rhodomyrtus tomentosa*

There was a significant difference in SOD levels between the C- and C+ groups based on ANOVA analysis with

Bonferroni post hoc test ($p < 0.05$) (Table 1). There was no statistically significant difference in SOD level when *R. tomentosa* was administered for cervical cancer at the lowest dose (100 mg/kg BW) ($p > 0.05$). However, substantial differences in SOD levels were observed with doses of 200 and 400 mg/kg BW ($p < 0.05$). Therefore, rats with cervical cancer had increased levels of SOD in their blood when given higher doses of *R. tomentosa*.

Table 1. Value of *Superoxide dismutase* by *Rhodomyrtus tomentosa* in carcinoma cervical.

No	Groups	Mean ± SD (pg/mL)
1	C-	20.21 ± 3.13
2	C+	13.01 ± 2.18$^{\#}$
3	CER100	15.91 ± 2.01
4	CER200	17.22 ± 1.90*
5	CER400	19.04 ± 1.23*

C-: Control, C+: Cervical cancer, CER100: Cervical cancer +100 mg/BW of *Rhodomyrtus tomentosa*, CER200: Cervical cancer + 200 mg/BW of *Rhodomyrtus tomentosa*, CER400: Cervical cancer + 400 mg/BW of *Rhodomyrtus tomentosa*. (##p < 0.01 versus C-, *p < 0.05 versus C+).

Analysis of malondialdehyde expression in rats with cervical carcinoma treated with *Rhodomyrtus tomentosa*

There was a significant difference in MDA level between the C- and C+ groups based on ANOVA analysis with Bonferroni post hoc test ($p < 0.05$) (Table 2). There was no statistically significant difference in MDA level when *R. tomentosa* was administered for cervical cancer at the lowest dose (100 mg/kg BW) ($p > 0.05$). However, significant differences in MDA levels were observed with doses of 200 and 400 mg/kg BW ($p < 0.05$). Therefore, MDA levels in the blood of rats with cervical cancer decreased with higher doses of *R. tomentosa*.

Table 2. Value of *Malondialdehyde* by *Rhodomyrtus tomentosa* in carcinoma cervical.

No	Groups	Mean ± SD (μM/L)
1	C-	7.23 ± 2.01
2	C+	12.09 ± 3.22$^{\#}$
3	CER100	9.24 ± 1.09
4	CER200	8.47 ± 1.11*
5	CER400	8.12 ± 2.10*

C-: Control, C+: Cervical cancer, CER100: Cervical cancer +100 mg/BW of *Rhodomyrtus tomentosa*, CER200: Cervical cancer + 200 mg/BW of *Rhodomyrtus tomentosa*, CER400: Cervical cancer + 400 mg/BW of *Rhodomyrtus tomentosa*. (##p < 0.01 versus C-, *p < 0.05 versus C+).

Analysis of TGFβ1 expression in rats with cervical carcinoma treated with *Rhodomyrtus tomentosa*

According to the Kruskal–Wallis analysis, there was a significant difference ($p = 0.000$) (Table 3). The expression of TGFβ1 was significantly different from C- based on the average value ($p < 0.01$). The lowest dose of *R. tomentosa* (100 mg/kg BW) produced a significant difference in TGFβ1 expression ($p < 0.05$), as did doses of 200 and 400 mg/kg BW ($p < 0.001$).

Table 3. Analysis of TGF β1 expression in carcinoma cervical.

Groups	Mean ± SD	Kruskal-Wallis	Mann-Whitney (p-value)				
			C-	C+	CER100	CER200	CER400
C-	10.92 ± 1.02	0.000		0.0001	0.001	0.001	0.040
C+	72.10 ± 8.22$^{\#\#}$				0.030	0.010	0.001
CER100	49.09 ± 4.09*					0.040	0.010
CER200	24.67 ± 4.81**						0.030
CER400	19.07 ± 3.92**						

C-: Control, C+: Cervical cancer, CER100: Cervical cancer +100 mg/BW of *Rhodomyrtus tomentosa*, CER200: Cervical cancer + 200 mg/BW of *Rhodomyrtus tomentosa*, CER400: Cervical cancer + 400 mg/BW of *Rhodomyrtus tomentosa*. (##p < 0.01 versus C-, *p < 0.05 versus C+, **p < 0.01 Versus C+).

The cervical cells in group C- had histologically normal epithelial lining and nuclei (Fig. 1a). Undifferentiated cells that could develop apoptotic characteristics were restricted to the lowest layer of the epithelium in the rats administered a 50-mg injection of benzopyrene (Fig. 1b). Changes in epithelial cells included thickening of the epithelium and increased TGFβ1 expression. As the dose of *R. tomentosa* increased (from 100 to 400 mg/kg BW), TGFβ1 expression in the tumour tissue decreased. Using immunohistochemical labelling, *R. tomentosa* was administered at various doses to decrease the quantity of brown-stained nuclei, which revealed a positive index of TGFβ1 expression in cancer tissues (Fig. 1c–e). The previously uncontrollable growth of carcinomas in the untreated group was effectively slowed down and stopped in the epithelium.

Analysis of VEGFR expression in rats with carcinoma cervical treated with *Rhodomyrtus tomentosa*

According to the Kruskal–Wallis test, there was a significant difference ($p = 0.000$) (Table 4). The expression of VEGFR was significantly different from C- based on the average value ($p < 0.01$). The lowest dose of *R. tomentosa* (100 mg/kg BW) did not produce a significant change in VEGFR expression ($p > 0.05$); however, doses of 200 and 400 mg/kg BW significantly altered VEGFR expression ($p < 0.01$ and $p < 0.05$, respectively). VEGFR expression was highest in the C+ and CER100 groups, and it was lowest in the CER400 group.

Table 4. Analysis of VEGFR expression in carcinoma cervical.

Groups	Mean ± SD	Kruskal-Wallis	Mann-Whitney (p-value)				
			C-	C+	CER100	CER200	CER400
C-	5.18 ± 4.71	0.000		0.001	0.001	0.050	0.040
C+	83.88 ± 6.55$^{\#\#}$				0.070	0.040	0.020
CER100	70.22 ± 7.29^{ns}					0.040	0.040
CER200	25.07 ± 7.09*						0.040
CER400	19.82 ± 4.02**						

C-: Control, C+: Cervical cancer, CER100: Cervical cancer +100 mg/BW of *Rhodomyrtus tomentosa*, CER200: Cervical cancer + 200 mg/BW of *Rhodomyrtus tomentosa*, CER400: Cervical cancer + 400 mg/BW of *Rhodomyrtus tomentosa* (#p < 0.05 versus C-, *p < 0.05 versus C+, ^{ns}p > 0.05 Versus C+).

Normal histological changes are shown in Fig. 2a; however, Fig. 2b shows a carcinoma with an uneven core that migrated to the pelvic wall. *R. tomentosa* produced a similar histological profile as the C+ group that received a dose

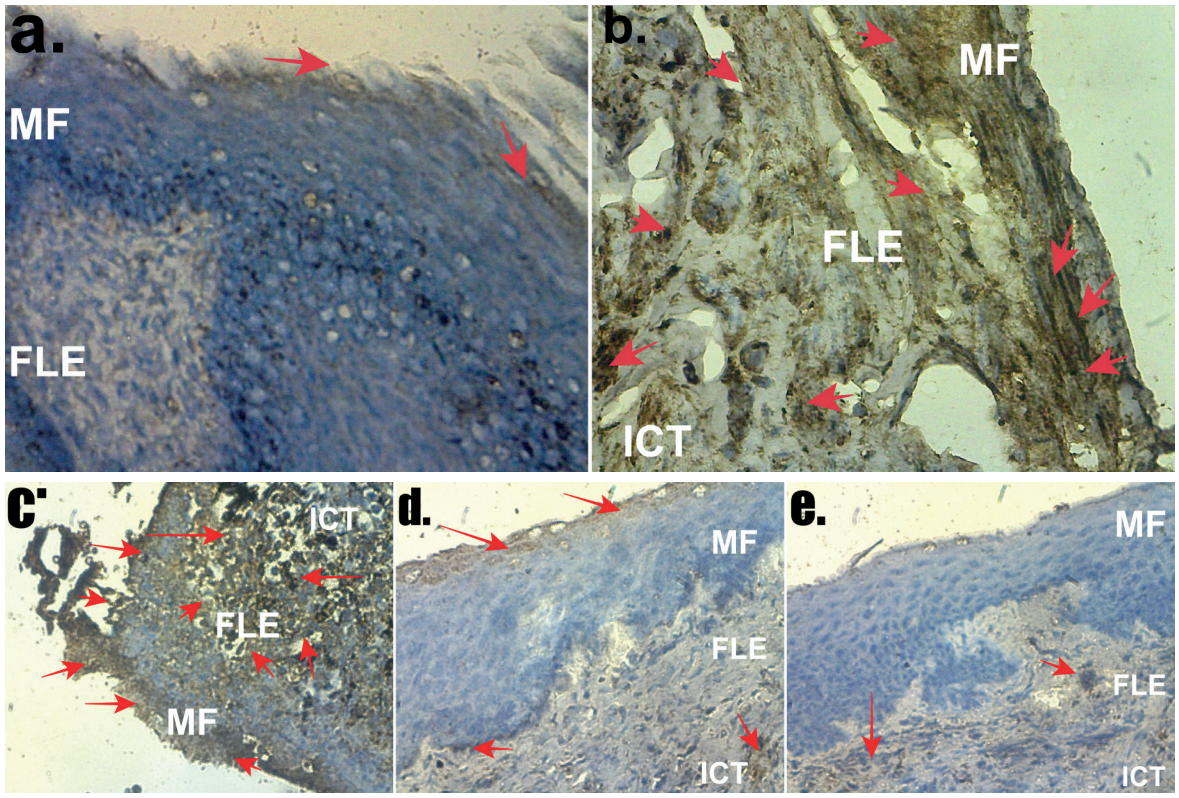

Figure 1. TGF β1 expression of Cervical cancer after given *Rhodomyrtus tomentosa*, **a.** Control (C-); **b.** Cervical cancer (C+); **c.** Cervical cancer +100 mg/BW of *Rhodomyrtus tomentosa* (CER100); **d.** Cervical cancer + 200 mg/BW of *Rhodomyrtus tomentosa* (CER200); **e.** Cervical cancer + 400 mg/BW of *Rhodomyrtus tomentosa* (CER400). Red arrows: Positive expression. MF: Mucous folds, FLE: Flattened layered epithelium, ICT: Interstitial connective tissue (40X).

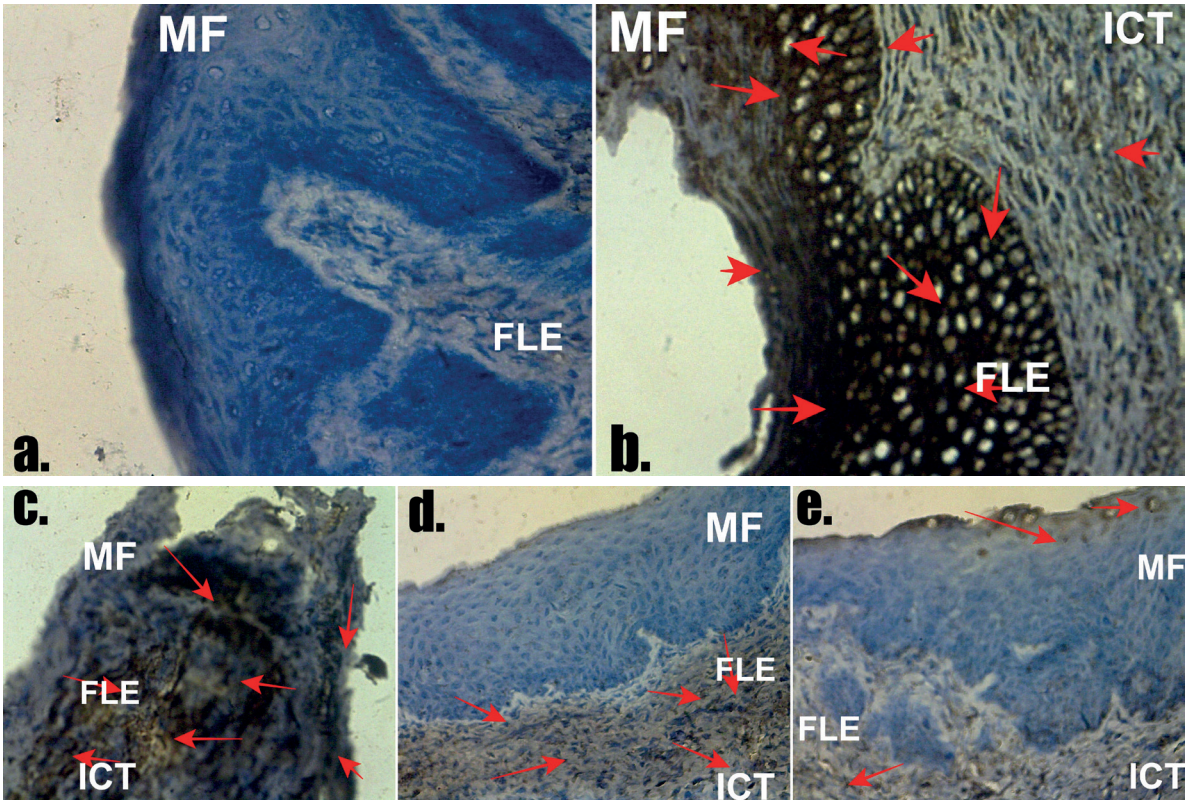

Figure 2. VEGFR expression of Cervical cancer after given *Rhodomyrtus tomentosa*, **a.** Control (C-); **b.** Cervical cancer (C+); **c.** Cervical cancer +100 mg/BW of *Rhodomyrtus tomentosa* (CER100); **d.** Cervical cancer + 200 mg/BW of *Rhodomyrtus tomentosa* (CER200); **e.** Cervical cancer + 400 mg/BW of *Rhodomyrtus tomentosa* (CER400). Red arrows: Positive expression. MF: Mucous folds, FLE: Flattened layered epithelium, ICT: Interstitial connective tissue (40X).

of 100 mg/kg BW, including large lesions. VEGFR expression at a dose of 200 mg/kg BW (Fig. 2d) revealed that the herb could drastically decrease VEGFR expression; at the maximum dose, cervical cancer stopped developing and the nucleus started to form normally (Fig. 2e).

Discussion

Increased MDA and decreased SOD levels were observed in rates with cervical cancer (Tables 1, 2). These two indicators are closely related. The administration of *R. tomentosa* leaf extract balanced SOD and MDA levels. Higher doses of *R. tomentosa* were associated with higher SOD levels and lower MDA levels.

R. tomentosa, when administered in various doses, may decrease the prevalence of brown-stained nuclei, which are indicative of TGFβ1 expression in cancer tissue. The growth of the previously uncontrollable malignancy in the untreated group was slowed down and stopped in the epithelium. Immune cells of varying types and numbers can be detected in nests of tumour cells that are surrounded by different densities of intratumoral stroma in cervical cancer (Principe et al. 2014). TGFβ1 production is prevalent in women with cervical cancer (Principe et al. 2014; Taylor et al. 2021). TGFβ1 in cervical cancer reveals that gene inactivation contributes to cervical carcinoma development (Wang et al. 2021). TGFβ1 expression was shown to decrease as the dose of *R. tomentosa* was increased. In addition to being a novel therapeutic drug that reduces cancer metastasis, induces cell cycle arrest, and increases death in gastric carcinoma (Tayeh et al. 2017; Zhang et al. 2020), rhodomyrtone, found in *R. tomentosa*, has been demonstrated to decrease cell migration, adhesion, and invasion of A431 cells (Tayeh et al. 2017; Zhang et al. 2020). The antioxidant significantly inhibited cancer metastases at subcytotoxic concentrations (0.5 and 1.5 g/ml) by decreasing A431 cell motility, cell adhesiveness, and cell invasion, with dose-dependent outcomes (Tayeh et al. 2017). The phosphorylation of a number of proteins, including protein kinase B (AKT), c-Raf, extracellular signal-regulated kinase 1/2 (ERK1/2), and p38 MAPK, which are involved in the downregulation of enzyme activity and the formation of matrix proteins, can also be prevented by rhodomyrtone. Matrix metalloproteinase 9 (MMP-9) and MMP-2 (Tayeh et al. 2017; Luo et al. 2021). Rhodomyrtone, a novel antimetastatic medication for the treatment of cancer cells, inhibits the production and phosphorylation of NF-κB in a dose-dependent manner (Xia et al. 2021). Previous studies have analysed the content of *R. tomentosa*, which contains high levels of antioxidants in nano- or micro-colloid sizes and has low toxicity (Situmorang et al. 2021; Simanullang et al. 2022a).

Cervical cancer development was halted at doses of 200 mg/kg BW to 400 mg/kg BW, indicating that this herb might be effective in suppressing VEGFR expression. An increase in VEGF expression in precancerous changes and cervical cancer is indicative of the role of this proangiogenic factor in the mechanism of neoangiogenesis (Tomao et al. 2014). The discovery of VEGF expression in tissues with poor histopathological characteristics highlights the significance of VEGF activity in the neoangiogenesis and progression of cervical cancer (Sulzmaier and Ramos 2013). VEGFR is sometimes referred to as a chemical that inhibits a blood vessel-forming enzyme (Sulzmaier and Ramos 2013); it is known as an inhibitor of the tyrosine kinase receptor for VEGF. One of the main therapeutic targets in the anti-angiogenic treatment of many malignancies is the VEGFR pathway (Shibuya et al. 2011; Rahmani et al. 2018). VEGFR is an endothelial mitogen that stimulates angiogenesis under both pathological and physiological conditions (Li et al. 2016). Because research on VEGF has gained popularity, it is possible that it serves purposes other than just promoting angiogenesis and vascular permeability (Li et al. 2016; Rahmani et al. 2018). Depending on the specific clinical condition, VEGF can interact with macrophages and T lymphocytes and produce abnormalities in the functional maturation of dendritic cells (Zhao et al. 2022). Furthermore, VEGF signalling, both autocrine and paracrine, supports cancer stem cells. Because *R. tomentosa* has been discovered to exhibit antioxidant, antibacterial, anti-inflammatory, and anticancer properties due to its biological action (Vo and Ngo 2019), increasing the dose of the herb decreases VEGFR expression. Rhodomyrtone, which is present in this herb, can enhance apoptotic bodies, nuclear fragmentation, and chromatin condensation (Tayeh et al. 2017). Rhodomyrtone can be employed as an anticancer treatment because it produces cell cycle arrest in the G1 phase according to flow cytometry studies (Tayeh et al. 2017). Antioxidants have been shown to reduce toxic side effects during cancer treatment (Situmorang et al. 2021). Antioxidant-containing plants, such as *R. tomentosa*, have been associated with cancer treatment with few side effects.

Conclusion

The elevated expression of TGFβ1 and VEGFR in cervical carcinoma cells with poor histological characteristics shows how important these proteins' actions are in the neoangiogenesis and progression of cervical cancer. *R. tomentosa* has been demonstrated to heal carcinogenic metastatic carcinoma tissue; it can be administered at various doses to decrease the number of brown nuclei that exhibit a positive index of TGFβ1 and VEGFR expression in cancer tissues.

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