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Research Article
Histological Alterations of Cervical Cancer Following *Zanthoxylum acanthopodium* DC Therapy in Relation to E7, pRb, EGFR and p16 Expression

[1]Rostime Hermayerni Simanullang, [2]Jekson Martiar Siahaan and [3]Putri Cahaya Situmorang

[1]Department of Nursing, Faculty of Nursing, Universitas Murni Teguh, Medan, Indonesia
[2]Department of Molecular Biology, Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia

Abstract

Background and Objective: Cervical cancer is the second most common cancer in Indonesia, where traditional herbal treatments like *Zanthoxylum acanthopodium* (andaliman) are culturally used. Investigating protein biomarkers such as E7, pRb, EGFR and p16 can help assess the efficacy of these treatments. **Materials and Methods:** There were 5 groups in this study: 2 control groups (C- and C+) and 3 treatment groups (each receiving one of three doses). Oral administration of andaliman was performed for 30 days in cancer model rats, after which the cervix was dissected, cervical tissue was taken and immunohistochemistry repair was performed. Statistical analysis was performed using the Kruskal-Wallis test with a $p<0.05$. **Results:** As *Zanthoxylum acanthopodium* DC dose rose, cervical tissue E7, EGFR and p16 expression decreased. However, greater doses of this plant increased cervical tissue pRb protein. Cervical cancer histology exhibited increased nuclear size, irregular cellular structure, atypical cell shape, higher nuclear-cytoplasmic ratio and various nuclear shape variants. This herb induced tissue to show well-organized non-hyperchromatic cells that resembled normal clusters. **Conclusion:** *Zanthoxylum acanthopodium* DC improved cervical tissue and balanced cervical cancer biomarker proteins such E7, EGFR, pRB and p16.

Key words: Cervical cancer, epidermal growth factor receptor protein (EGFR), Human Papillomavirus (HPV), retinoblastoma protein (pRB), *Zanthoxylum acanthopodium* DC

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Corresponding Author: Putri Cahay Situmorang, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The number of people diagnosed with cervical cancer ranks second, with 36,633 instances or 9.2% of the overall number of cancer cases in Indonesia[1]. Hormone therapy is an option for patients who are undergoing treatment for cervical cancer. Surgical methods, chemotherapy, or radiation therapy are available to accomplish this treatment[1]. Since their forefathers' time, some Indonesians have ingrained the practice of drinking certain plants as a complementary treatment[2]. Molecular approaches are important as protein biomarkers to investigate the efficacy of herbal treatments, especially in cervical cancer[3]. Understanding the relationship between the expression of these proteins and the constituents of herbal plants is crucial and one way to achieve this is by applying molecular pathways in the medical field, specifically through the use of herbs[2,3]. The E7, pRb, EGFR and p16 are some of the biomarker proteins that are associated with cervical cancer.

Human Papillomavirus (HPV) is responsible for HPV-induced carcinogenesis because it encodes two oncoproteins, E6 and E7[4]. The E7 protein like the "active" form of the retinoblastoma gene produces pRb that is less phosphorylated. The pRb inhibits cell cycle progression via E2F target genes, acting as a central tumor suppressor[4]. Cells infected with high-risk strains of the human papillomavirus create an oncogenic protein that can cause cancer if it attaches to and inactivates pRb[5]. The tyrosine kinase receptor EGFR is frequently overexpressed in malignant tumors[6]. The epidermal growth factor receptor (EGFR) protein functions in cell signaling pathways that regulate cell division and survival[7].

Because herbal medicine is inexpensive and has minimal adverse effects, Indonesians opt for it in addition to conventional medicine[8]. Andaliman (*Zanthoxylum acanthopodium*), one of Indonesia's botanical fruits, binds free radicals to reduce oxidative damage. Traditional Batak dishes use the fruit of this plant for flavor[8]. Additionally andaliman fruit can treat cervical cancer[9,10], placenta cells[11], preeclampsia[12] and diabetic wounds[13]. Cervical tissue was examined in animal models for cancer marker proteins including E7, pRb, EGFR, p16 and Ki67. The objective of this research was to examine the effect between E7, pRb, EGFR and p16 expression and the histological alterations in cervical cancer following *Zanthoxylum acanthopodium* DC treatment as herbal medicine, especially in cancer treatment, is so expected to play a significant role.

MATERIALS AND METHODS

Study area: The study took place between August, 2022 and August, 2023. The research was performed at the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara's, Medan, Indonesia.

Ethics statement: All procedures regarding animal care and experiments are fully in accordance with the guidelines provided by the FMIPA USU Medan Medical Research Ethics Committee (No. 0909/KEPH-FMIPA/2023). Every effort was made to minimize the rats suffering.

Reagents: The antibodies utilized for Immunohistochemistry were sourced from Biorbyt Ltd., Cambridge, United Kingdom and included rabbit polyclonal antibodies to HPV16 E7 (orb184006). Rabbit monoclonal to EGFR (ab32562), rat monoclonal to CDKN2A/p16INK4a (ab241543) and rabbit monoclonal to pRb (ab181616) are all products of Abcam in the US. Plus, a PBS buffer solution containing 50% glycerol and 1% bovine albumin (BSA) (Cat. No. BS-0812R) was utilized.

Plant materials: The plant was sent for identification to the Plant Systematics Laboratory, Medanense Herbarium at USU. It was collected from the Sihalus and Sihorbo Samosir areas in Samosir Regency, which is located at an Altitude of 1600-1700 m above sea level. *Zanthoxylum acanthopodium* DC (1325/MEDA/2023) is the plant's official identification.

Preparation of the *Zanthoxylum acanthopodium* DC fruits: After removing the fruit from the branches, the andaliman is mixed and dried in the oven for one day. A 5 L container is filled with 500 g of andaliman and soaked in 96% ethanol. After the solution has been mixed until it is uniform, it is allowed to stand for one night so that it can settle. It is necessary to extract the uppermost layer or portion of the solvent and filter it using filter paper. After removing the top layer of the solvent or a portion of it, the substance is transferred to the evaporating flask and then connected to the evaporator (Büchi® Rotary Evaporator Model R-200, Merck, 2010, Germany). Following the heating of the series of evaporating flasks, evaporators and rotating evaporators in a water bath maintained at a temperature of 90°C, an electric current is then connected to the apparatus. It will take some time for the ethanol to become separated from the active material as you wait for the solution to trickle into the holding flask. The products of the extraction were placed in plastic bottles and placed in the freezer for safekeeping.

Animal handling: The thirty female Wistar strain rats (*Rattus norvegicus*) from the Animal House, FMIPA, USU. Female rats weigh between 180 and 200 g and reach sexual maturity at 12 to 14 weeks of age. Each animal cage consisted of a total of five adult female rats. To acclimate the female rats to laboratory conditions, the rats were kept at a temperature of 25.3°C and humidity levels ranging from 35 to 60% for 3 weeks. The rat cage was in light conditions for 12 hrs and then in darkness for 12 hrs. Water and rodent feed (standard feed, CitraFeed Ratbio, Company: PT. Citra Ina Feedmill, Indonesia) were freely available to the rats throughout the experiment and the rats were weighed twice a week.

Cancer model rats: The rats were injected with 5 mg/kg b.wt., of xylazine to the rats after two weeks of acclimatization to induce stupor. The anesthetic chemical was injected into a 1 mL bottle using a 26G×1/2 short syringe. The rat model was designed to mimic human conditions from a physiological and pathological perspective. Benzo(a)pyrene was injected into the cervix of the rats at a dose of 50 mg/kg b.wt., after they had stupor. Every week, the cancerous nodules caused by benzo(a)pyrene were palpated (touched) to determine whether the rats had developed cancer. This injection was given to all groups of female rats, except group C, which served as the untreated group. After three to four months, a fairly large growth had been detected in the cervix of the rats, measuring 8-20 mm in diameter (using a caliper). Then a biopsy was performed and the tissue samples were sent to the Anatomical Pathology Laboratory to confirm whether the mass was indeed a tumor. If the investigation found tumor tissue, the investigation was continued by giving herbal medicine.

Research design: Group C+ rats were given benzo(a)pyrene, whereas group C- rats were used as normal, negative controls. Both groups of rats received the same doses of benzo(a)pyrene and *Z. acanthopodium* fruits: C1 rats received 100 mg/kg b.wt., C2 rats received 200 mg/kg b.wt. and C3 was a 300 mg/kg b.wt., dose of *Zanthoxylum acanthopodium* DC fruits. Oral administration of *Z. acanthopodium* was continued for a full month. On the 31st day, anesthesia was administered to the rat using 4% isoflurane in 1.5 L/min of oxygen in a Perspex chamber. The anesthesia was then maintained using 2% isoflurane in 0.5 L/min of oxygen. Isoflurane is chosen due to its ability to induce anesthesia quickly and allow for a safe recovery while causing minimal side effects. Next, the cervical organ is taken and preserved in formalin in preparation for the immunohistochemistry stage.

Tumor volume: The formula $V = \pi/6$ (L×W×W) was used to determine the tumor volume, where L represents the tumor's length and W represents its width[14]. To determine the size of the tumor using a computerized caliper (Mitutoyo, Aurora, Illinois, USA, 2007) with a resolution of 0.01 mm.

Immunohistochemistry: The Anatomical Pathology Laboratory at USU performed the immunohistochemistry and Hematoxylin and Eosin (H&E) preparations. Xylol I/II was allowed to soak into the cervix tissue for 10 min. Steps I through III were repeated with increasing concentrations of absolute alcohol for 5 min each: I, with 95% alcohol; II, with 80% alcohol and III, with 70% alcohol. The tissue was rinsed twice with 2 min of deionized water. The preparations were soaked in a citric acid buffer (0.01M, pH 6.0). Without removing the tissue, the medium was heated in the microwave for 10 min while it boiled. The container for soaking was allowed to cool. After the preparation cooled, it was taken out and washed three times for 3 min in PBS (pH 7.4). Cell peroxidase activity was reduced using 3% H_2O_2. The slides were rinsed thrice with PBS after being immersed in 30% H_2O_2 for 15 min.

Five percent normal serum and a secondary antibody of the same or a comparable species were added after cleaning the absorbent paper with PBS. The slides were blocked at 37°C for half an hour. The primary antibody, diluted with water, was dropped onto the tissue after it had been dried using absorbent paper. The PBS was placed in the control area if there was no negative control. The slides were incubated overnight at 4°C in a damp container with the diluted primary antibodies (E7, pRb, EGFR and p16). After 2, 3 min washes with PBS, the slides were dried with absorbent paper and HRP-conjugated secondary antibody was added, followed by incubation at 37°C for 30 min. The slides were washed for 3 min, repeated 4 times, dried with absorbent paper and DAB substrate was added to each tissue before inspection under a microscope. Positive signals typically appeared brown or yellowish brown. To prevent the response from becoming too dark, it could be interrupted using flowing water. The material could be stained if it was first soaked for 30 sec to a minute in a solution of 1% alcohol and hydrochloric acid. After rinsing with water, a second wash was optional. The slide was set aside to soak in a mixture of xylol and alcohol at different concentrations (70, 80, 90 and 95% absolute alcohol I and II, etc.). Each solution was placed in a fume cupboard to dry after 2 min of soaking. Under a cover glass, the slide was viewed using a microscope (Olympus Microscope Binocular, Cx-23 Olympus, Tokyo, Japan, 2015)[15].

Statistical analysis: The statistical analysis was performed using Kruskal-Wallis with PASW 18 (SPSS, Inc., Chicago, Illinois, USA) and XLSTAT version 2011.4.02 (Addinsoft SARL, France) with a $p<0.05$.

RESULTS

Tumor volume after being given *Zanthoxylum acanthopodium* DC: There were no statistically significant variations ($p>0.05$) in the cervical volumes of the rats before andaliman treatment, as shown in Table 1. Table 1 shows that the cervical volume of the C+ group of rats was significantly larger than that of the C- group. But when compared to group C, neither C1 nor C2 differed significantly. The tumor volume was largest in group C+ and lowest in group C3. Rats in Group C did not have any malignancies, however they did have a higher cervical volume. At the highest dose of *Zanthoxylum acanthopodium* DC, there was a significant reduction in cervical tumor volume.

Histological alterations in cervical carcinoma with andaliman fruits treatments: Andaliman can decrease the presence of cancerous tissue in the cervix during histological investigation using Hematoxylin and Eosin (H&E) staining. The flat stratified epithelium undergoes alterations in cancer histology (Fig. 1), which can result in enlargement, cellular maturation and detachment of epithelial cells. Epithelial tissue serves as a diagnostic tool for assessing body metabolism and can indicate the condition of both healthy cells and cancerous cells. Figure 1 also displays typical mucosal folds and areas of flattened epithelium and interstitial connective tissue. The cervical tissue is often lined with squamous epithelium or flat stratified epithelium. The cervical mucous layer contains glands that secrete mucus. Group C- exhibited a typical tissue

Table 1: Effect of *Zanthoxylum acanthopodium* DC in cervix size

	Cervical volume (Mean±SD (mm^3))	
Group	Before treatment	After treatment
C-	12.10±1.51	12.14±2.01
C+	13.11±3.67^{ns}	620.23±23.33$^{###}$
C1	13.23±2.21^{ns}	603.67±34.22^{ns}
C2	12.39±3.45^{ns}	576.45±41.23^{ns}
C3	13.67±3.66^{ns}	431.34±32.56*

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman, $^{###}p<0.001$ vs C-, *$p<0.05$ vs C+, $^{ns}p>0.05$ and ±: Represents the margin of error or the standard deviation

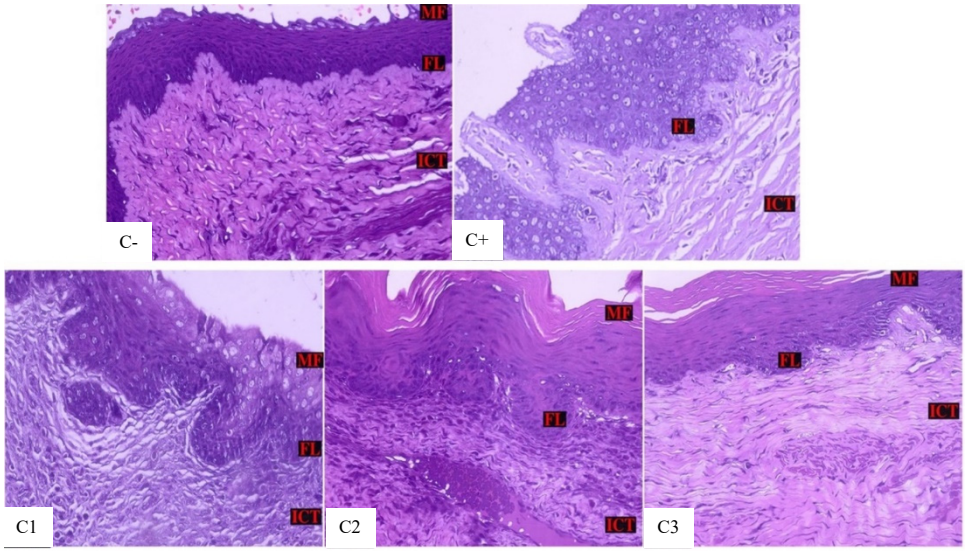

Fig. 1: Histological alterations in cervical carcinoma with *Zanthoxylum acanthopodium* DC (andaliman) treatments

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman, MF: Mucous folds, FL: Flattened layered epithelium and ICT: Interstitial connective tissue (400×)

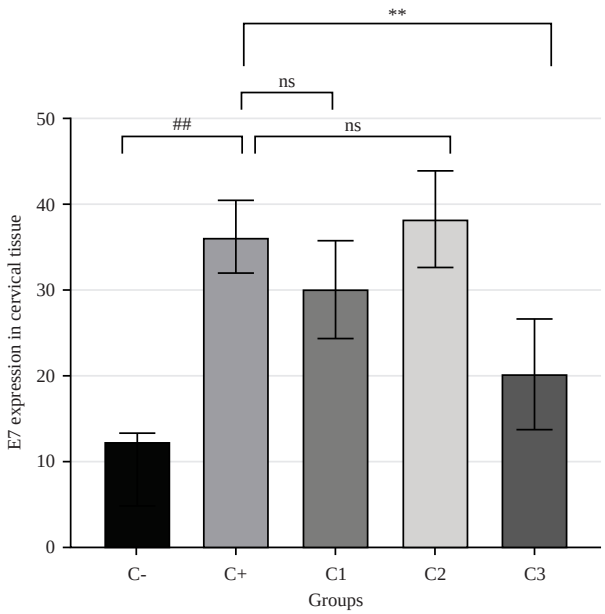

Fig. 2: E7 expression in cervical tissue after administration of *Zanthoxylum acanthopodium* DC

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., Andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., Andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., Andaliman, $^{\#\#}p<0.01$ vs C-, $^{**}p<0.01$ compared C+ and $^{ns}p>0.05$

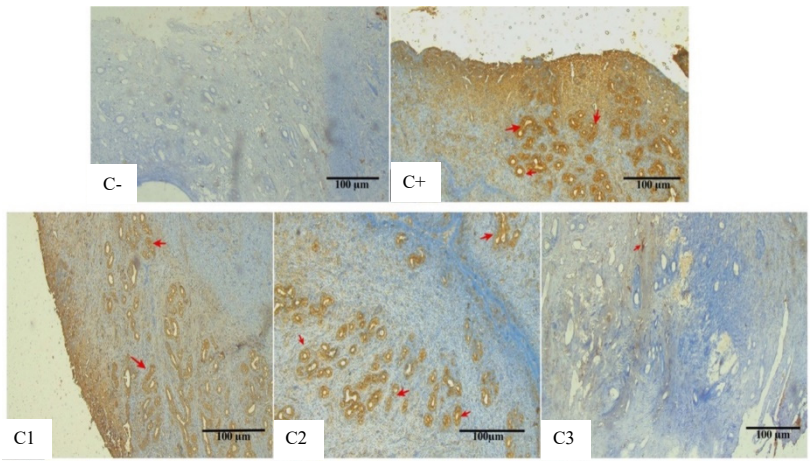

Fig. 3: Expression of E7 on cervix histological changes after administration of *Zanthoxylum acanthopodium* DC in Benzo[a]pyrene-induced rats

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman and red arrows: Positive expression (200×)

architecture in contrast to Group C+. The C+ refers to cancerous tissue resulting from benzopyrene injection. The mucosal folds exhibit irregularities and the interstitial connective tissue is elongated. The administration of C3 groups results in a restoration of the cervix's form, whereas the administration of C1 and C2 has no discernible impact.

Impact of *Z. acanthopodium* on cervical E7 expression in histology: The difference between the C- and C+ categories was significant. The maximum levels of E7 expression were found in group C+ and C1, while the lowest levels were found in group C- and C3 (Fig. 2). The expression of E7 in cervical tissue decreases as the dose of *Zanthoxylum acanthopodium* DC increases. On histology, it can be seen that in the group without benzo[a]pyrene (C-) induction, the normal epithelium of the cervical portion undergoes gradual maturation, vacuolization in the cytoplasm and a single layer of basal cells where the nucleus is perpendicular to the basal lamina but

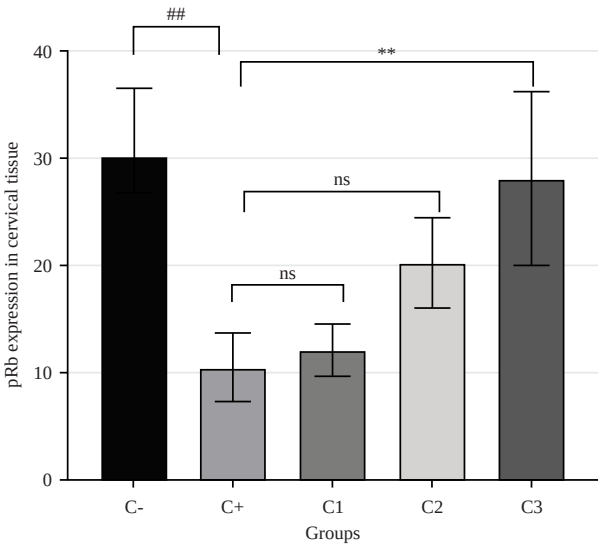

Fig. 4: pRb expression in cervical tissue after administration of and *Zanthoxylum acanthopodium* DC

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., Andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., Andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., Andaliman, $^{\#\#}p<0.01$ vs C-, $^{**}p<0.01$ compared C+ and $^{ns}p>0.05$

when induction of benzo[a]pyrene can be seen that the epithelium has shrunk and the nucleus is irregular (Fig. 3). *Zanthoxylum acanthopodium* DC began to enhance the histological structure of cervical tissue to the point where the C3 group or the highest dose nearly resembled the histology of the control group.

Impact of *Z. acanthopodium* on cervical pRb expression in histology: There was a notable distinction between the C- and C+ groups, but no distinction was observed between the C2 and C3 groups. The group C- and C3 exhibited the highest level of pRb expression, whereas the group C+ and C1 showed the lowest level (Fig. 4). There is a direct correlation between the dosage of *Zanthoxylum acanthopodium* DC and the amount of pRb expression in cervical tissue. Specifically, as the dosage of *Zanthoxylum acanthopodium* DC increases, the level of pRb expression in cervical tissue also increases. Cervical histology identifies aberrant cells by distinct features such as an enlarged nucleus, unregulated cell structure, uneven cell shape, a high ratio of nucleus to cytoplasm and several changes in nucleus shape (C+). Normal cells displayed typical cell morphologies, intact cell structures and non-hyperchromatic cell nuclei at the maximum dosage of *Zanthoxylum acanthopodium* DC (Fig. 5).

Impact of *Z. acanthopodium* DC on cervical EGFR expression in histology: The administration of *Zanthoxylum acanthopodium* DC can alter EGFR expression in cervical histology. There was a significant difference between all categories except between C1 and C+. The highest expression of EGFR was observed in groups C+ and C1, while the lowest expression was observed in groups C- and C3 (Fig. 6). The expression of EGFR in cervical tissue is reduced proportionally to the dose of *Zanthoxylum acanthopodium* DC. On cervical histology, 50 mg of benzopyrene in maize oil induced in group C+ harmed the structure of epithelial cells with abnormal nuclei, which began to ameliorate when *Zanthoxylum acanthopodium* DC was administered (Fig. 7).

Impact of *Z. acanthopodium* on cervical p16 expression in histology: The expression of p16 in cervical histology may be altered by the administration of *Zanthoxylum acanthopodium* DC. All groups exhibited significant differences, except for the comparison between C1 and C+ ($p>0.05$). Group C+ and C1 had the highest level of p16 expression, whereas group C- and C3 demonstrated the lowest level (Fig. 8). Upon histological evaluation of cervical tissue, notable disparities in p16 expression were observed between Group C+ and C-. In C+ rats, the cancer had spread to the pelvic wall, filling the space between the tumor and the pelvic wall and causing the nuclei to become disorganized. After being treated with *Zanthoxylum acanthopodium* DC, the presence of p16-associated brown spots decreased, leading to an improvement in cancer and abnormal nucleus conditions. Higher concentrations of *Zanthoxylum acanthopodium* DC decrease the expression of p16 in cervical tissue, as seen in Fig. 9.

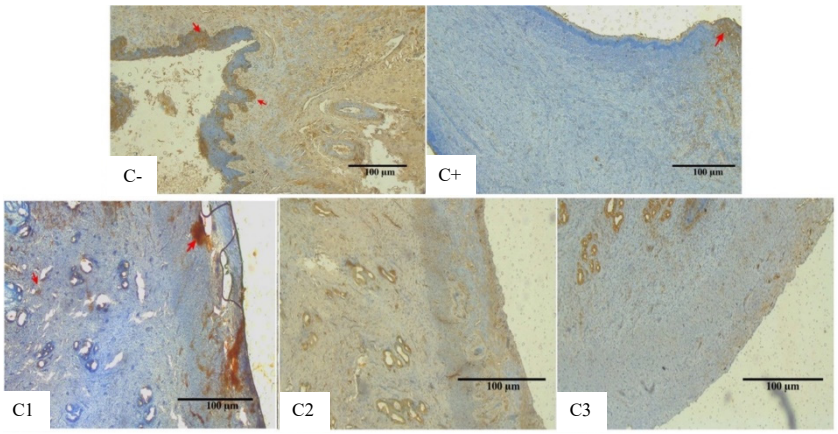

Fig. 5: Expression of pRb on cervix histological changes after administration of *Zanthoxylum acanthopodium* DC in Benzo[a]pyrene-induced rats

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman and red arrows: Positive expression (200×)

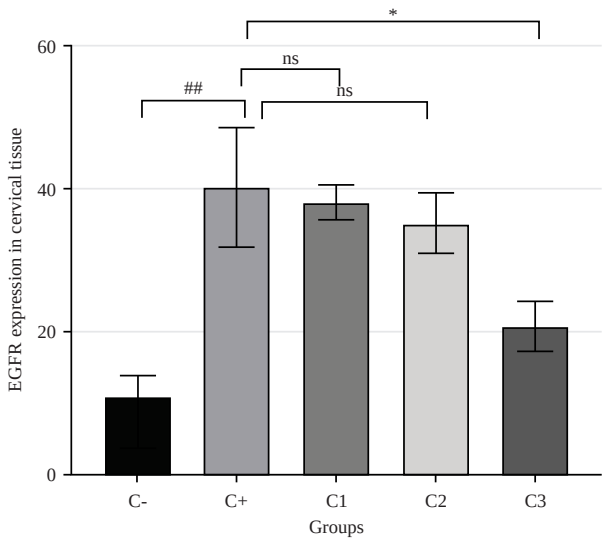

Fig. 6: EGFR expression in cervical tissue after administration of and *Zanthoxylum acanthopodium* DC

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman, $^{\#\#}p<0.01$ vs C-, $^{*}p<0.05$ compared C+ and $^{ns}p>0.05$

DISCUSSION

The excessive generation of free radicals due to the carcinogenic effects of benzo[pyrene] (C+ groups) leads to the structural destruction of cervical tissue. Oxidative stress arises from an excessive presence of reactive oxygen species (ROS) when there is an insufficient amount of antioxidants[17]. Oxidative stress is a significant contributor to the processes of aging and the development of cancer. Oxidative stress occurs when there is an imbalance between free radicals and antioxidants[16,17]. Oxidative stress impairs the balance of ions inside cells, reduces enzyme activity, disturbs the integration of cell membranes and ultimately impairs cell function, leading to cell damage[18]. The C+ group exhibits anomalous tissue forms of various shapes, such as oval, tadpole, irregular, caudate and spherical. Andaliman possesses antioxidant, anti-inflammatory and anticancer characteristics that facilitate the regeneration of cervical epithelial cells through the processes of mitosis, elongation and desquamation during sexual reproduction[19]. Cell proliferation is essential for regulating the overall growth of the body. The epithelium undergoes cell renewal every 4-5 days. The presence of

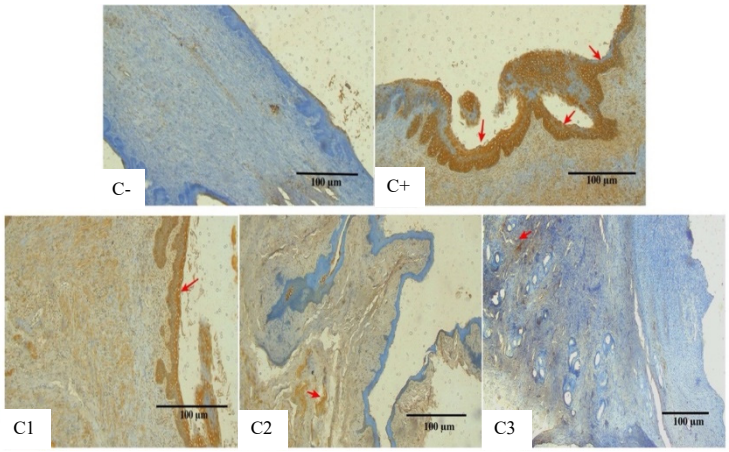

Fig. 7: Expression of EGFR on cervix histological changes after administration of *Zanthoxylum acanthopodium* DC in Benzo[a]pyrene-induced rats

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman and red arrows: Positive expression (200×)

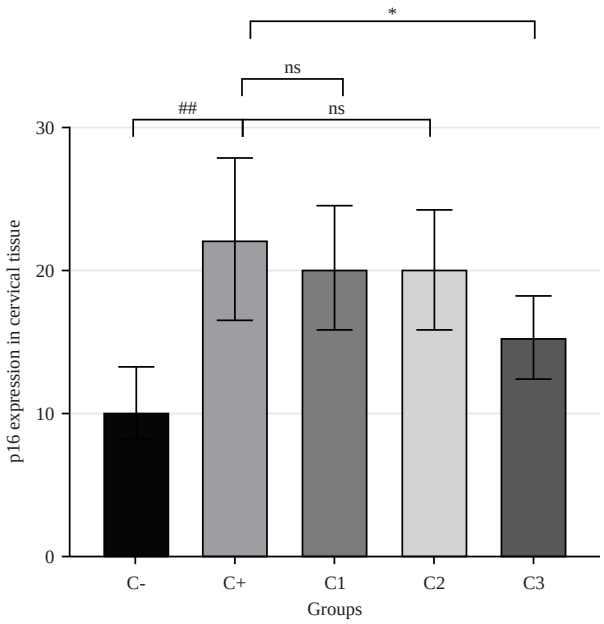

Fig. 8: p16 expression in cervical tissue after administration of and *Zanthoxylum acanthopodium* DC

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman, $^{\#\#}p<0.01$ vs C-, $^{*}p<0.05$ compared C+ and $^{ns}p>0.05$

brown spots, which serve as a molecular biomarker, decreased following the administration of andaliman. Additionally, there was observed improvement in both cancer and abnormal nuclei. Andaliman 300 mg/kg b.wt., lowered E7 expression. This herb's antioxidants suppress angiogenesis, COX-2, lipoxygenase, adhesion molecules, proinflammatory cytokines and E7[20]. By binding to the proapoptotic proteins p53 and pRb, oncogenes E6 and E7 trigger the aggressive proliferation of infected cells, leading to the development of precancerous lesions that eventually progress to cancer[5]. Tumor suppressor gene products form complexes with E6 and E7 enzymes[21,22].

The level of pRb expression in cervical tissue is directly related to the dosage of andaliman. Cervical histology can detect abnormal cells by looking for certain features, such as an enlarged nucleus, an uncontrolled cell structure, an uneven cell shape, a high nucleus to cytoplasm ratio and multiple changes in nucleus shape (C+). Consistent cell morphologies,

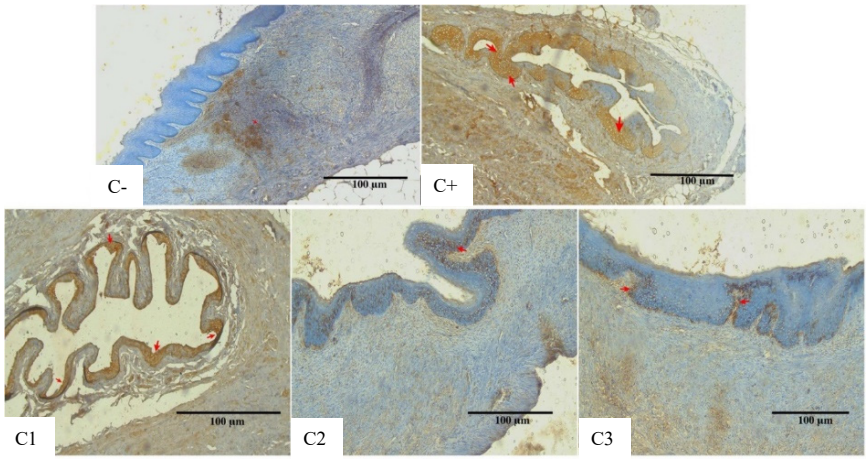

Fig. 9: Expression of p16 on cervix histological changes after administration of *Zanthoxylum acanthopodium* DC in Benzo[a]pyrene-induced rats

C-: Untreated groups, C+: Rats induced by Benzo[a]pyrene, C1: Rats induced Benzo[a]pyrene+100 mg/kg b.wt., andaliman, C2: Rats induced Benzo[a]pyrene+200 mg/kg b.wt., andaliman, C3: Rats induced by Benzo[a]pyrene+300 mg/kg b.wt., andaliman and red arrows: Positive expression (200×)

undamaged cell structures and non-abnormally darkened cell nuclei were observed in the normal cells at the highest dosage of andaliman. The andaliman levels are positively correlated with pRb expression levels in cervical tissue. There are essential oils in these plants that make them even more anti-inflammatory and antioxidant. Terpenoids found in andaliman have a suppressive effect on cancer cells by reducing cyclin D1 expression, increasing p53 expression and inhibiting NF-κB phosphorylation. As a result, NF-κB activity is inhibited, leading to a drop in pro-survival proteins like XIAP[23]. It can enhance programmed cell death in cancer cells and amplify the pro-death activity of TRAIL[23,24]. During cervical cancer cell proliferation, a critical event in the signaling cascade occurs when E7 interacts with the pRb-E2F complex. When E2F expression is high during the G1/S phase, the proteasome breaks down pRb[5]. The application of Andaliman therapy led to a decrease in the expression of EGFR and the mitigation of tissue damage induced by carcinogenic substances. The EGFR controls the growth and maintenance of epithelial tissues[25]. Because EGFR amplifications or secondary mutations are often observed in response to medication or herbal ingredient pressure, it is being widely accepted as a biomarker of resistance in tumors[6]. Chemotherapy fails to treat some cancers because cancer cells become resistant to anticancer medications due to this overexpression[26]. The HPV associations with p16 overexpression in cervical cancer. Depending on the nature and origin of the tumor, overexpression of p16 could be a diagnostic indicator in metastatic carcinoma and a factor in tumor formation. An upregulation of p16 by immunohistochemistry indicates that HPV[26,27]. The p16 expression is associated with a greater histological grade of the tumor but not with cervical perineural invasion[26].

The secondary metabolites of herbs are what give them their medicinal value. *Zanthoxylum acanthopodium* DC provides antioxidant and anticancer effects due to its alkaloids, flavonoids, terpenoids and lignin content. However, additional research is required before these compounds can be utilized in functional foods, nutraceuticals or innovative pharmaceuticals[28]. Andaliman contains phytochemicals such as phenols, flavonoids, alkaloids, glycosides, lignin and tannins, which are naturally present in the plant[28]. This extract's high phenol and flavonoid content suggests that these plants could be employed in pharmacology and phytotherapy as antioxidants to decrease cell oxidation[8,9]. By neutralizing free radicals like peroxides and hydroperoxides, it reduces the risk of cancer and other degenerative diseases.

CONCLUSION

The expression of E7, EGFR and P16 in cervical tissue decreased with higher doses of *Zanthoxylum acanthopodium* DC, leading to improved cervical histology. The untreated group showed normal cervical epithelium, while benzo[a]pyrene exposure caused tissue damage and irregular cellular structure. Increasing the herb dosage improved tissue organization, though pRb expression increased with higher doses. Histological features such as abnormal nuclear size and shape were mitigated, with treated tissues resembling the normal cervical structure.

SIGNIFICANCE STATEMENT

This study demonstrates that *Zanthoxylum acanthopodium* DC extract has therapeutic potential for cervical carcinoma by influencing key molecular biomarkers such as E7, pRb, EGFR and p16. By reducing the expression of E7, EGFR and p16 while increasing pRb, the extract exhibits promising effects in improving cervical histology and mitigating cancerous changes. The primary objective of this research is to explore the molecular role of *Zanthoxylum acanthopodium* DC in regulating these proteins, contributing to cancer therapy development. The findings suggest that this plant may modulate tumor growth and progression at the molecular level. Future research could expand on these results by exploring the herb's influence on other signaling pathways, potentially leading to new avenues in cancer drug development and therapeutic strategies for cervical and other cancers.

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