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Research Article
Correlation of Myc Expression with Wee1 Expression by *Zanthoxylum acanthopodium* in Cervical Carcinoma Histology

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Abstract

Background and Objective: Natural herbs and molecular therapy can be used to treat cervical cancer. The Myc and Wee1 control tumour cell fate and microenvironmental changes like angiogenesis activation and host immune response suppression. The study aims to know about the correlation of Myc and Wee1 expressions as a molecular therapy given by *Zanthoxylum acanthopodium*. **Materials and Methods:** There are five rat groups: Group K^- is the untreated group, Group K^+ is the rats injected with benzopyrene, Group P_1 is the administration of *Zanthoxylum acanthopodium* 100 mg kg^{-1} b.wt., Group P_2 is the administration of *Zanthoxylum acanthopodium* 200 mg kg^{-1} b.wt. and Group P_3 is the administration of *Zanthoxylum acanthopodium* 400 mg kg^{-1} b.wt. The rats are dissected 30 days after receiving *Zanthoxylum acanthopodium*. To stain the cervical tissues, immunohistochemistry is performed. **Results:** *Zanthoxylum acanthopodium* administration caused epithelial thickening and decreased Myc expression in previously uncontrolled carcinomas from untreated malignancies, which now slowed and stopped growing into the normal epithelium. Wee1 expression revealed that this herb could repair tissue by drastically reducing Wee1 expression at a dose of 100-400 mg kg^{-1} b.wt. Similarly, at the highest dose, cervical carcinoma stops growing and the nucleus begins to form normally ($p<0.01$). **Conclusion:** The higher Myc expression on andaliman administration in cervical carcinoma decreases Wee1 expression in cervical carcinoma so these two proteins have a strong and significant correlation. *Zanthoxylum acanthopodium* can be administered at various dosages to lower the number of positive indexes of Myc and Wee1 expression in cervical carcinoma.

Key words: Cervical cancer, immunohistochemistry, molecular therapy, Myc, Wee1, *Zanthoxylum acanthopodium*, alkaloids, glycosides

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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

One of the most preventable forms of cancer is cervical cancer. But in 2020, cervical cancer killed an estimated 341,800 women worldwide[1]. Between 2018 and 2030, cervical cancer diagnoses are anticipated to increase from 570,000-700,000, while annual mortality is anticipated to increase from 311,000-400,000[2]. Due to a lack of access to cervical cancer prevention, screening and treatment, the majority of these deaths occur in LMICs, or low- and middle-income countries[3,4]. The Asia-Pacific Regional Office created a series of summaries on Cervical Cancer Elimination to help guide countries in the region, including Indonesia. Molecular therapy can be used to cure cancer while utilizing natural herbs[5]. In cancer treatments, including chemotherapy, certain herbs help minimize their side effects[5].

North Sumatra is home to a wild population of the Indonesian spice andaliman (*Zanthoxylum acanthopodium*)[6]. Antioxidants present in *Zanthoxylum acanthopodium* work as bacterial, anti-inflammatory and natural preservatives. These antioxidants include alkaloids, glycosides, tannins, phenols and flavonoids[7,8]. The MMP9, GLUT1, IL-10, IL1, VEGFR1 and TGFbeta1 expressions can all be reduced by Andaliman[9,10]. This plant can also improve placental tissue histology, rat kidney and liver hypertension, human trophoblast Hes1 and NOTCH1 expression and diabetes burn[11-16].

A lot of human malignancies are influenced by the MYC oncogene. Recent discoveries indicate that this protein may lead to new cancer therapeutic options[17]. Drug-like compounds that contain antioxidants can prevent bromodomain proteins from activating MYC, which inhibits tumour growth *in vivo*[18]. Additionally, tumour growth can be controlled by pharmacologically separating Myc-induced cellular biomass accumulation from metabolic pathways requiring glucose or glutamine[19]. Targeting Myc-Max dimerization or Myc-induced microRNA production is another method for preventing Myc-induced malignancy. The Ser/Thr kinase family of proteins, which includes Wee1, is mostly found in the fission yeast *Schizosaccharomyces pombe*[20].

Wee1 is a crucial regulator of cell cycle progression and has a 96 kDa molecular mass[17]. By preventing entry into mitosis via Cdk1, it decreases cell size. Many other organisms, including mammals, have Wee1 homologues. By phosphorylating Cdk1 twice, at Tyr15 and Thr14, Wee1 inhibits Cdk1[21]. For the cyclin-dependent component of several cell cycle checkpoints, Cdk1 is necessary[21]. For Wee1 to prevent Cdk1, at least three checkpoints are necessary: Checkpoint G2/M: Further cell growth may take place in *S. pombe* because Wee1 phosphorylates the Tyr15 and Thr14 amino acids in Cdk1, decreasing Cdk1 kinase activity and blocking mitotic entrance[21]. Wee1-mediated Cdk1 inactivation is highly sensitive to substrate competition. Several regulators reduce Wee1 activity during mitotic entry, resulting in increased Cdk1 activity[22].

The findings of the study on the correlation of Myc and Wee1 expressions in the herb's potential as a molecular therapy treatment for cancer provide strong support for its potential therapeutic use in modern medicine. It was determined to first investigate how *Zanthoxylum acanthopodium* altered the expression of Myc and Wee1 expression in rat cervical histopathology before employing human cells. It is intended to use this plant to produce drugs for human molecular cancer therapy.

MATERIALS AND METHODS

Materials: *Zanthoxylum acanthopodium* originated in the Kabanjahe region of North Sumatera Province, Indonesia. Corn oil contains 50 mg of benzopyrene (MFCD00003602, Merk, Vietname Ltd.).

Preparation of *Zanthoxylum acanthopodium*: Fruits from *Zanthoxylum acanthopodium* (andaliman) have any dirt or dust removed from them. The fruit methanol extract is made in the following three phases: (1) Drying the crude: Before blending, the andaliman fruit is cleaned and drained, (2) andaliman extract is made by macerating Andaliman fruit for one night in 96% methanol. The mixture is then allowed to percolate until clear. Evaporating the concentrated liquid yields powder extracts, (3) Because the andaliman extract does not completely dissolve in water. To create a homogenous mixture, it is mixed with a suspending agent, CMC 1.5-1.0%, or 1 mL in 150 mL of distilled water. Before being transferred to a closed container and stored for two days in a cold, dark environment, the dregs are cleaned with a 96% methanol solvent[10].

Experimental animals: The research took place in the animal physiology Laboratory, Pathology and Anatomy Laboratory of the Faculty of Medicine at the University of Sumatera Utara (USU) from January to August, 2022.

A total of 25 female rats were used in this experiment. There were five rat groups of rats (a total of 25 female rats) that receive the herbs for 30 days.

Group K^- is the untreated group, Group K^+ is the rats injected with benzopyrene, Group P_1 is the rats injected with benzopyrene+*Zanthoxylum acanthopodium* 100 mg kg^{-1} b.wt., Group P_2 is the rats injected with benzopyrene+ *Zanthoxylum acanthopodium* 200 mg kg^{-1} b.wt. and Group P_3 is the rats injected with benzopyrene+*Zanthoxylum acanthopodium* 400 mg kg^{-1} b.wt.

The rats are dissected 30 days after receiving *Zanthoxylum acanthopodium* to stain the cervical tissues, immunohistochemistry is performed[10].

Rats model of cervical cancer: Through the vagina, the rats were injected with 50 mg of benzopyrene diluted with maize oil. A bulge was then ignored for three months until it was thought to be a malignancy. The rats were given *Zanthoxylum acanthopodium* for 1 month after 3 months and then they were dissected to collect the tumour tissue. The Faculty of Mathematics and Natural Sciences at USU's Ethics Committee for Handling Experimental Animals with Ethical Clearance approved this study.

Making paraffin blocks: The cervix organs are taken out of storage after being fixed in formalin and immersed in xylol for 15 min. The tissues were submerged in 96 and 70% pure alcohol for five minutes each, then rinsed with distilled water. After being exposed to hematoxylin dye for 5 min. For 3 min, the tissues were rinsed in distilled water. Eosin dye is used for one minute. Before being submerged in xylol, the slides were dried in 70, 96 and 100% alcohol. Light microscope observations were then done[9].

Immunohistochemistry: Slices of the cervix that had been embedded in paraffin and were 5 m thick were deparaffinized and given a 30 min treatment with 1% H_2O_2 in methanol to lower endogenous peroxidase activity. After that, the slides were washed with 0.01 M Tris-buffered saline (TBS pH 7.4). The antibodies and antigen affinity-purified polyclonal antibody were treated with tissue slices (eBioscience Inc, San Diego, USA). Additionally, the Vectastain Elite ABC kit from Vector Laboratories in the US detected immunoreactivity, which Mayer's hematoxylin neutralized[9].

Data analysis: The categorical data (ordinal) or numerical data that were not normally distributed, the Kruskal-Wallis and Mann-Whitney Tests were run.

RESULTS

Myc expression on andaliman administration in cervical carcinoma: The results of the Kruskal Wallis Test are shown in Table 1 and the p-value indicates that the difference is statistically significant. Based on the mean value, Myc expression differed significantly from K^- ($p<0.01$). When compared to the control group, the difference between the lowest dose of *Zanthoxylum acanthopodium* (100 mg kg^{-1} b.wt.) and the highest dose (200 mg kg^{-1} b.wt.) was not significant ($p>0.05$) (K^-). On the other hand, the dose of 400 mg kg^{-1} b.wt., rose and differed substantially from the K^+ group ($p<0.05$). On histology, cervical cells from the K^- group had a healthy epithelial lining with prominent Myc expression (Fig. 1a). The cells in the epithelial lining, on the other hand, begin to break down regularly, with abnormal nuclei. In the network section, the Myc expression is visible. It is possible to conclude that giving mice 50 mg of benzopyrene in corn oil increased Myc expression and damaged epithelial cell structure (Fig. 1b). Epithelial thickening and enhanced Myc expression are markers of epithelial cell alterations. As the *Zanthoxylum acanthopodium* dose increased, Myc expression in tumour tissue decreased (Fig. 1c). The decrease in Myc expression at a dose of 200 mg kg^{-1} b.wt., began to improve as the tissue histology improved (Fig.1d). Andaliman was anticipated to lessen brown nuclei at the maximum dose, which showed a positive index of Myc expression in cancer tissue with almost the same form as the K^- group (Fig. 1e). The previously uncontrollable group of carcinomas resulting from untreated malignancies has now slowed and stopped growing into the normal epithelium.

Wee1 expression on andaliman administration in cervical carcinoma: The results of the Kruskal-Wallis Test are shown in Table 2 and the difference has a p-value of 0.00, which indicates that it is statistically significant. Using the median value, Wee1 expression was substantially different between the K^- and K^+ groups ($p<0.05$) in the K^- group. The lowest dose of *Zanthoxylum acanthopodium* (100 mg kg^{-1} b.wt.) did not differ significantly ($p>0.05$) from K^+. There was an increase in Wee1 expression and a slight decrease in the last dose at a dose of 200 mg kg^{-1} b.wt. Figure 2a shows normal histological changes with weak Wee1 expression, but Fig. 2b shows that the carcinoma with an uneven core has migrated to the pelvic wall and almost all tissues are stained brown, indicating increased Wee1

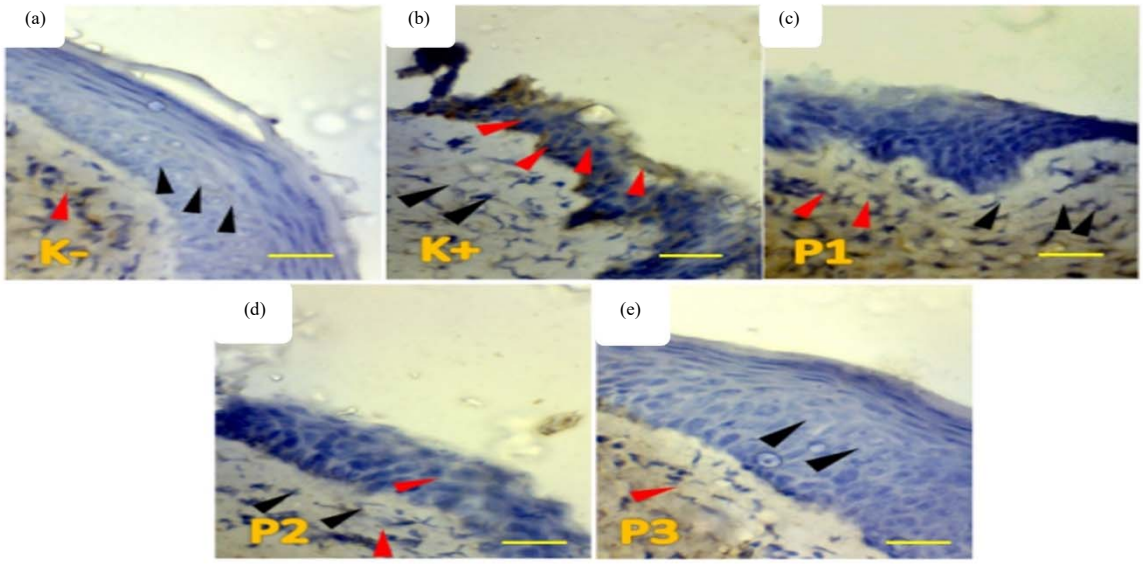

Fig. 1(a-e): Histology of carcinoma cervix on Myc expression by *Zanthoxylum acanthopodium*

K^-: Untreated, K^+: Rats injected with benzopyrene, P_1: Rats injected with benzopyrene+100 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_2: Rats were injected with benzopyrene+200 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_3: Rats were injected with benzopyrene+400 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, Yellow arrow: Negative expression and Red arrow: Positive expression

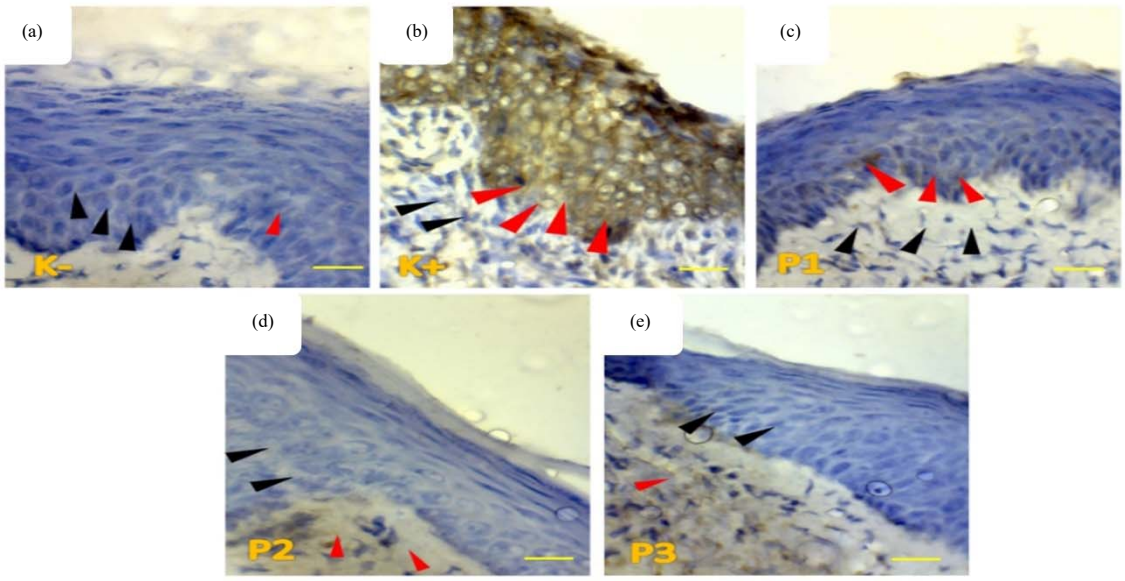

Fig. 2(a-e): Histology of carcinoma cervix on Wee1 expression by *Zanthoxylum acanthopodium*

K^-: Untreated, K^+: Rats injected with benzopyrene, P_1: Rats injected with benzopyrene+100 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_2: Rats were injected with benzopyrene+200 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_3: Rats were injected with benzopyrene+400 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, Yellow arrow: Negative expression and Red arrow: Positive expression

expression. *Zanthoxylum acanthopodium* (shows a histological profile similar to the K^- group in cervical cancer histology after a dose of 100 mg kg^{-1} b.wt. (Fig. 2c). The data in Fig. 2d depicts Wee1 expression at a dose of 200 mg kg^{-1} b.wt., indicating that this herb can repair tissue by significantly lowering Wee1 expression. Similarly, the nucleus started to form correctly and cervical cancer ceased growing at the maximum dose (Fig. 2e).

Correlation of Myc vs Wee1 on andaliman administration in cervical carcinoma: According to Kendall's tau b analysis of Myc vs Wee1 expression in cervical tissue (Table 3), there is a

Table 1: Analysis of Myc expression using Kruskal Wallis and Mann-Whitney for cervical histology

				Mann-Whitney				
Group	n	Mean rank	Kruskal-Wallis	K^-	K^+	P_1	P_2	P_3
K^-	5	5.40	0.001		0.008**	0.008**	0.572	0.021
K^+	5	22.50				0.033*	0.008**	0.008**
P_1	5	17.70					0.013*	0.033*
P_2	5	7.10						0.077
P_3	5	12.30						

K^-: Untreated, K^+: Rats injected with benzopyrene, P_1: Rats injected with benzopyrene+100 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_2: Rats were injected with benzopyrene+200 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_3: Rats were injected with benzopyrene+400 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, **p<0.01 and *p<0.5

Table 2: Analysis of Wee1 expression using Kruskal Wallis and Mann-Whitney for cervical histology

				Mann-Whitney				
Group	n	Mean rank	Kruskal-Wallis	K^-	K^+	P_1	P_2	P_3
K^-	5	3.80	0.000		0.032*	0.016*	0.006**	0.008**
K^+	5	9.00				0.268*	0.006**	0.011*
P_1	5	11.50					0.007**	0.014*
P_2	5	23.00						0.007
P_3	5	17.70						

K^-: Untreated, K^+: Rats injected with benzopyrene, P_1: Rats injected with benzopyrene+100 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_2: Rats were injected with benzopyrene+200 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, P_3: Rats were injected with benzopyrene+400 mg kg^{-1} b.wt., *Zanthoxylum acanthopodium*, **p<0.01 and *p<0.5

Table 3: Kendall's tau b analysis of Myc vs Wee1 expression in cervical tissue

Correlation							
						Myc	Wee1
Kendall's tau b	Myc	Correlation coefficient				1.000	-0.661**
		Significant (2-tailed)				0.000	0.001
		N				16.000	16.000
		Bootstrap[c]	Bias			0.000	0.002
			Standard error			0.000	0.124
			95% confidence interval		Lower	1.000	-0.870
					Upper	1.000	-0.379
	Wee1	Correlation coefficient				-0.661**	1.000
		Significant (2-tailed)				0.001	0.000
		N				16.000	16.000
		Bootstrap[c]	Bias			0.002	0.000
			Standard error			0.124	0.000
			95% confidence interval		Lower	-0.870	1.000
					Upper	-0.379	1.000

**Correlation is significant at the 0.01 level (2-tailed) and [c]Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

significant relationship (p<0.01) with a fairly strong correlation strength with a value of -0.0661 approaching -1 with a correlation direction of minus (-), which means that higher Myc expression on andaliman administration in cervical carcinoma decreases Wee1 expression in cervical carcinoma, implying that these two proteins have a strong and significant correlation.

DISCUSSION

Zanthoxylum acanthopodium administration caused epithelial thickening and decreased Myc expression in previously uncontrolled carcinomas from untreated malignancies, which now slowed and stopped growing into the normal epithelium. According to numerous studies, Myc appears to have a tissue-specific function in tissue stem cells and is necessary for commitment to terminal differentiation, in contrast to Myc's role in pluripotency. By promoting stem cells and suppressing cellular senescence and differentiation, Myc controls the fate of tumour cells[17,23]. In addition, Myc controls host immune response suppression and angiogenesis promotion in the tumour microenvironment. Cancer cells need nucleotides to maintain their unchecked proliferative and metabolic reprogramming[24]. Uncontrolled MYC expression can stimulate p53-mediated apoptosis by activating the oncogene stress pathway signalling through the

p14ARF/MDM2 pathway[24]. The Myc levels in normal cells, on the other hand, are usually insufficient to activate the tumour suppressor p14AR[25]. The Myc promotes nucleotide synthesis by inducing several genes involved in the process, as well as preparing cells for cell cycle transition[24,25]. Due to the delivery of the antioxidants included in andaliman, it has been demonstrated that the involvement of Myc in cancer treatment reduces hazardous side effects. The antioxidant intervention is motivated by the fact that antioxidant-rich plants, such as andaliman, have been linked to cancer treatment with minimal side effects[8].

Wee1 expression showed that this herb could repair tissue by significantly lowering Wee1 expression beginning at a dose of 100-400 mg kg^{-1} b.wt. Similarly, at the highest dose, cervical carcinoma stops growing and the nucleus begins to form normally. The higher the expression of Myc in cervical carcinoma after administration of andaliman, the lower the expression of Wee1 in cervical carcinoma, indicating a strong and significant correlation between these two proteins. Wee1 regulates the activity of cyclin-dependent kinases 1 and 2 by suppressing the phosphorylation of conserved tyrosine15 residues on both kinases, hence regulating mitosis and DNA replication during the S phase[26]. Wee1 is also important in the precise timing of cell division. CDK1 activity could be caused by Wee1 inhibition. When cells move past the G2/M checkpoint without sufficiently repairing DNA damage, which causes catastrophic mitosis and cell death (apoptosis)[27]. Apoptosis has been connected to elevated levels of ROS generation and oxidative stress. Apoptosis thus contributes to the aetiology and pathophysiology of cancer[26]. Antioxidants have a close relationship with cancer because they have become a widely accepted therapeutic approach[7]. Rather than increasing antioxidants, most chemotherapeutic agents and radiation killers kill tumour cells by increasing free radicals, which cause irreversible tissue damage. Cancer can be treated with appropriate antioxidant inhibitors, such as andaliman. This plant may one day be used to create a cervical cancer treatment candidate. Because andaliman contains potent antioxidants, it can be employed as a component of an efficient cancer therapy plan that also uses appropriate antioxidant inhibitors and/or free radical-producing substances[9,10]. Zanthoxylum herbs have anti-inflammatory, analgesic, antinociceptive, antioxidant, antibacterial, hepatoprotective, antiplasmodial, cytotoxic, antiproliferative, anthelmintic, larvicidal, antiviral and anticancer effects in addition to their antioxidant content[7,8].

The implications of this study include the examination of Myc and Wee1 in cervical carcinoma, which is necessary for the discovery of cancer drugs. *Zanthoxylum acanthopodium* should be studied further for the expression of other target genes. Cancer markers such as Myc and Wee1 expression in cervical carcinoma have a strong and significant correlation that is important in the process of protection from cancer.

CONCLUSION

The importance of the action of these proteins in the process of cervical cancer neoangiogenesis and its growth is demonstrated by the affect expression of Myc and Wee1 in poor histological characteristics. Because *Zanthoxylum acanthopodium* contains antioxidants, which have been demonstrated to heal carcinogenic metastatic carcinoma tissue. The higher Myc expression on andaliman administration in cervical carcinoma decreases Wee1 expression in cervical carcinoma so these two proteins have a strong and significant correlation. *Zanthoxylum acanthopodium* can be administered at various dosages to lower the number of positive indexes of Myc and Wee1 expression in cervical carcinoma.

SIGNIFICANCE STATEMENT

This study discovers that *Zanthoxylum acanthopodium* extract can as a herbal for carcinoma cervical problem therapy molecularly. This study will help the researcher to uncover the role of *Zanthoxylum acanthopodium* in the molecular signalling of other target proteins for drug development in cancer. Thus, a new theory on the role of *Zanthoxylum acanthopodium* extract in the cervical cancer animal model may be arrived at.

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