Effects of Zanthoxylum acanthopodium on MMP-9 and GLUT-1 Expression and Histology Changes in Rats with Cervical Carcinoma

by Turnitin ®

Effects of *Zanthoxylum acanthopodium* on MMP-9 and GLUT-1 Expression and Histology Changes in Rats with Cervical Carcinoma

Running title : Effects of *Zanthoxylum acanthopodium* on MMP-9 and GLUT-1 expression in Cervical Carcinoma histology

Abstract

Cervical cancer is one of the most common cancers in Indonesia. It can be treated with molecular therapies targeting Matrix metallopeptidase 9 (MMP-9) and Glucose transporter (GLUT-1), which are enzymes that are involved in tumour cell invasion, metastasis and angiogenesis. *Zanthoxylum acanthopodium* (andaliman) is an Indonesian herb with anti-cancer properties. This study aimed to investigate the histological changes andaliman treatment caused in MMP-9 and GLUT-1 expression. This study used five groups of rats: control (C-), cancer model (C+), cancer-bearing rats with a 100-mg dose of *Zanthoxylum acanthopodium* methanol extract (ZAM)/BW (ZAM100), cancer-bearing rats with a 200-mg dose of ZAM /BW (ZAM200) and cancer-bearing rats with a 400-mg dose of ZAM/BW (ZAM400). Immunohistochemical methods were used to stain cervical tissue with MMP-9 and GLUT-1 antibodies, and a TUNEL assay was performed to investigate cell apoptosis. *Zanthoxylum acanthopodium* methanol extract administration did not affect rat body weight but had a significant effect on cervical cancer growth. There was an increase in MDA levels associated with SOD deficiency in tumour tissue. SOD activity increased due to ZAM administration, allowing cells to be protected from oxidant disruption and oxidative stress. ZAM ameliorated cervical carcinoma tissue damage and reduced the expression of MMP-9, GLUT-1 and apoptosis in serum and tissue ($p < 0.01$) In short, the higher the ZAM dose, the lower the expression of MMP-9, GLUT-1 and apoptosis, indicating that ZAM is effective to treat cervical cancer. .

Keywords: Apoptosis; Cervical Cancer; GLUT-1; MMP-9; *Zanthoxylum*

Introduction

Cervical cancer is the second-most-frequent cancer among Indonesian women, after breast cancer, with a 23.4/100,000 incidence rate and a 13.9/100,000 mortality rate (Afiyanti *et al*., 2019; Simanullang and Sitopu, 2020). According to current estimates from the Indonesian Ministry of Health, 90–100 new women are diagnosed with cervical cancer per 100,000 people, and 40,000 cases are diagnosed each year (Simanullang, 2018).

Matrix metalloproteinase 9 (MMP-9) is a proteolytic enzyme that is thought to play an important role in the progression from precancerous lesions to cervical cancer (Gobin *et al*., 2019). MMP-9 levels above a certain threshold accelerate cervical tissue degradation and facilitate cancer cell invasion (Mondal *et al*., 2020). MMPs are normally formed only at the time and location of tissue remodelling (Knapinska *et al*., 2019), but also form and contribute to pathological conditions such as tumour cell invasion, metastasis and angiogenesis. In pathological processes, such as cancer, MMP activation largely bypasses the normal activating process (Knapinska *et al*., 2019). MMP-9 gelatinase or collagenase is very effective in the gelatinolytic process that degrades collagen, fibronectin and elastin, increasing MMP-9 expression in the inflammatory and tumour malignancy processes (Isaacson *et al*., 2017).

Glucose transporter-1 (GLUT-1) is a protein found in most normal tissues. GLUT-1 is normally undetectable in normal epithelial tissue or benign epithelial tumours. Glucose is the most important source of energy for cells (Barbosa *et al*., 2020), and cancer cells frequently have higher glucose metabolism values than normal cells to support their proliferative ability (Zambrano *et al*., 2019). In many cancers, GLUT-1 overexpression is a significant limiting factor in the rate of glucose transport in tumour cells. Overexpression of glucose transporters (GLUTs), a protein family responsible for glucose uptake, increases cancer's aerobic glycolysis (Botha *et al*., 2021).

The Indonesian spice andaliman (*Zanthoxylum acanthopodium*) grows wild in the North Sumatra region (Djati and Christina, 2019). *Zanthoxylum acanthopodium* contains alkaloids, glycosides, tannins, phenols and flavonoids, which are antioxidant, anti-inflammatory and antibacterial agents (Wijaya *et al*., 2019: Li *et al*., 2020). In vitro, *Zanthoxylum acanthopodium* can also change the Mcf-7 cell line and mend the tissue (Arsita *et al*., 2019; Simanullang *et al*., 2021a). In addition, andaliman fruit can increase Hes1 and Notch1 gene expression in human trophoblasts in vitro (Situmorang *et al*., 2021a). *Zanthoxylum acanthopodium* fruit in nano herbal form can reduce tissue damage such as diabetic wounds and renal and liver hypertension (Situmorang *et al*., 2019a; Situmorang *et al*., 2019b; Situmorang *et al*., 2019c; Situmorang et al., 2021b; Manurung *et al*., 2021).

This study sought to investigate the histological changes in cervical cancer tissue in terms of MMP-9 and GLUT-1 expression, as well as apoptosis, after *Zanthoxylum acanthopodium* treatment in vivo.

Materials and Methods

Reagents and chemicals

Z. acanthopodium fruits (family Rutaceae) were collected from Kabanjahe Regency, Berastagi Indonesia (30° 17′ 50″ to 3° 18′ 39″ N and 98° 36′ 0″ to 98° 36′ 36″ E.). The voucher was identified and authenticated by Dr N. Pasaribu (an Indonesian botanist), and deposited in the Herbarium Medanense (registration number 5940/MEDA/2022), at Universitas Sumatera Utara, Indonesia. MMP-9 (matrix metalloproteinase 9) ELISA Kit, catalogue number: E-EL-R3021 (Elabsciences, Houston, Texas, United State); rabbit polyclonal GLUT1 IHC antibody, catalogue number: IW-PA1120 (IHC WORLD, LLC Ellicott City, MD 21042, USA); rabbit polyclonal MMP9 antibody (ab237782), catalogue number: EPR22140-154 and BSA- and azide-free rabbit polyclonal antibody for cellular apoptosis susceptibility/CSE1L (ab96755) (Abcam, Cambridge Biomedical Campus Cambridge CB2 0AX, UK) were used in this study.

Extract preparation

Zanthoxylum acanthopodium (andaliman) fruits were cleaned and then dried in the drying room and ground to a powder. The extract was prepared by macerating 10 kg of dried *Zanthoxylum acanthopodium* fruit in 10 litres of 96% methanol for 1 night. Then, it was filtered and evaporated to produce the dry extract. The phytochemical analysis results for *Zanthoxylum acanthopodium* were confirmed by subsequent studies (Situmorang *et al*., 2020; Wijaya *et al*., 2019).

Animals

The University of Sumatera Utara's Animal House Laboratory provided 36 female rats (*Rattus norvergicus*) of the Wistar strain, weighing 180–200 g (8–12 weeks old) for this study. During the study, the rats were fed standardized food pellets and given sufficient water every day. They were acclimated to laboratory settings for 4 weeks. Cervical cancer was induced by injecting benzopyrene 50 mg/kg BW into their cervices and then the tumour was allowed to grow for 3 months (Simanullang *et al.,* 2022).

Experimental design

The research was conducted at the University of Sumatera Utara's Biology Laboratory, the Pathology and Anatomy Laboratory of the Faculty of Medicine, Universitas Methodist Indonesia and STIKes Murni Teguh. Doses of 100, 200 and 400 mg/kg were selected based on the acute toxicity test results of previous studies (Situmorang *et al*., 2020) and other researchers (Alam *et al.,* 2020). There were five treatment groups consisting of 6 rats each: the control group (Group C-), the cancer model group (Group C+) without treatment, the ZAM100 group of cancer-bearing rats given a dose of 100 mg/kg BW of ZAM, the ZAM200 group of cancer-bearing rats given a dose of 200 mg/kg BW of ZAM and the ZAM400 group of cancer-bearing rats given a dose of 400 mg/kg BW of ZAM.

Each group was given its respective dose of extract orally for 30 days. On day 31, the rats were euthanised with chloroform and then dissected to collect blood and the cervix. Immunohistochemistry and a TUNEL assay were used to stain cervical tissue. The Ethics Committee for Handling Experimental Animals, Faculty of Mathematics and Natural Sciences USU approved this study (ethical clearance: No. 0262/KEPH-FMIPA/2022).

Measurement of superoxide dismutase (SOD) and malondialdehyde (MDA)

The analysis was conducted at Universitas Methodist Indonesia. First, 2–4 mg/ml of blood and SOD standard sample was added to the reaction mixture in the presence and absence of 1mM cyanide to measure SOD activity. Second, lipid oxidation was identified with the thiobarbituric acid reactive test (TBARS), which measures the products of the unsaturated fatty acid endoperoxides produced by lipid oxidation using an ELISA reader at 450 nm.

Immunohistochemistry

A microtome was used to cut 4-micrometre-thick slices of the paraffin-embedded cervical tissue (Qin *et al*., 2018). For pre-treatment, the tissue was heated in citrate buffer at a pH of 6.0. After a phosphate-buffered saline (PBS) wash, the tissue was incubated with antibodies at 37 °C according to the manufacturer's instructions before being treated with avidin-biotin-peroxidase. 3,3-diaminobenzidine (DAB) hydrochloride was used for the chromogenic visualisation reaction. The slices were then stained with haematoxylin, after Mayer (Situmorang *et al*., 2021a). The stained cervical tissue score was calculated by multiplying the positive result by the staining intensity, where 0 indicated that less than 10% of the cells were stained, 1 indicated that 10–25% of the cells were stained (negative), 2 indicated that 25–50%

of the cells were stained (weak), 3 indicated that 50–75% of the cells were stained (moderate) and 4 indicated that more than 75% of the cells were stained (strong).

TUNEL assay

To study apoptotic cells, the cervical tissue was stained with the TUNEL assay technique (Kyrylkova *et al*., 2012). Slides with mounted cervical slices were immersed in xylene for 5 minutes, then rehydrated with graded ethanol (70–100%) and rinsed with 0.85% PBS. Following the manufacturer's instructions for antigen retrieval, the slides were rinsed in TBS-Tween (TBST) for 1 minute. A working solution of Proteinase K was applied to the slides and they were incubated for 10 minutes before being rinsed in 1X TBST for 1 minute. The diluted rTdT reaction mixture before endogenous peroxidase synthesis, the slide was submerged in citrate buffer at a pH of 6.0 for 15 minutes at room temperature and then rinsed with PBS. The tissue was then incubated at room temperature for 30 minutes. Then, 3,3-diaminobenzidine hydrochloride (DAB) was utilized for the chromogenic imaging reaction. The slides were immersed in ethanol and xylene before being covered with glass. The researcher used a light microscope to observe five fields of view.

Data analysis

A one-way ANOVA test was used for data analysis. If the *p*-value was less than 0.05, there was a significant difference between groups and if the *p*-value was higher than 0.05, there was no difference between groups. A Kruskal–Wallis test (for non-parametric data) was used to analyse the data with *SPSS V*.22.

Results and Discussion

Body and cervix weight in cancer model rats

Data on body and cervical weight from each experimental group are shown in Table 1. There was an insignificant difference ($p > 0.05$) between groups before the injection of 50 mg/kg BW benzopyrene in the cervix. After benzopyrene injection, there was a significant difference between group C- and C+ ($p = 0.04$). Table 1 also shows that cervical weight was significantly different in C- vs. C+ ($p = 0.004$) or in C+ vs. ZAM100 ($p = 0.03$), ZAM200 ($p = 0.03$) or ZAM400 ($p = 0.004$).The injection of benzopyrene and ZAM treatment affected body weight and cervical weight significantly.

No significant difference in the body weight of cervical-cancer-bearing rats was found between groups after the administration of ZAM at doses of 100 to 400 mg/kg BW. Tumour cells can proliferate indefinitely and exhibit excessive angiogenesis. However, in the cervical-cancer-bearing rats, the impact was not significant (Clarke *et al*., 2018). This could be due to the small size of cervical cancer, rats' fat or excessive activity (Clarke *et al*., 2018). Cervical tumours could influence cervical weight in both the control and ZAM groups.

Table 1. Body and Cervical Weight after ZAM treatment

Treatment	Body Weight (BW)		Mass difference (g)	Cervical Weight (g)
	Before (g)	After (g)		
C-	189.20 ± 7.8.22	200.98 ± 12.70	11.78	0.30 ± 0.03
C+	200.23 ± 9.21^{ns}	210.21 ± 9.59$^{\#}$	9.98^{ns}	1.66 ± 0.14$^{\#\#}$
ZAM100	201.73 ± 11.89^{ns}	212.22 ± 11.25^{ns}	10.49^{ns}	1.07 ± 0.06*
ZAM200	201.77 ± 21.88^{ns}	221.44 ± 11.33^{ns}	19.67^{ns}	0.69 ± 0.12*
ZAM400	199.42 ± 23.22^{ns}	212.93 ± 12.10^{ns}	13.51^{ns}	0.32 ± 0.10**

C-: Control, C+: rats with cancer ZAM100: cancer-bearing ratswith a dose of 100mg/BW of ZAM, ZAM200: cancer-bearing rats with a dose of 200 mg/BW of ZAM, ZAM400: cancer-bearing rats with a dose of 400 mg/BW of ZAM (#p<0.05 vs. C-, ##p<0.01 vs. C-, *p<0.05 vs. C+, **p<0.01 Vs. vs. C+, ^{ns}p>0.05).

Measurement of superoxide dismutase (SOD) and malondialdehyde (MDA) in rats with cervical cancer

There was a significant difference in SOD and MDA levels in C+ rats compared to the C- group (*p* <0.05), with cancer-bearing rats having lower SOD levels. SOD levels increased in cancer-bearing rats given a ZAM dose of 100 mg/kg BW, but decreased under a dose of 400 mg/kg BW (Figure 1a). This contrasts with the increased MDA levels in cancer-bearing rats, for which there was a significant difference between C- and C+ (*p* >0.01) (Figure 1b), and ZAM administration at doses ranging from 100 to 400 reduced MDA levels in cancer-bearing rats. ZAM more effectively altered SOD levels in rats with cervical cancer at doses of 100 and 200 mg/kg BW and MDA levels at 200 and 400 mg/kg BW. Increased lipid peroxidation due to antioxidant deficiency is linked to increased circulating levels of MDA and decreased SOD activity in tissues in general, including in cervical cancer tissue (Sherif *et al.*, 2018; Thakur *et al.*, 2015). Furthermore, elevated MDA levels in tumour tissue may be linked to SOD deficiency. Long-term, superoxide anions, which are highly radical and can penetrate membranes, accumulate and cause negative effects away from the tumour (Sherif *et al.*, 2018). The decreased MDA levels and increased SOD activity are thought to be because the andaliman fruit has certain substances that can affect their expression. Cells can be protected from oxidant interference and oxidative stress via ZAM administration, increasing SOD activity and reducing the effects of diseases, including cancer.

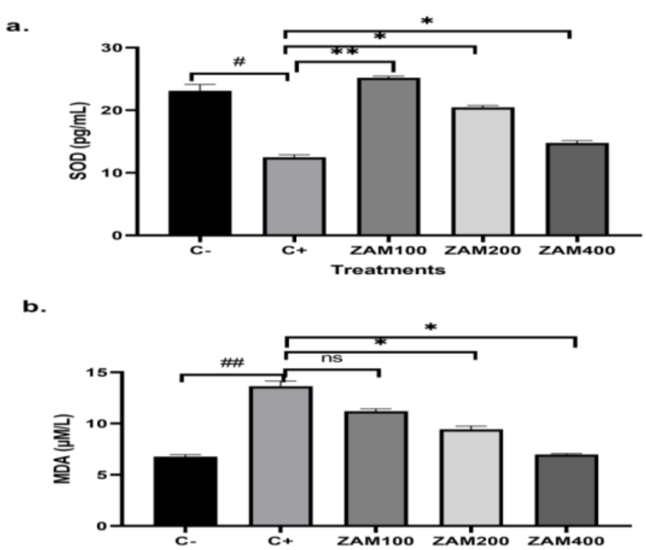


Figure 1. Levels of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) in cancer model rats. a. SOD, b. MDA. C-: Control, C+: cancer-bearing rats ZAM100: cancer-bearing rats with a dose of 100mg/BW of ZAM, ZAM200: cancer-bearing rats with a dose of 200 mg/BW of ZAM, ZAM400: cancer-bearing rats with a dose of 400 mg/BW of ZAM (#p<0.05 vs. C-, ##p<0.01 vs. C-, *p<0.05 vs. C+, **p<0.01 Vs. C+, ^{ns}p>0.05).

MMP-9 expression in cervical cancer after ZAM administration

As shown in Table 2, a statistically significant difference (p <0.00) appeared among the treatment groups. According to the average, there was a significant difference in MMP-9 expression (p <0.05) compared to C-. The lowest dose of ZAM (100 mg/kg BW) had no significant effect (p >0.05), but doses of 200 and 400 mg/kg BW were significant (p <0.05). Cervical cells in group C- had normal epithelial and nuclear layers (Figure 2a). In contrast, as Figure 2b (C+ group) shows, undifferentiated cells were confined to the lower layers of the epithelium and developed mitotic features. Lower epithelial cell changes were characterised by epithelial thickening and increased MMP-9 expression. Additionally, MMP-9 expression in cancer tissue decreased as the ZAM dose increased. ZAM (Figure 2c–2e) administration at different doses reduced the number of nuclei that were stained brown by immunohistochemistry, indicating a positive index of MMP-9 expression in cancer tissue. Carcinomas that were uncontrollably spreading in the C+ group slowed and no longer developed into the epithelium. Figure 3 shows that serum MMP-9 levels in cancer-bearing rats differed significantly (p <0.05) from C- rats, but doses of 100 and 200 mg/kg BW did not result in significant differences (p >0.05) from C+ rats. The highest dose (400 mg/kg BW) produced significant results. As Table 2 and Figure 2 show, administering ZAM, particularly at 400 mg/kg BW, can suppress the expression of MMP-9 cervical cancer histological changes.

Table 2. Kruskal Wallis and Mann-whitney analysis of MMP-9 expression in cervical carcinoma

Groups	Mean ± SD	Kruskal-Wallis test	Mann-Whitney test (p-value)				
			C-	C+	ZAM100	ZAM200	ZAM400
C-	14.80 ± 4.11	0.00		0.001	0.001	0.020	0.056
C+	42.30 ± 7.21$^{##}$				0.070	0.030	0.001
ZAM100	40.80 ± 7.19^{ns}					0.030	0.020
ZAM200	22.67 ± 4.71*						0.040
ZAM400	19.41 ± 3.22**						

C-: Control, C+: cancer-bearing rats ZAM100: cancer-bearing rats with a dose of 100mg/BW of ZAM, ZAM200: cancer-bearing rats with a dose of 200 mg/BW of ZAM, ZAM400: cancer-bearing rats with a dose of 400 mg/BW of ZAM (#p<0.05 vs. C-, ##p<0.01 vs. C-, *p<0.05 vs. C+, **p<0.01 Vs. C+, ^{ns}P>0.05).

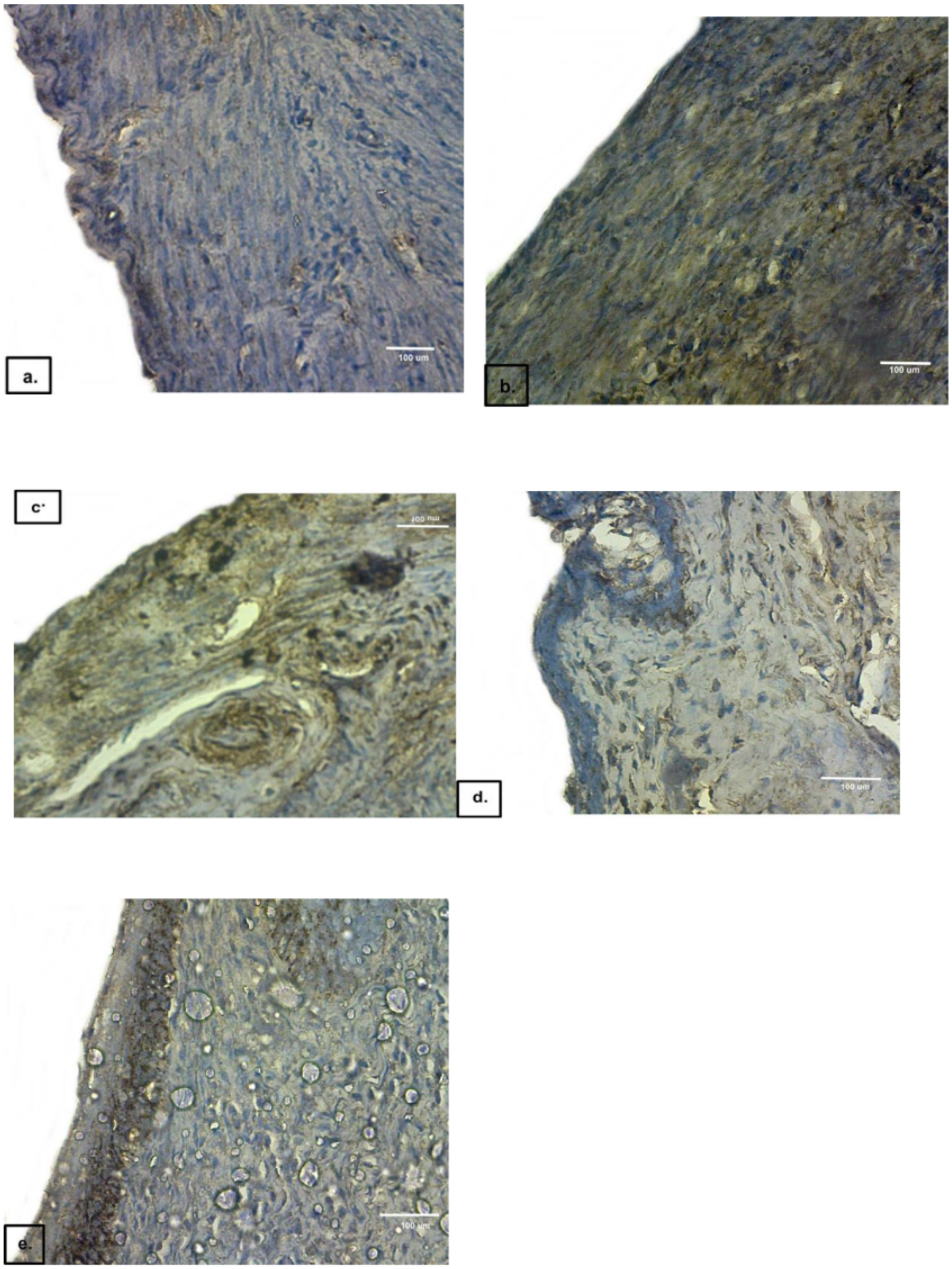


Figure 2. MMP-9 Expression and Histology changes of cervical cancer model in rats. **a.** Control (C-), **b.** cancer-bearing rats (C+), c. cancer-bearing rats with a dose of 100mg/BW of ZAM (ZAM100), **d.** cancer-bearing rats with a dose of 200 mg/BW of ZAM (ZAM200), **e.** cancer-bearing rats with a dose of 400 mg/BW of ZAM (ZAM400). MMP-9 expression is indicated by a brown-black color in histology (400x).

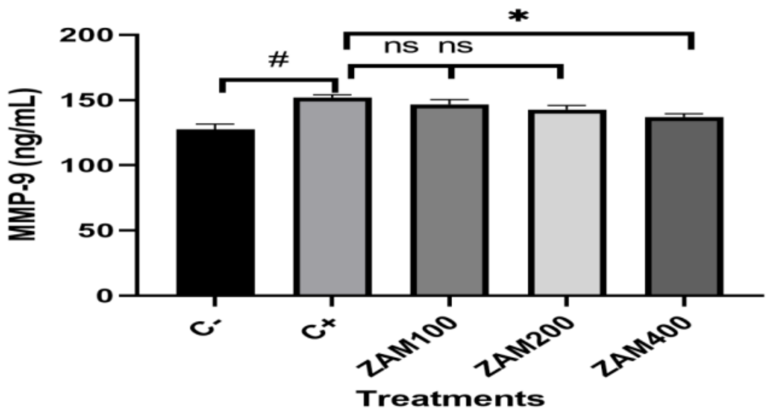


Figure 3. MMP-9 Expression in serum of cervical cancer model rats. C-: Control, C+: cancer-bearing rats ZAM100: cancer-bearing rats with a dose of 100mg/BW of ZAM, ZAM200: cancer-bearing rats with a dose of 200 mg/BW of ZAM, ZAM400: cancer-bearing rats with a dose of 400 mg/BW of ZAM (#$p<0.05$ vs. C-, *$p<0.05$ vs. C+, $^{ns}p>0.05$).

ZAM, particularly at a dose of 400mg/kg BW, can suppress the expression of MMP-9 in cervical cancer. The greater the immunohistochemical positivity of MMP-9, measured as intensity and quantity, the more severe the cervical neoplastic lesions (Mondal *et* al., 2020). MMP-9 immunohistochemical expression is elevated in cervical tumours and contributes to carcinogenesis. Cervical carcinomas can be polypoid or infiltrative on the macroscopic level. Unlike polypoid tumours, infiltrative tumours will invade and damage the surrounding tissue. Microscopically, their enlargement, rough chromatin and prominent nucleoli can be seen throughout the thickness of the squamous epithelial layer (Tanaka *et al*., 2019). The body's ability to degrade MMP depends strongly on the balance of active enzymes and natural inhibitors. MMP damages the basement membranes of blood vessel walls, allowing tumour cells to enter and exit the bloodstream (intravasation and extravasation) (Rajesh and Mandal, 2017; Tanaka *et al*., 2019). MMPs also affect the modification of new microenvironments at metastasis sites, aiding metastatic tumour cell growth (Mondal *et al*., 2020). *Zanthoxylum acanthopodium* contains alkaloids, glycosides, tannins, phenols and flavonoids that act as antioxidant, anti-inflammatory and antibacterial substances (Wijaya *et al*., 2019: Li *et al*., 2020). These substances are suspected to drive ZAM's MMP-9 expression control in the tissues and serum of cancer-bearing mice. Effective cancer treatment strategies may include applying appropriate antioxidant-containing compounds as inhibitors of free-radical-generating compounds.

GLUT-1 expression in histological changes of cervical cancer after ZAM administration

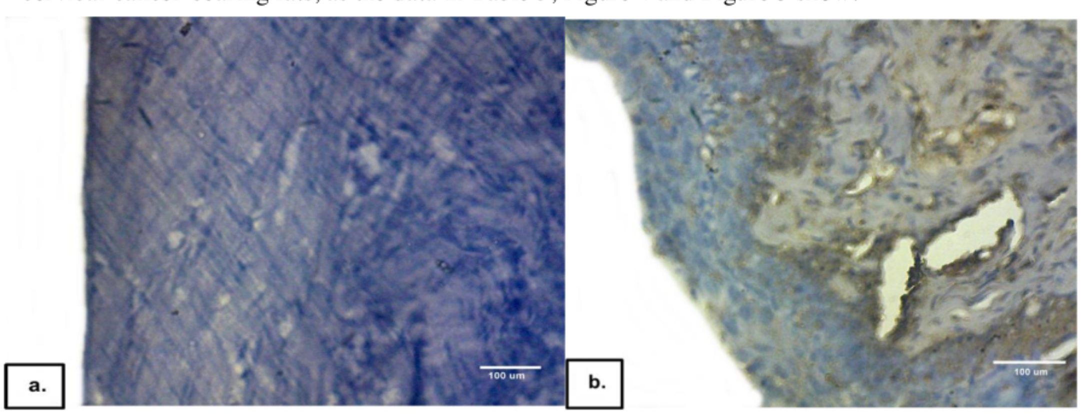
Table 3 shows that both the Kruskal–Wallis test and the Mann–Whitney follow-up test revealed significant differences among groups. According to the mean values, there was a significant difference (p <0.001) in GLUT-1 expression between the C+ and C- groups. The difference was not significant at the lowest ZAM dose (100 mg/kg BW) but was significant at 200 and 400 mg/kg BW (p <0.01). The C+ group had the highest level of GLUT-1 expression, while the C- group had the lowest level, occasionally showing no expression at all. Figure 4a shows that C+ rats exhibited normal histological changes, but the carcinoma had spread to the pelvic wall, there was no clear space between the tumour and the pelvic wall and the core was irregular (Figure 4b). Cancer cell metabolism is reprogrammed to promote cancer cell proliferation (Sharen *et al*., 2017). Cancer cells frequently have high glucose metabolism values compared to normal cells to support their proliferative ability (Pragallapati and Manyam, 2019). This contrasts starkly with the C- group's histology, which showed that the cervical tissue still contained normal cells (Figure 4a). The lesions in the group at the lowest ZAM dose (Figure 4c) were larger than in the control group, but GLUT-1 expression began to decrease. The reduction in GLUT-1 expression at doses of 200 and 400 mg/kg BW (Figure 4d–4e) shows that andaliman could significantly reduce GLUT-1 expression. The space between tumours was reduced, the carcinoma stopped developing and the nuclei began to form normally. Figure 5 shows that serum GLUT-1 levels in cancer-bearing rats were significantly different (p <0.01) from C- rats, but not significantly different (p >0.05) from C+ at 100 mg/kg BW.

Table 3. Kruskal Wallis and Mann-whitney analysis of GLUT-1 expression in carcinoma cervical

Groups	Mean ± SD	Kruskal-Wallis	Mann-Whitney (p-value)				
			C-	C+	ZAM100	ZAM200	ZAM400
C-	1.45 ± 0.12	0.000		0.000	0.001	0.001	0.001
C+	30.92 ± 2.25$^{\#\#\#}$				0.056	0.002	0.002
ZAM100	22.80 ± 3.11^{ns}					0.040	0.040
ZAM200	12.67 ± 2.21**						0.056
ZAM400	10.42 ± 1.34**						

C-: Control, C+: cancer-bearing rats ZAM100: cancer-bearing rats with a dose of 100mg/BW of ZAM, ZAM200: cancer-bearing rats with a dose of 200 mg/BW of ZAM, ZAM400: cancer-bearing rats with a dose of 400 mg/BW of ZAM (#p<0.05 vs. C-, ###p<0.001 vs. C-, **p<0.01 Vs. C+, ^{ns}p>0.05).

ZAM administration can reduce GLUT-1 expression and improve histology in cervical cancer. The expression of GLUT-1 in cancer can indicate a tumour's metabolic and vascular requirements, which have clinical implications for survival and treatment plans. Given the importance of GLUT-1 in oncogenesis, several studies have been conducted to investigate its prognostic value in tumours (Pragallapati and Manyam, 2019). GLUT-1 overexpression may be linked to increased glucose metabolism in cancer cells (Zambrano *et al*., 2019). Andaliman doses from 200 to 400 mg/kg BW produced significant results, and serum GLUT-1 analysis affirms that ZAM can reduce GLUT-1 expression in cervical cancer. Thus, ZAM doses of 200 and 400 mg/kg BW can reduce GLUT-1 expression in the serum and improve histology in cervical-cancer-bearing rats, as the data in Table 3, Figure 4 and Figure 5 show.



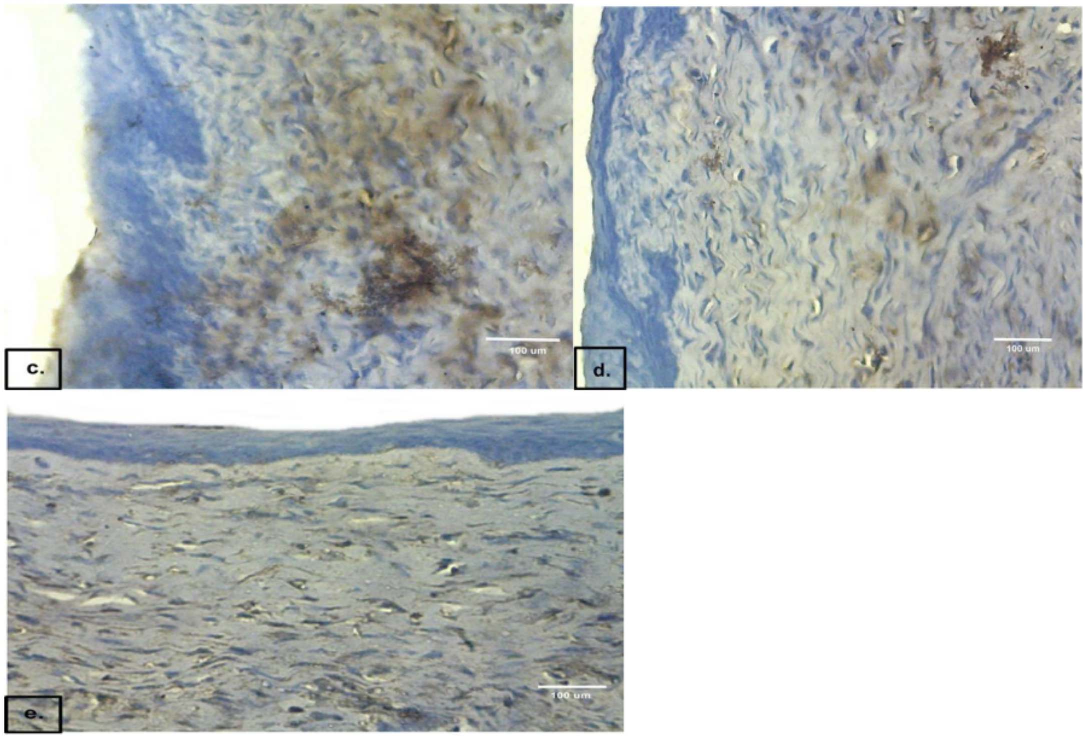


Figure 4. GLUT-1 Expression in Histology changes of cervical cancer model rats. a. Control (C-), b. cancer-bearing rats (C+), c. cancer-bearing rats with a dose of 100mg/BW of ZAM (ZAM100), d. cancer-bearing rats with a dose of 200 mg/BW of ZAM (ZAM200), e.cancer-bearing rats with a dose of 400 mg/BW of ZAM (ZAM400). GLUT-1 expression is indicated by a brown-black color in histology (400x).

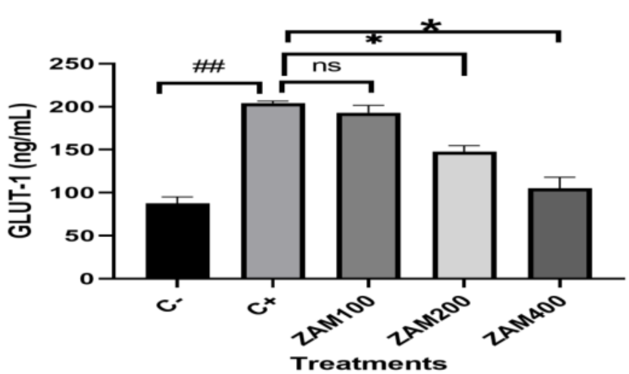


Figure 5. GLUT-1 Expression in serum of cervical cancer model rats. C-: Control, C+: cancer-bearing rats ZAM100: cancer-bearing rats with a dose of 100mg/BW of ZAM, ZAM200:

cancer-bearing rats with a dose of 200 mg/BW of ZAM, ZAM400: cancer-bearing rats with a dose of 400 mg/BW of ZAM (##p<0.01 vs. C-, *p<0.05 vs. C+, ^{ns}p>0.05).

Apoptotic cells in cervical cancer after ZAM administration

The data on apoptotic cells from each experimental group is presented in Table 4. They show that both the Kruskal–Wallis test and the Mann–Whitney follow-up test revealed significant differences. There was a significant difference (p <0.05) in the mean values of apoptotic cells between the C+ and C- groups. The difference was not significant at 100 mg/kg BW ZAM but was significant at 200 mg/kg BW (p < 0.01) and 400 mg/kg BW (p <0.001). The C+ group had the highest level of apoptotic cell expression, while the C- group had the lowest level.

Figure 6 depicts the apoptotic histology of the rat cervix after benzopyrene injection and ZAM administration at various doses. The histology of cervical tissue in group C- (Figure 6a) was healthy and normal, but it changed dramatically after benzopyrene injection in group C+. Figure 6b depicts the histology of C+ cervices with irregular cell nuclei forming bubbles known as apoptotic bodies. Apoptosis has been linked to increased ROS production and oxidative stress, thus contributing to cancer pathogenesis and aetiology (Situmorang dan Ilyas, 2018). The environment within the cell nucleus appears disjointed and karyorrhexis occurs. Because the protein structures that comprise the cytoskeleton are digested by a specific peptidase enzyme (caspase) that is activated in the cell, the cell becomes circular (red arrow). The absence of apoptotic regulation lengthens cancer cells' lifespans and allows more time for mutations to accumulate, which can increase invasiveness during tumour progression, induce angiogenesis, deregulate cell proliferation and interfere with differentiation (Chen *et al*., 2004). Apoptosis can be triggered with herbal medicine to repair tissue (Situmorang dan Ilyas, 2018). Histological features differed significantly between treatments (p <0.05). According to the statistical data (Table 4), ZAM100 and ZAM200 rats showed higher apoptosis than ZAM400 rats (Figure 6c–6e). This is because the 400 mg/kg BW dose was the highest, so there was little apoptosis in the tissue.

Table 4. Kruskal Wallis and Mann-whitney analysis of TUNEL expression on cervical tissue

Groups	Mean ± SD	Kruskal-Wallis	Mann-Whitney (p-value)				
			C-	C+	ZAM100	ZAM200	ZAM400
C-	7.30 ± 0.12			0.040	0.045	0.03	0.06
C+	18.30 ± 2.25$^{\#}$				0.06	0.04	0.002
ZAM100	12.80 ± 3.11^{ns}	0.000				0.04	0.04
ZAM200	10.67 ± 2.21*						0.05
ZAM400	9.42 ± 1.34**						

C-: Control, C+: cancer-bearing rats ZAM100: cancer-bearing rats with a dose of 100mg/BW of ZAM, ZAM200: cancer-bearing rats with a dose of 200 mg/BW of ZAM, ZAM400: cancer-bearing rats with a dose of 400 mg/BW of ZAM (#p<0.05 vs. C-, *p<0.05 vs. C+, **p<0.01 Vs. C+, ^{ns}p>0.05).

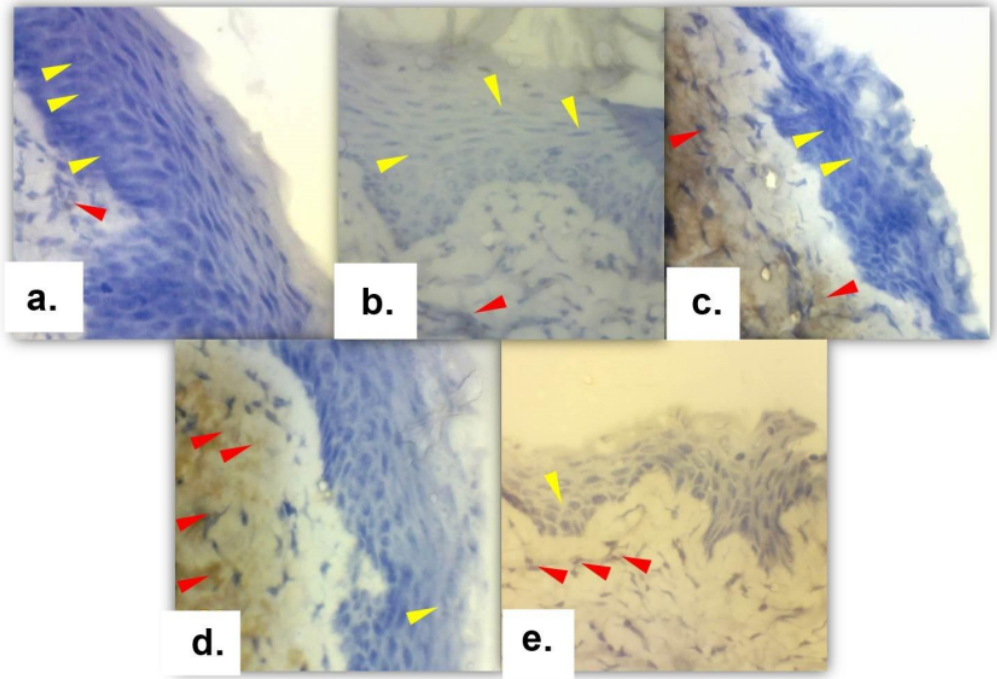


Figure 6. Apoptotic cells in Histology changes of cervical cancer model rats. a. Control (C-), b. cancer-bearing rats (C+), c. cancer-bearing rats with a dose of 100mg/BW of ZAM (ZAM100), d. cancer-bearing rats with a dose of 200 mg/BW of ZAM (ZAM200), e.cancer-bearing rats with a dose of 400 mg/BW of ZAM (ZAM400). Yellow arrows indicate low expression of apoptosis in tissues, while red arrows indicate high expression (400x).

Conclusion

In summary, this study found evidence that *Zanthoxylum acanthopodium* methanol extract (ZAM) significantly ameliorated cervical carcinoma tissue damage and also reduced the expression of MMP-9 and GLUT-1 and apoptosis in serum and tissue ($p < 0.010$). In vitro studies of MMP-9 and GLUT-1 genes in human cervical cancer cells are recommended to further confirm the effects of *Zanthoxylum acanthopodium*..

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References

Afiyanti Y, Wardani IY, Martha E (2019) The Quality of Life of Women with Cervical Cancer in Indonesia: A Cross-Sectional Study. Nurse Media Journal of Nursing 9(2):128-140. https://doi.org/10.14710/nmjn.v9i2.26014

Alam F, Din KM, Rasheed R, Sadiq A, Jan MS, Minha, AM, Khan, A (2020) Phytochemical investigation, anti-inflammatory, antipyretic and antinociceptive activities of *Zanthoxylum armatum* DC extracts-in vivo and in vitro experiments. Heliyon 25;6(11):e05571. doi: 10.1016/j.heliyon.2020.e05571.

Arsita EV,Saragih DE, Aldrin K (2019) Anticancer Potential From Ethanol Extract of *Zanthoxylum acanthopodium* DC. Seed too Against Mcf-7 Cell Line. IOF Conference Series Earth Environmental Sciences 293:012016. doi: 10.1088/1755-1315/293/1/012016

Barbosa AM, Martel F(2020) Targeting Glucose Transporters for Breast Cancer Therapy: The Effect of Natural and Synthetic Compounds. Cancers 12(1): 154. doi:10.3390/cancers12010154

Botha H, Farah CS, Koo K, Cirillo N, McCullough M, Paolini R,Celentano A (2021) The Role of Glucose Transporters in Oral Squamous Cell Carcinoma. Biomolecules 11(8): 1070. doi:10.3390/biom11081070

Chen D,Carter TH, Auborn KJ (2004) Apoptosis in cervical cancer cells: implications for adjunct anti-estrogen therapy for cervical cancer. Anticancer Research 24(5A):2649-56

Clarke MA, Fetterman B, Cheun LC, Wentzensen N, Gage JC, Katki HA, Dermaco M, Schulees K, Kinney WK, Raine-Bennet TR, Lorey TS, Poitras NE, Castle PE, Schiffman M (2018) Epidemiologic Evidence That Excess Body Weight Increases Risk of Cervical Cancer by Decreased Detection of Precancer. Journal Clinical oncology 36(12): 1184–1191. Doi: 10.1200/JCO.2017.75.3442.

Djati MS, Christina YI (2019) Traditional Indonesian Rempah-rempah as a Modern Functional Food and Herbal Medicine. Functional Foods in Health and Disease 9(4):241-264. doi:10.31989/ffhd.v9i4.571

Gobin E, Bagwell K,Wagner J,Mysona D,Sandirasegarane S,Smith N, Bai S, Sharma A, Scheleifer R, Xiong-She J (2019) A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential BMC Cancer. 19: 581. https://doi.org/10.1186/s12885-019-5768-0

Isaacson KJ, Martin, JM, Subrahmanyam NB, Ghandehari H (2017) Matrix-metalloproteinases as targets for controlled delivery in cancer: An analysis of upregulation and expression Journal Controlled Release 259:62–75. doi:10.1016/j.jconrel.2017.01.034

Ilyas S, Murdela F, Hutahaean S, Situmorang PC (2019). The Effect of Haramounting Leaf Ethanol Extract (*Rhodomyrtus tomentosa (Aiton)* Hassk.) on the Number of Leukocyte Type and Histology of Mice Pulmo (*Mus Musculus* L.) Exposed to Electronic Cigarette. Open Access Macedonia Journal of Medical Science 13:7(11):1750-1756. doi: 10.3889/oamjms.2019.467.

Knapinska AM,Fields GB (2019) The Expanding Role of MT1-MMP in Cancer Progression. Pharmaceuticals 12(2):77. doi:10.3390/ph12020077

Kyrylkova K, Kyryachenko S, Leid M, Kioussi K (2012) Detection of apoptosis by TUNEL assay. Methods in Moleculer Biology 887:41-7. doi: 10.1007/978-1-61779-860-3_5

Li M, Hassan FU,Tang Z, Peng L, Liang X, Li L ,Peng K, Xie F,Yang C (2020) Mulberry Leaf Flavonoids Improve Milk Production, Antioxidant, and Metabolic Status of Water Buffaloes. Front veterinary sciences 7: 599. https://doi.org/10.3389/fvets.2020.00599

Mondal S,Adhikari N,Banerjee S,Amin SA,Jha T T., (2020) Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview. European Journal of Medical Chemistry194:112260. doi:10.1016/j.ejmech.2020.112260

Pragallapati S,Manyam R (2019) Glucose transporter 1 in health and disease. Journal of oral and maxillofacial pathology: Journal of Oral and Maxillofacial Pathology 23(3): 443–449. https://doi.org/10.4103/jomfp.JOMFP_22_18

Qin C, Bai Y, Zeng Z, Wang L, Luo Z, Wang S, Zou S S., (2018) The Cutting and Floating Method for Paraffin-embedded Tissue for Sectioning. Journal of Visualized Experiments 5(139):58288. doi: 10.3791/58288.

Rajesh Y, Mandal M (2017) Regulation of Extracellular Matrix Remodeling and Epithelial-Mesenchymal Transition by Matrix Metalloproteinases: Decisive Candidates in Tumor Progression. Proteases in Physiology and Pathology 159–194. doi:10.1007/978-981-10-2513-6_9

Sharen G, Peng Y, Cheng H,Liu Y, Shi Y, Zhao J (2017) Prognostic value of GLUT-1 expression in pancreatic cancer: results from 538 patients. Oncotarget 8(12): 19760–19767. doi: 10.18632/oncotarget.15035

Sherif MH,Abas AM, Zaitoun LA (2018) Lipid peroxidation is crucial in the regulation of cell division. Low concentrations of oxygen free radicals caused by a lack of antioxidants can stimulate cell proliferation while also inducing cytotoxicity and cell death. Research Journal of Pharmacy and Technology 11(12): 5439-5448. doi : 10.5958/0974-360X.2018.00992.7

Simanullang RH (2018) Impact of Health Education Intervention on Knowledge of Cervical Cancer Prevention Among Women in Bahorok's Village, North Sumatra Indonesia. Belitung Nursing Journal 4(6):591-595. https://doi.org/10.33546/bnj.452

Simanullang RH, Sitopu SD (2020) Effect of Health Education on Women's Knowledge Level about Pap Smear's Early Detection of Cervical Cancer Prevention. Asian Journal Oncology6:65-71. doi:10.1055/s-0040-1709365.

Simanullang RH, Situmorang PC, Herlina M, Noradina, Silalahi B, Manurung SS (2022) Histological changes of cervical tumours following *Zanthoxylum acanthopodium* DC treatment, and its impact on cytokine expression. Saudi Journal of Biological Sciences 29(4): 2706-2718.https://doi.org/10.1016/j.sjbs.2021.12.065

Simanullang RH, Ilyas S, Hutahaean S, Rosidah, Manurung RD, Situmorang PC (2021a). Effect of Andaliman Fruit Extract on Cervical Cancer Rat's Histology. IOP IEEE Explore 1-5. doi: 10.1109/InHeNce52833.2021.9537186

Simanullang RH, Ilyas S, Hutahaean S, Rosidah (2021b) Effect of andaliman (*Zanthoxylum acanthopodium*) methanol extract on rat's kidney and liver histology induced by benzopyrene. Pakistan Journal of Biological Sciences 24(2):274-281. doi: 10.3923/pjbs.2021.274.281

Situmorang PC, Ilyas S, Hutahaean S (2019a) Study of Combination of Nanoherbal Andaliman (*Zanthoxylum acanthopodium*) and *Extra Virgin Olive Oil* (EVOO) Effects in the Expression of Malondialdehyde (MDA), Heat Shock Protein-70 (Hsp70) and Placental Histology of Preeclamptic Rats Pharmaceutical Sciences 25(3): 205-220. Doi: 10.15171/PS.2019.37

Situmorang PC, Ilyas S, Hutahaean S (2019b) Effect of Combination of Nano Herbal Andaliman (*Zanthoxylum acanthopodium* DC.) and *Extra Virgin Olive Oil* (EVOO) to Kidney Histology of Preeclampsia Rats. IOP Conferences Series Earth Environmental Sciences 305: 012081. Doi:10.1088/1755-1315/305/1/012081

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2019c) Effect of Nanoherbal Andaliman *(Zanthoxylum acanthopodium)* and Extra Virgin Olive Oil Combination on Preeclamptic Rats Liver Histology. Open Access of Macedonian Journal Medical Science.7(14):2226-2231. Doi:10.3889/oamjms.2019.651

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2021a) Effect of Nano Herbal Andaliman (*Zanthoxylum acanthopodium*) Fruits in NOTCH1 and Hes1 Expressions to Human

Placental Trophoblasts. Pakistan Journal of Biological Sciences 24: 165-171. Doi: 10.3923/pjbs.2021.165.171

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2021b) Histological changes in placental rat apoptosis via FasL and cytochrome c by the nano-herbal *Zanthoxylum acanthopodium*. Saudi Journal of Biological Science 28(5): 3060–3068. doi: 10.1016/j.sjbs.2021.02.047

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R, Manurung RD (2020) Acute toxicity Test and Histological Description of Organs after Giving Nano Herbal Andaliman (*Zanthoxylum acanthopodium)*. Rasayan Journal of Chemistry13(2):780-788. doi:10.31788/RJC.2020.1325621

Situmorang PC, Ilyas S(2018) Review: germinal cell apoptosis by herbal medicine. Asian Journal of Pharmaceutical and Clinical Research 11(9):24-31. Doi:10.22159/ajpcr.2018.v11i9.26400

Tanaka T, Tera Y, Ohmichi M (2019) Association of matrix metalloproteinase-9 and decorin expression with the infiltration of cervical cancer. Oncology Letters 17: 1306-1312. https://doi.org/10.3892/ol.2018.9713

Thakur VS, Deb G, Babcook MA,Gupta S (2014) Plant phytochemicals as epigenetic modulators: role in cancer chemoprevention. The American Association of Pharmaceutical Scientists 16: 151–163. https://doi.org/10.1208/s12248-013-9548-5

Wijaya CH, Napitupulu FI, Karnady V,Indariani S (2019) A review of the bioactivity and flavor properties of the exotic spice "andaliman" (*Zanthoxylum acanthopodium* DC.). Food Reviews International 35:1: 1-19. https://doi.org/10.1080/87559129.2018.1438470

Zambrano A,Molt M, Uribe E, Salas MM (2019) Glut 1 in Cancer Cells and the Inhibitory Action of Resveratrol as A Potential Therapeutic Strategy. International Journal of Molecular Sciences 20(13): 3374. doi:10.3390/ijms20133374

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