The Cytokines Expression on Histological Changes of Cervical Cancer Tissue by Andaliman (Zanthoxylum acanthopodium)

by Herma Yerni

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Abstract

Cervical cancer is the second most lethal cancer in Indonesia, after breast cancer. Cytokines play an important role in cancer treatment because they are an inflammatory response product that plays an important role in the immune system, such as cytokines IL-β1, IL-10, TGF1, and VEGFR1. *Zanthoxylum acanthopodium* is an Indonesian herb rich in antioxidants with anti-inflammatory and anti-cancer properties. The study's goal was to look at the histological changes in andaliman treatment, as well as the expression of cytokines like IL-10, IL-1, VEGFR1, and TGFβ1 in tissue and serum from cervical cancer rat models. For 30 days, rats were divided into five groups: the control group (C-), the cancer model group (C+), cancer rats with a dose of ZAM 100mg/BW (ZAM100), cancer rats with a dose of ZAM 200 mg/BW ZAM (ZAM200), and cancer rats with a dose of ZAM 400 mg/BW ZAM (ZAM400). The blood was prepared for ELISA analysis, and cervical tissue was stained with immunohistochemistry using antibodies against IL-10, IL-1, VEGFR1, and TGFβ. As a result, administration of ZAM had no significant effect on rat body weight and cervical cancer weight ($p>0.05$). However, it had an effect on hematological value in cervical cancer rats ($p<0.05$). Elevated MDA levels may be linked to SOD deficiency in tumor tissue. ZAM significantly decreased the expression of IL-β1, TGFβ1, and VEGFR1 ($p<0.01$). ZAM also aids IL-10 in inhibiting the proliferation of abnormal cells that continue to differentiate. Finally, ZAM may be targeted in molecular cytokines therapy for cervical cancer.

1. Introduction

Cervical cancer is the world's second most frequent cancer, behind breast cancer, and it is particularly prevalent in developing nations such as Indonesia. According to the International Agency for Research on Cancer (IARC), breast cancer affects 40 out of every 100,000 women, and cervical cancer affects 26 out of every 100,000 women. HPV infection and cervical lesions are caused by a combination of intrinsic and extrinsic causes. In this context, environmental influences as well as the involvement of immunoregulatory mechanisms are crucial.

Cytokines are inflammatory response products that play a crucial part in the immune system's fight against viral infections. The IL-1 family of proteins, which includes IL-10 and IL-1β, is a crucial component of the body's innate immune system. At plasma IL-1 concentrations, the IL-1β genotype can be functionally changed to contribute to the etiology of cervical cancer in women. IL-1β has been demonstrated to regulate gene expression, cytokine production, cellular adhesion and migration, angiogenesis, and immunological responses, among other things. The amount of IL-1β generated, the type of cell that produces it, the microenvironment (immune cells or fibroblasts), the stage of cancer, and anti-cancer therapy may all have a role in IL-1β's effects. Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine found in cancer cells. Increased IL-10 expression in normal and abnormal cervix was observed to correspond with the severity of squamous intraepithelial lesions. IL-10, also known as an immunoregulatory cytokine, has the primary biological role of restricting and terminating the inflammatory response as well as

suppressing tumor development. Overexpression of IL-10 in the tumor microenvironment can result in immunological rejection of the cancer.

Transforming growth factor-beta (TGFβ) is a multifunctional cytokine that has a role in cell proliferation and differentiation, angiogenesis, immunosuppression, cell motility, apoptosis, wound healing, embryonic development, and cancer pathogenesis, among other things. Because of its ability to interrupt the cell cycle and induce apoptosis, it operates as a tumor suppressor gene during the early stages of carcinogenesis. Cervical cancer has been connected to TGFβ1, one of several cytokines that regulate cell development, maturation, and differentiated differentiation. *Vascular endothelial growth factor* (VEGF) is a protein released by cancer cells or tumor microenvironment cells that stimulates the creation of blood vessels in some cancers. One of three tyrosine kinases for VEGF, which is a key regulator of cancer angiogenesis, is the vascular endothelial growth factor receptor-1 (VEGFR-1). Although VEGFR was previously thought to be only expressed on endothelial (EC) cells, new research has shown that VEGFR-1 is present in malignant non-EC type cancer cells. VEGFR-1 is found in cancer cells and has the ability to enhance MAPK signaling, migration, and invasion. Furthermore, because carcinoma cells produce VEGFR-1 ligands, this effect may be autocrine-regulated.

Andaliman (*Zanthoxylum acanthopodium*) is an Indonesian spice that grows wild in the dense forests of Toba Samosir, North Tapanuli, and Dairi in North Sumatra's region. Alkaloids, glycosidia, tannins, phenols, and flavoids are antioxidants found in Andaliman that have the ability to act as natural preservatives, anti-inflammatory, and antibacterial agents. Andaliman can also alter CDK4 expression in the histology of cervical cancer tissue and improve the histology of cervical cancer tissue. Apart from cervical cancer, this plant can improve the histology of placental tissue, hypertensive rats' kidneys and livers, human trophoblasts' Hes1 and Notch1 genes, and diabetic burns. The goal of this study was to look at the histological changes in cervical cancer tissue after andaliman treatment, as well as the expression of cytokines likes IL-10, IL-1β, VEGFR1, and TGFβ1 in tissue and serum in cervical cancer rat models. So it is hoped that this research on cancer cells will continue and that this plant will be developed into cervical cancer drugs in the future using cytokines therapy.

2. Material and Methods

2.1. Materials

Andaliman was originally from Dairi, North Sumatera Province. ELISA kit for IL-1β Polyclonal Antibody, Catalog #13-7112-81, and IL-10 Monoclonal Antibody (JES3-9D7) Catalog #16-7108-81, TGF β-1 Monoclonal Antibody, Catalog #MA1-169 (B11-4C3). VEGFR1 (soluble) Polyclonal Antibody (Catalog # 36-1100). (Company:eBioscience, Inc. San Diego, USA). IL-10 Polyclonal Antibody with tested dilution 1:400, storage buffer: PBS with 50% glycerol, 1% BSA(Catalog # BS-20373R), IL-1 Beta Polyclonal Antibody with tested dilution 1:200, storage buffer : PBS with 50% glycerol, 1% BSA (Catalog # BS-0812R), VEGF Receptor 1 Polyclonal Antibody with tested dilution 1:50, storage buffer: PBS (Catalog # PA1-21731) and TGF beta 1 Polyclonal Antibody with tested dilution 1:500,storage buffer: PBS with 50% glycerol, 1% BSA (Catalog # BS-0086R) (Company: Thermo Fisher, in Waltham, Massachusetts).

2.2. Preparation of Zanthoxylum acanthopodium methanol extract (ZAM)

Zanthoxylum acanthopodium or andaliman fruits are cleaned of any soil or dust that has adhered to the fruit. The following three steps are used to create the fruit extract: (1), Drying of the crude drug: the andaliman fruit is cleaned and drained dry before being mashed in a blender. (2), Andaliman extract is made by macerating the fruit of Andaliman in 96 percent methanol for one night. It is then percolated until it becomes clear. The concentrated liquid is then evaporated to obtain the powder extracts (3), Because the extract of Andaliman does not dissolve completely in water, a homogeneous mixture is obtained by using a suspending agent CMC 1.5 percent to 1.0 percent or 1 ml in 150 ml of distilled water. The dregs are washed with 96 percent methanol solvent before being transferred to a closed container and stored in a cool, dark place for two days.

2.3. Experimental Animals

From January to October 2021, the research was carried out at the University of Sumatera Utara's Biology Laboratory and the Pathology and Anatomy Laboratory of the Faculty of Medicine's Faculty of Medicine. There are five groups of rats, the control group is Group C-, the cancer model group is Group C+, the ZAM100 group is cancer rats with a dose of 100mg/BW of ZAM, the ZAM200 group is cancer rats with a dose of 200 mg/BW of ZAM, and the ZAM400 group is cancer rats with a dose of 400 mg/BW of ZAM, during 30 days administration. The rats are dissected on the 30th day after ZAM administration. The blood is then prepared for ELISA analysis, and cervical tissues are stained with Immunohistochemistry. The Ethics Committee for Handling Experimental Animals, Faculty of Mathematics and Natural Sciences, USU, approved this research (Ethical Clearance: No. 0262/KEPH-FMIPA/2021).

2.4. Rats model of cervical cancer

The Animal House Laboratory at the University of Sumatera Utara provided 36 rats (*Rattus norvergicus*) weighing 180-200g for this investigation. The rats are fed standardized rat pellets and provided plenty of water before being acclimatized to laboratory conditions for four weeks prior to the trial. By injecting benzopyrene 50 mg/BW into the cervix and allowing the cancer to grow for three months, the rats are formed in an animal model of cancer.

2.5. Measurement of hematology

Rat blood was drawn with a sterile syringe and was never exposed to water, to avoid hemolysis. Blood is drawn through the heart after the animal has been sedated. A total of 0.5 mL was placed in a microcentrifuge tube with 10 L/1 mL of EDTA anticoagulant. The Medan City Health Laboratory performed a hematology examination. Each sample was run three times according to the manufacturer's instructions.

2.6. Measurement of SOD, MDA and NGAL

The sample was homogenized for 5 minutes in a homogenizer tissue with 10 ml of PBS pH 7.4, the supernatant was obtained, and the levels of NGAL, SOD, and MDA were determined using an ELISA reader at 450 nm.

2.7. ELISA

Enzo Life Sciences' ELISA test was used to perform quantitative cytokine analysis. In this study, the IL-1β (rabbit) ELISA kit, the IL-10 (rat) ELISA kit, the Tgf1 (rat), and the VEGFR1

(rabbit) ELISA kits were used. Each sample was run three times according to the manufacturer's instructions.

2.8. Immunohistochemistry

A microtome was used to cut paraffin cervical tissue with a thickness of 4-6 microns. The tissue was heated in citrate buffer at pH 6.0 and 350W for pre-treatment. After washing with PBS, the tissue was incubated with IL-10, IL-1β, VEGFR1, and TGFβ1 antibodies at 37 °C, then washed with PBS again before being treated with avidin-biotin peroxidase. For the chromogenic visualisation reaction, 3,3-Diaminobenzidine (DAB) hydrochloride was used, which was then stained with haematoxylin Mayer. The cervical tissue on the slide was stained with hematoxylin, and the score was calculated by multiplying the positive result by the staining intensity, 0 indicates that less than 10% of the cells were stained, 1 indicates that 10% to 25% of the cells were stained, 2 indicates that 25% to 50% of the cells were stained, 3 indicates that 50% to 75% of the cells were stained, and 4 indicates that more than 75% of the cells were stained. The staining intensity was graded as 1 (weak), 2 (moderate), and 3 (strong).

2.9. Statistical analysis of data

The Anova test and the *Kruskal Wallis* test (for non-parametric data) were used to analyze the data in the SPSS 22 program.

3. Results

3.1. The Effect of ZAM on Cancer Rat Body Weight and Cervical Weight

Figure 1a, cervical cancer model rats had an insignificant weight before treatment (P>0.05), but when injected with benzopyrene 50 mg/BW (Figure 1b), there was a significant difference (P0.05, p=0.040) compared to the control group. So there was a significant difference between cancer rats and healthy rats. However, when compared to the C+ group, there was no significant difference in the group that received ZAM at a dose of 100 to 400mg/Kg BW. According to Figure 1, administration of ZAM at a dose of 100 to 400mg/Kg BW had no effect on the body weight of cervical cancer rats.

When the cervical organs in all groups were weighed, it was discovered that there was a significant difference in the weight of cervical cancer with the control group (C-) with p value = 0.01. There were also significant differences in the groups given 100mg/kg BW (p0.05, p=0.045), 200mg/kg BW (p0.05, p=0.040), and 400mg/kg BW (p0.01, p=0.002). According to Figure 2, cervix tumors can affect cervical weight in both the control group and the ZAM administration.

3.2. The Effect of ZAM on Hematology value of cancer rat

The administration of ZAM at a dose of 100 to 400 mg/KgBW can change the hematological value in cervical cancer rats, as shown in Table 1. Hematological data such as hemoglobin, leukocytes, lymphocytes, eosinophils, monocytes, SGOT, SGPT, and creatinine were significantly different from the control rat group (p<0.05), while neutrophils, hematocrit, and urea were not (p>0.05) in comparison to the C- group and the ZAM group in comparison to the C+ group. The administration of ZAM at a dose of 100 to 400 mg/KgBW can impact the hematological value in cervical cancer rats, except for neutrophils, hematocrit, and ureum, according to the hematological data in table 1. The neutrophils, hematocrit, and ureum are still classified as normal.

3.3. The Effect of ZAM on SOD, MDA and NGAL in cancer Rats

In comparison to group C-, there were significant differences in SOD, MDA, and NGAL levels in cancer rats (p0.05, p=0.040). SOD levels in cancer rats administered ZAM at doses of 100 and 200 mg/kgBW similarly decreased. At the highest dose, however, it was not significant (P>0.05, p=0.06). This is in contrast to MDA levels, which show a significant difference between the highest and lowest doses (p>0.05, p = 0.060). The effects of ZAM treatment on NGAL levels were not significant at dosages of 100 and 200mg/kgBW, but they were significant at the highest dose. Based on biochemical data for SOD, MDA, and NGAL in cancer rats (Table 2), it is known that providing ZAM at dosages of 100 and 200 mg/Kg BW for SOD levels, 200 and 400 mg/Kg BW for MDA levels, and 400 mg/Kg BW for NGAL levels in cervical cancer rats is more effective.

3.4. IL-1β expression in histological changes of cervical cancer after ZAM administration

The Kruskal Wallis test, with a p value of 0.00, reveals a significant difference in Table 3. It is known that there is a significant difference (p0.01, p=0.0060) in IL-β1 expression when compared to the C- group based on the average value. At the lowest dose of ZAM (100mg/kgBW), it was not significant; however, at doses of 200 and 400 mg/kgBW, it was significant. The C+ group had the highest IL-β1 expression, while the C- group had the lowest, and the ZAM dose was 400mg/Kg Bw.

The presence of positive IL-β1 expression was identified in the nucleus and cytoplasm by brownish black staining, as shown by the red arrow (Figure 3). The most crucial information for diagnosis is the nucleus and cytoplasm of the cell, while the background and stroma are eliminated. The squamous epithelium can tell you if a cell is normal (Figure 3a) or abnormal (Figure 3b). Small cancer cells that can only be detected using a microscope have spread to adjacent lymph nodes. Enlargement of the cell nucleus (Figure 3b and 3c), uncontrolled cell development, uneven cell shape, a significant ratio of cell nucleus to cytoplasm, and various variations in the form of the nucleus are all signs of cell abnormalities. When given ZAM (Figure 3d and 3e), however, the expression of IL- β1 began to drop, with the nucleus dyed black, the cell shape began to become irregular, the nucleus to cytoplasm ratio began to balance, and the histology of cervical tissue began to improve, similar to the C- group. This is consistent with serum IL-β1 values obtained using an ELISA reader (Figure 4). At a dose of 100mg/kg BW, the cancer rats had significantly increased serum IL-β1 expression (p<0.01, p=0.0050) than the control animals. In cervical cancer, ZAM at doses of 200 and 400 mg/kg BW improved and suppressed serum IL-β1 expression (p<0.05). According to Table 3, Figure 3, and Figure 4, ZAM treatment can suppress the production of IL-β1, which promotes cancer cell growth in rats.

3.5.IL-10 expression in histological changes of cervical cancer after ZAM administration

It is known that there is a significant difference ($p<0.01$, $p=0.0030$) in IL-10 expression when compared to the C- group based on the average value. At the lowest dose of ZAM (100mg/kgBW), it was not significant; however, at doses of 200 and 400 mg/kgBW, it was significant. The C- group had the highest IL-10 expression, while the C+ group had the lowest. IL-10 expression characterized by black or brown nuclei (Figure 5a) was increasing in the C- group in immunohistochemical examination (Figure 5). Because IL-10 activity was lowered in a hypoxic cell environment, nuclei stained black by immunohistochemical dyes were reduced in the cancer animals. From the lowest to the highest dose, there was a significant reduction in cervical squamous cell cancer. Enlargement of the cell nucleus indicates cell abnormalities (Figure 5b and 5c). There were no significant changes in the histological classification of cervical tumors after ZAM treatment (Figure 5c-5e). Using an ELISA reader, this was consistent with serum IL-10 (Figure 6). The serum IL-10 expression in cancer rats was lower (p0.05, p=0.040) than in the control group. In cancer rats, administration of ZAM at the lowest and highest doses can significantly increase IL-10 expression. According to Table 4, Figure 5 and Figure 6, ZAM administration can increase IL-10 expression in cervical cancer rats, thereby suppressing the growth of cervical cancer.

3.6. TGFβ1 expression in histological changes of cervical cancer after ZAM administration

The Kruskal Wallis test, as shown in Table 5, reveals a significant difference with a p value of 0.00. Based on the average value, it is known that there is a significant difference in TGFβ1 expression ($p<0.01$, $p=0.0030$) when compared to the C- group. The lowest dose of ZAM (100mg/kgBW) was not significant, but doses of 200 and 400 mg/kgBW wereCervical cancer cells in group C were made up of normal epithelial and nuclear layers (Figure 7a). Undifferentiated cells in the C+ group, on the other hand, were confined to the lower layer of the epithelium and developed mitotic features. Cellular changes confined to the lower epithelium were characterized by epithelial thickening and increased TGFβ1 expression (Figure 7b). TGFβ1 expression in cancer tissue decreases as the dose of ZAM increases. ZAM (Fig. 7c-7e) administration at various doses reduced the number of nuclei stained brown by immunohistochemistry, indicating a positive index of TGFβ1 expression in cancer tissue. Carcinomas that previously spread uncontrollably in the untreated group of cancers have now been slowed and no longer develop into epitheliumThe findings of this histology were consistent with the findings of an ELISA reader analysis of serum TGFβ1 cancer rats (Figure 8). TGFβ1 serum expression was higher in the cancer rats ($p<0.05$, $p=0.040$) than in the control group, but this difference was not significant when ZAM doses of 100 and 200mg/Kg BW were administered. The highest dose of ZAM (400mg/kg BW) did, however, significantly suppress TGFβ1 expression ($p<0.05$). According to Table 5, Figure 7 and Figure 8, administration of ZAM, specifically at a dose of 400mg/kg BW, can suppress TGF1 serum expression and histology in cervical cancer rats.

3.7. VEGFR1 expression in histological changes of cervical cancer after ZAM administration

The Kruskal Wallis test and the Mann-Whitney follow-up test both show a significant difference in Table 6. Based on the average value, it is clear that there is a significant difference ($p<0.01$, $p=0.0040$) in VEGFR1 expression between the C+ and C- groups. It was, however, not significant at the lowest ZAM dose (100mg/kgBW) and significant at the 200 and 400 mg/kgBW doses. The C+ group had the highest VEGFR1 expression, while the C- group had the lowest, and the ZAM dose was 400mg/Kg Bw. Histological examination revealed that the carcinoma had spread to the pelvic wall, that there was no clear space between the tumor and the pelvic wall, and that the core was irregular (Figure 9b). This was in stark contrast to the histology in the C-group, where cervical tissue still contained normal cells (Figure 9a). This was in stark contrast to the histology in the C-group, where cervical tissue still contained normal cells (Figure 9a). At the lowest ZAM dose (Figure 9c), lesions were larger than in the control group, but VEGFR1 expression began to decrease. The reduction in VEGFR1 expression at doses of 200 and 400 mg/kg BW demonstrated that this herb can reduce VEGFR1 expression because the empty space between tumors was reduced, the carcinoma stopped expanding, and the nucleus began to take on a normal shape (Figure 9d-9e). This was confirmed by comparing the VEGFR1 serum of cancer rats to the control group using an ELISA reader (Figure 10). However, at a dose of 100mg/kg BW, this is not the case. In cervical cancer rats, ZAM at doses of 200 and 400 mg/kg BW improved of cervical histology and suppressed VEGFR1 serum expression ($p<0.05$). So, according to Table 6, Figure 9 and Figure 10, Administration of ZAM can lower serum expression of VEGFR1 and enhance histology in cervical cancer rats.

4. Discussion

ZAM at doses ranging from 100 to 400mg/Kg BW had no effect on the body weight of cervical cancer rats. Body weight may be a significant factor in cancer survivors. However, it was not significant in cervical cancer rats. This could be due to the small size of cervical cancer, fat in rats, or the overactivity of rats. Cervical tumors can have an effect on cervical weight in both the control and ZAM groups. Tumor cells can grow indefinitely and undergo excessive angiogenesis. When this happens in specific areas of the tumor, there is a lack of nutrients, including oxygen. In this state, the tumor cells enter a resting phase in which oxygen is depleted, causing the cells to become hypoxic or even anoxic, and necrosis to occur. Because of the presence of abnormal cells that continue to proliferate and form cancerous tissue that interferes with the body's metabolic system, this process can increase the volume of the cervix.

Except for neutrophils, hematocrit, and urea, giving ZAM at a dose of 100 to 400 mg/KgBW can affect the hematological value in cervical cancer rats. Only patients with an eosinophil cell infiltrate in the tumor were found to have metastases. Table 1 shows that changes in the number of hemoglobin, leukocytes, lymphocytes, eosinophils, and monocytes in cervical cancer are very reasonable because the cervix has an endocervical mucosal layer

containing mucus-producing columnar epithelium in a thick lamina propria that is connected to the vagina. In this section, stratified columnar epithelium gives way to stratified squamous epithelium. The middle layer of the cervix is deeper and has less smooth muscle and more dense connective tissue. This section contains a large number of lymphocytes and other leukocytes that help to strengthen the body's defense against microorganisms and abnormal cells like cancer. Because ZAM contains strong antioxidants that can counteract free radicals, it can be used to control the state of these cells.

In cervical cancer rats, ZAM was more effective at doses of 100 and 200 mg/kg BW for SOD levels, 200 and 400 mg/kg BW for MDA levels, and 400 mg/kg BW for NGAL levels. In cervical cancer, increased lipid peroxide due to antioxidant deficiency was associated with increased levels of Malondialdehyde (MDA) and circulating neutrophil gelatinase associated lipocalin (NGAL) and decreased Superoxide dismutase (SOD) activity. Furthermore, elevated MDA levels in tumor tissue may be associated with SOD deficiency. If this continues, superoxide anion accumulates, which is highly radical and capable of penetrating membranes, causing negative effects far from the tumor. Antioxidants found in andaliman can lower levels of MDA and serum NGAL, thereby increasing SOD activity. By increasing SOD activity as a result of ZAM administration, it is possible to protect cells from oxidant disorders and oxidative stress, which can lead to a variety of diseases, including cancer.

In rats, ZAM administration can reduce the expression of IL-1, which promotes the proliferation of cancer cells. In cervical tissue cells, innate immune system cells such as macrophages, Langerhans cells (LC), dendritic cells (DC), neutrophils, Natural Killer (NK) cells, T lymphocytes, and keratinocytes recognize foreign structures not found in the host via receptors such as Toll. -like receptors (TLRs) that signal the expression of inflammatory cytokines and chemokines such as interleukin (IL) IL1β, , IL6, IL8, IL12, tumor necrosis factor (TNFα) and interferon (IFN) -, -β and -γ.

Immune cells are taken up, antimicrobial factors are secreted, and the innate and adaptive immune systems are linked. Because these immune cells don't always perform at their best, they need help from the outside world, such as more antioxidants. ZAM, an antioxidant found in plasma and erythrocyte membranes, can modify responses that affect messenger seconds and arachidonic acid cascade products, both of which have a significant impact on cell proliferation. Biological reactions such as oxidative stress caused by a lack of antioxidants in cells can harm cellular components, resulting in a variety of diseases. The formation and progression of cancer is caused by DNA damage. Antioxidants and cancer have a very close relationship because antioxidants have become a widely accepted therapeutic approach. The mechanism underlying the majority of chemotherapy and radiation agents that kill tumor cells is not an increase in antioxidants, but rather an increase in free radicals that cause irreversible tissue damage. Appropriate antioxidant inhibitors and/or free radical-producing compounds can be an effective cancer treatment strategy. In addition to antioxidants, the herb genus *Zanthoxylum* has anti-inflammatory, analgesic, antinociceptive, antioxidant, antibiotic,

hepatoprotective, antiplasmodial, cytotoxic, antiproliferative, anthelmintic, larvicidal, antiviral, and anticancer properties.

Giving ZAM to cervical cancer rats increases the expression of IL-10, which suppresses the growth of the cancer. In cancer patients, ZAM administration has been shown to protect against benzoapyrene-induced oxidative stress. In this regard, cancer may cause a decrease in IL-10 by reducing benzoapyrene-induced oxidative stress. Increased ROS production and oxidative stress have been linked to apoptosis. As a result, apoptosis plays a role in cancer pathogenesis and etiology. The role of phytochemicals as antioxidants or modulators of other carcinogenic and cancer-prevention processes. Antioxidants in ZAM stimulate IL-10, which inhibits or eliminates the inflammatory response and regulates the development and differentiation of abnormal cells. IL-10 is a cytokine secreted widely by monocytes that has pleiotrophic effects on the immune system and inflammation. T cells, monocytes, and macrophages can be inhibited in their activity and effector function by IL-10. In the presence of ZAM, IL-10 acts as the primary anti-inflammatory in the natural and adaptive immune response, preventing an excessive inflammatory response by inactivating macrophages and local and systemic inflammatory mediators. Actually, the body produces large amounts of these cytokiness, making them easily detectable in serum. However, if abnormal cells proliferate in large numbers, the performance of IL-10 is disrupted, necessitating the use of antioxidants from outside the body.

ZAM administration, particularly at 400mg/kg BW, can suppress TGFβ1 serum expression and histology in cervical cancer rats. TGFβ1 dysregulation is a major driver of tumor development, including angiogenesis, tissue invasion, metastasis, and immune suppression. TGF can actually maintain tissue homeostasis and prevent pre-cancerous tumors from progressing to malignancy by regulating not only cellular proliferation, differentiation, survival, and adhesion, but also the cellular microenvironment TGF signaling, on the other hand, promotes tumor growth and invasion, immune evasion, and cancer cell spread and metastasis as a genetically unstable entity, as in cervical cancer cells. In this study, ZAM was given to suppress tissue invasion, metastasis, and immunity. TGFβ1 enforces homeostasis and suppresses tumor development in normal cells (C-group) either directly through cell autonomic tumor suppressor effects or indirectly through suppression of inflammation and stromal-derived mitogens. When abnormal cells become cancerous, these cytokines lose their tumor suppressive response. Cancer cells use TGFβ1 to their advantage by initiating immune evasion, producing growth factors, differentiating into invasive phenotypes, and establishing and expanding metastatic colonies. As a result, administering antioxidants is critical for restoring TGFβ1's initial function in cells.

In cervical cancer rats, ZAM administration can suppress VEGFR1 serum expression and improve histology. In cancer, high VEGFR1 expression can be caused by lipid peroxidation due to a lack of antioxidants. 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. Giving ZAM can improve histology in cervical cancer rats because antioxidants found in Andaliman, such as

alkaloids, glycosidia, tannins, phenols, and flavoids, have anti-inflammatory and anticancer properties.

The use of antioxidants during cancer treatment has been shown to reduce toxic side effects. Antioxidant interventions stem from the fact that plants, such as the herbal andaliman, contain antioxidants and are associated with cancer treatment with few side effects. Herbs' ability to protect against DNA damage can be demonstrated by measuring the damage. The estimated background level in cells for DNA oxidation varies by more than threefold depending on the method used. It was possible to demonstrate a reduction in oxidative damage after supplementation with antioxidants from herbs using a biomarker assay for DNA oxidation.

ZAM administration reduced the expression of Il-β1, TGFβ1, and VEGFR1 in serum and cervical cancer histology, suggesting that it could be used as molecular cytokines therapy in cervical cancer. ZAM can also assist IL-10 in inhibiting the proliferation of differentiated abnormal cells. ZAM may be effective in molecular cytokines therapy for cervical cancer, but cancer therapy success is dependent on a variety of factors, including clinical stage, tumor histological type and differentiation, cellular immune response, and apoptosis. Furthermore, factors outside of the tumor, such as chemoradiation, immunotherapy, micronutrients and antioxidants consumed, and genetic susceptibility, all have an impact on treatment success.

5. Conclusion

In Conclusion, ZAM administration had no effect on the body weight of cervical cancer rats. Increased circulating MDA and NGAL in cervical cancer, on the other hand, were associated with decreased SOD activity after ZAM administration. Furthermore, elevated MDA levels in tumor tissue may be associated with SOD deficiency. After ZAM administration, there was a significant decrease in the expression of Il-β1, TGFβ1, and VEGFR1 in serum and cervical cancer histology ($p<0.01$). ZAM can also assist IL-10 in inhibiting the proliferation of differentiated abnormal cells. ZAM could be in molecular cytokines therapy for cervical cancer.

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