

# EFFECT OF TOMATO (*Lycopersicum commune*) JUICE ON VAGINAL WALL ELASTICITY IN MENOPAUSAL RATS

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# **EFFECT OF TOMATO (*Lycopersicum commune*) JUICE ON VAGINAL WALL ELASTICITY IN MENOPAUSAL RATS**

## **ABSTRAK**

Masa menopause merupakan kondisi hipoestrogen. Pada masa ini terjadi atrofi vagina akibat penurunan kadar estrogen yang menyebabkan penurunan elastisitas vagina. Tomat banyak mengandung fitoestrogen yang meningkatkan kadar estradiol dalam darah. Namun efek jus tomat terhadap elastisitas dinding vagina belum diketahui. Tujuan penelitian ini meneliti efek jus tomat (*Lycopersicum commune*) terhadap elastisitas dinding vagina tikus ovariektomi. Jenis Penelitian yang digunakan adalah jenis penelitian eksperimen laboratoris. Rancangan penelitian ini adalah Post test Only Control Group Design. Jumlah sampel yang diperlukan pada penelitian ini adalah 24 ekor tikus yang dibagi dalam 4 kelompok, 1 kelompok kontrol, 3 kelompok perlakuan dengan dosis 110 mg/kgBB/hari, 220 mg/kgBB/hari dan 330mg/kgBB/hari yang diberikan selama 28 hari. Hasil penelitian didapatkan rerata kepadatan kolagen pada kelompok kontrol berada pada rentang antara 64-72% dengan nilai simpangan baku antara + 2,82, Pada kedua kelompok perlakuan 1 kepadatan kolagen berada pada rentang antara 70-82% dengan nilai simpangan baku antara + 4,57. Pada kedua kelompok perlakuan 2 kepadatan kolagen berada pada rentang antara 80-92% dengan nilai simpangan baku antara + 4,72. Pada kedua kelompok perlakuan 3 kepadatan kolagen berada pada rentang antara 90-96% dengan nilai simpangan baku antara + 2,63. Hasil uji statistik dengan One Way Anova didapatkan terdapat perbedaan yang signifikan kepadatan kolagen antara kelompok kontrol dan kelompok perlakuan. Jus tomat (*Lycopersicum commune*) meningkatkan elastisitas vagina tikus Wistar pasca ovariektomi pada dosis pemberian 110 mg/kgBB/hari, 220 mg/kgBB/hari dan 330 mg/kgBB/hari. (FMI 2015;51:132-136)

Kata kunci: Menopause, elastisitas dinding vagina, kepadatan kolagen, tomat, fitoestrogen

## **ABSTRACT**

Menopausal period is a hipoestrogen condition. In menopausal periode, some woman get vaginal atrophy because of decreasing estrogen level. It can cause a decline of vagina elasticity. Tomatoes are known as rich of lignan phytoestrogen. Phytoestrogen can increase estradiol level on blood. However the effect of tomato juice toward the elasticity of vaginal wall of ovariectomized rats is still unknown. The purpose of this research was to study the effect of tomato juice (*Lycopersicum commune*) toward elasticity of the vaginal wall of ovariectomized rats. This study was a laboratory experiment using Post test Only One Control Group design. This study used 24 female-ovariectomized Rattus norvegicus Wistar strain randomly divided into 4 groups, one control group (K) and three treatment groups three difference dosage of tomato juice that is 110 mg/kgBW/day (P1), 220 mg/kgBW/day (P2) and 330 mg/kgBW/day (P3) for 28 days. The results showed that there was an increase of collagen synthesis in vagina wall of ovariectomized rats. The Control group showed the lowest level of estradiol = 67.43% + 2.82., while the highest result was found on group P3 93.29% ± + 2.63. One-way ANOVA test showed a significant difference between control group and all treatment groups. Hence the study shows that the application of tomato juice indeed increase the elasticity of the vaginal wall during menopause. (FMI 2015;51:132-136)

Keywords: menopause, tomato juice, elasticity of the vaginal wall

## INTRODUCTION

Menopause is the permanent cessation of menstruation periods due to reduced or loss of ovarian activity. At menopause, the ovaries no longer secrete progesterone and  $17\beta$  estradiol in significant quantities. Estrogen levels decline which was originally more than 120 pg/ml to only 18 pg/ml (Bachmann & Nevadunski 2000). Declining levels of estrogen cause a burning sensation at the vulva complaint, dysuria, pruritus, dyspareunia and pain due to a decrease in elasticity and thickness of the wall of the vagina (vaginal atrophy) due to a decrease in collagen in the vaginal wall.

Seventy-five to eighty-five percent of postmenopausal women experience vaginal atrophy (Immanuel et al 2010). Forty-eight percent of them complained of painful intercourse due to vaginal atrophy. Painful intercourse can have a significant impact on sexual activity and the effect on the decrease in the quality of life of menopausal women and can disrupt the harmony of the marital relationship (Ferrero et al 2008).

Thinning of the vaginal epithelium will increase the vaginal pH becomes more alkaline which result in increased risk of vaginal infections or atrophic vaginitis. Giving estrogen vaginal cream aims to improve the experience vaginal epithelium atrophy due to menopause, but it can affect systemic administration, which would increase the risk of endometrial hyperplasia and carcinoma (BPOM RI 2008). The use of alternative sources of natural estrogen for vaginal atrophy overcome need to be developed so that it can be used as a concept of hormone replacement therapy for menopausal women in the future, especially for prevention and treatment of vaginal atrophy. The levels of phytoestrogens in high tomato fruit and tomato is a plant that is very easy to find in Indonesia it is necessary to study the effect of tomato juice on the amount of collagen and thickness of the vaginal wall on the state of menopause. This study uses a model of rats *Rattus norvegicus* Wistar strain which ovariectomized consideration of ethics in order to get a picture resembling a state of menopause in humans. The purpose of this research was to study the effect of tomato juice (*Lycopersicon commune*) against vaginal elasticity Wistar rats after ovariectomy.

Compounds in tomatoes are among solanine (0.007%), saponins, folic acid, malic acid, citric acid, bioflavonoids (including lycopene,  $\alpha$  and  $\beta$ -carotene), proteins, fats, vitamins, minerals and histamine (Canene-Adams et al 2005). Lycopene is one of the most widely chemical content in tomatoes, 100 grams of tomatoes on average contain as much as 3-5 mg lycopene (Giovannuci 1999).

Phytoestrogens are chemicals found in plants and has a chemical structure and function that resemble estrogen (Bustamam 2008). Phytoestrogens can be interpreted as natural compounds from plants to affect estrogenic activity of the body (Lukitaningsih 2010). Phytoestrogen compound fill empty estrogen receptor sites and produce estrogenic effects similar to endogenous estrogen. These phytoestrogens can compete with endogenous estrogen in the body occupying the estrogen receptor. This can help reduce the overall estrogenic effects in the body, due to the effects of phytoestrogens tends to be lighter than the endogenous estrogen

(Lukitaningsih 2010). In the state of high estrogen levels, although very weak binding power of phytoestrogens will bind to the receptor and block estrogen from binding to the receptor, this means that phytoestrogens can be antiestrogenic (antagonist). In the state of estrogen deficiency at menopause, phytoestrogens dominant and will bind to estrogen receptors and cause estrogenic effects empty.

Phytoestrogens modulate concentrations of steroid hormones by binding to enzymes involved in the synthesis and metabolism of steroid (Johnston & Williamson 2003). Phytoestrogens will inhibit the reaction catalyzed by the enzyme  $17\beta$  HSOR II so that the levels of estradiol  $17\beta$  will increase (Comitee on Toxicity 2003). Research has shown a strong correlation between the decrease in collagen with the state of estrogen deficiency in menopause (Brincat et al 2005). Thickening and elasticity of the vaginal wall is influenced by an increase in collagen synthesis and proliferation of epithelial maturation of the vaginal wall. Giving estrogen can increase the amount of collagen, (Fan 2012). Increased collagen density increases tissue elasticity. The hypothesis in this study is the tomato juice (*Lycopersicum commune*) improve vaginal elasticity Wistar rats after ovariectomy.

## MATERIALS AND METHODS

The type of research that is used is a kind of experimental laboratory research that aims to determine the possibility of a causal relationship to provide treatment in the experimental group and compared to the control group. The design of this study is the Post Test Only Control Group Design.

Population and the subjects in this study were white rats (*Rattus norvegicus*) adult females over the age of 3-4 months with a weight of 150 grams  $\pm$  10 grams. The number of samples required in this study were 24 male rats were divided into 4 groups, each group consisting of 6 animal. To avoid the shortcomings of the samples because of death, then the sample size of each group plus 18 so the overall sample is 28 tail. The independent variable in this study is the tomato juice with a dose of 110 mg/kg/day, 220 mg/kg/day and 330mg/kg/day given for 28 days. The dependent variable in this study is the elasticity of the vagina as indicated by the density of collagen, which is a time of collagen fiber in each specific area (100 x 100  $\mu$ m<sup>2</sup>) and expressed in percent In this examination collagen density data is the average percentage of five field of view different.

This study using female white rats *Rattus Wistar norvegicus* aged 8-10 weeks and has a weight of 150 grams  $\pm$  10 grams. Materials used in this study is tomato juice, distilled water, pellets, ether, hematoxylin eosin (HE). Materials used for the treatment is tomato juice, while the control group was given distilled water only Tomato juice is given in this study is the result of freeze dry from the inside of a tomato that has been separated from the seeds so that the inside of the tomato powder obtained which is then dissolved in distilled water. Total tomato powder tailored to the needs of each animal based dose treatment and weight of each animal, then add distilled water up to 2 cc for each animal. Dosing based on that consumption of fruit for menopausal women of at least 400 grams/day so that by the conversion formula, the required dose of fresh tomatoes in mice is at 7.2 grams/day. Results 1 gram of freeze dry the inside of the fresh tomatoes obtained 6 mg tomato powder, tomato so that the dose required is estimated a minimum of 110 mg/kg/day. Animal acclimatization prior to the study carried out for two weeks and vaginal smear is also performed to ensure that the mice are not pregnant, then do oovarektomi. Two weeks after vaginal smear oovarektomi performed on all animals try and

obtain the entire animal at diestrus phase which means that already in a state of menopause. In this study there is a control group of mice that carried oovarektomi groups but not given treatment and three groups treated with different doses. Each group was placed in a separate cage. In the treatment group was given the provision of tomato juice in different doses for 28 days. After day 28, the experimental animals were sacrificed and taken part vagina for examination by histopathology with HE smear. The data obtained by analyzing statistical test One Way Anova.

## RESULTS

Figure 1 shows that the average density of the highest collagen in the treatment group 3 (Tomato juice dose of 330 mg/kg for 28 days) with a mean of 93.29% and the lowest was in the control group (using distilled water) with a mean density of collagen 67.43%.

In table 1 can be explained that the average density of collagen in the control group were in the range between 64-72% with a standard deviation value between + 2.82. In both treatment groups 1 collagen density in the range between 70-82% with a standard deviation value between + 4.57. In both treatment groups 2 collagen density in the range between 80-92% with a standard deviation value between + 4.72. In both treatment groups 3 collagen density in the range between 90-96% with a standard deviation value between + 2.63.

In Table 1 we can also see the results of homogeneity tests levene on animal collagen levels is obtained with significant value 0.296 which is where the results of > 0.05, so that based on these results the animal collagen levels in the control group and the treatment group is homogeneous.

Figure 1. Diagram of the mean density of collagen in experimental animals in each group after the administration of tomato fruit extract for 28 days.

Table 1. Analysis of collagen density

Aquadest

Treatment Groups 1 tomato juice dose of 110 mg/kg

Treatment Groups 2 tomato juice dose of 220 mg/kg

Treatment Groups 3 tomato juice dose of 330 mg/kg Minimal 64% 70% 80% 90% Maximal 72% 82% 92% 96% Mean 67.43% 74.57% 84.71% 93.29% ± SD ± 2.82 ± 4.57 ± 4.72 ± 2.63

Homogeneity test 0.296

Table 2. Analysis of the levels of collagen by using oneway ANOVA test

Sum of Squares

df

Mean Square

F Sig.

Between Groups

2703.714 3 901.238 62.103 .000



Within Groups

348.286 24 14.512

Total 3052.000 27

From the table above the ANOVA test results to the density of animal collagen which aims to analyze whether there are significant differences in collagen density in the control group, the treatment group 1, group 2 and 3 treatment groups, the calculation results showed the significance of which 0.000. There are significant differences in collagen density in the control group, the treatment group 1, group 2 and 3 treatment groups.

## DISCUSSION

Menopause is the permanent cessation of menstruation periods due to reduced or loss of ovarian activity. At menopause, the ovaries no longer secrete progesterone and  $17\beta$  estradiol in significant quantities. Hypoestrogen state inhibits fibroblast proliferation in the vaginal wall as a result of collagen synthesis and elastin fibers decreases resulting in thinning of the vaginal wall passage and a decrease in elasticity of the vagina.

The inside of the tomato fruit contains compounds that resemble estradiol  $17\beta$  as many as 1536 pg/g. Tomatoes are one type of phytoestrogen lignans. Phytoestrogens modulate concentrations of steroid hormones by binding to enzymes involved in the synthesis and metabolism of steroid (Johnston & Williamson 2003). The structure of phytoestrogens has some similarities with endogenous estrogen, so as to have the ability to bind to estrogen receptors (Johnston & Williamson 2003). Phytoestrogens bind to estrogen receptors in the nucleus then induces a conformational change which will cause the dimerization with a section of DNA that is Estrogen Response Element (ERE), then the process transactivation which stimulates RNA polymerase resulting in gene transcription. The process of producing mRNA transcription. The newly formed messenger RNA binds to ribosomes in the cytosol denngan and experienced translation.

Translation process produces several proteins, among others, CDK and cyclin that play a role in cell division cycle that also stimulates mitogenesis of fibroblasts. Fibroblast proliferation induced by Transforming growth factor  $\beta$  (TGF $\beta$ ) (Leask & Abraham 2004). Phytoestrogens contained in tomato given in dosis 110 mg/kg/day, 220 mg/kg/day and 330 mg/kg/day could be expected to induce an increase in TGF  $\beta$  the vagina resulting in the proliferation of fibroblasts in the vaginal wall experimental animals. Fibroblasts are the cells most widely in connective tissue and in charge of synthesizing extracellular matrix components. Fibroblasts synthesize collagen, elastin, glycosaminoglycans, proteoglycans and glycoproteins multiadhesif. Fibroblasts involved in the production of growth factors and cell differentiation (Junqueira 2002).

This study showed that administration of tomato juice can induce proliferation of fibroblasts resulting in increased synthesis of collagen in the vaginal wall. The higher dose consumed by the animal collagen is synthesized also increased. Increased collagen synthesis is indicated by an increase in collagen density collagen fiber that time on any particular area (100 x 100  $\mu$ m<sup>2</sup>) is expressed in percent. Estrogen affects the synthesis and metabolism of collagen in the vagina (Elghany 2011). Estrogen contained in tomatoes especially the tomato seed mucilage is able to trigger an increase in the synthesis and metabolism of collagen in the vagina. Increased

collagen density in the vaginal wall can improve the elasticity of the vaginal walls, so that when applied to humans tomato juice consumption is expected to overcome the problem of sexual disorders in menopause due to decreased vaginal elasticity due hipoestrogen condition at menopause.

#### CONCLUSION

Tomato juice (*Lycopersicum commune*) improves vaginal elasticity Wistar rats after ovariectomy on the dosage of 110 mg/kg/day, 220 mg/kg/day and 330 mg/kg/day

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