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the effect of ajwa dates fruit extract on ovarian follicle development in female rats exposed to arsenic

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THE EFFECT OF AJWA DATES FRUIT EXTRACT ON OVARIAN FOLLICLE DEVELOPMENT IN FEMALE RATS EXPOSED TO ARSENIC

ABSTRACT

Arsenic trioxide (As_2O_3) is a form of inorganic arsenic which is very toxic in the environment. Arsenic exposure can cause reproductive health problems, one of which is the ovaries, by interfering with the follicle development. Arsenic exposure causes oxidative stress in the ovaries due to the accumulation of excessive *Reactive Oxygen Species* (ROS) and an imbalance between ROS and antioxidants in the body. The powerful antioxidants contained in dates can protect the effects of free radicals due to arsenic exposure. The group in this study was divided into a negative control group (without arsenic exposure), positive control (3 mg / kg BW arsenic), T1 (arsenic 3 mg / kg BW + date extract 2mL/ kg BW), T2 (arsenic 3 mg / kg BW + date extract 4ml / kg BW), and T3 (arsenic 3 mg / kg BW + date extract 8ml / kg body weight). The administration of Ajwa date extract could significantly provide antioxidant protection in the treatment group with an increase in follicle count on this study.

KEY WORD : Arsenic trioxide, Arsen,Ovary, Free Radical, *Reactive Oxygen Species*, Oxidative Stress

INTRODUCTION

Exposure to heavy metals, one of which is arsenic, which is a group *metalloid*, can cause reproductive dysfunction in women (Mendola *et al*., 2008), even residual heavy metal exposure in the body can affect women's reproductive health (Bloom *et al*., 2011). Arsenic toxicity has become a major concern due to increased contamination of exposure to this compound in the air, water, and soil (Flora, 2014). *The Agency for Toxic Substances and Disease Registry* United States (ATSDR) has designated arsenic as No. 1 on the Priority List of Hazardous Substances over the lead, mercury and *Vinyl Chloride* (ATSDR, 2019) (Shen *et al*., 2013). Currently, the number of humans exposed to arsenic through the air, food, water, and soil is increasing all the time. Arsenic toxic effects are very common in developing countries. Studies on the toxicity of arsenic exposure in women have been carried out in several countries. In a study conducted on women in Taiwan by Lei *et al*. (2015) arsenic levels in infertile women were higher than in pregnant women. A case study conducted on women in China who were exposed to arsenic found that arsenic can cause ovarian insufficiency /*Premature Ovarian Insufficiency* (Pan *et al*., 2020). A drastic decrease in the number of follicles due to environmental factors such as heavy metal exposure, namely arsenic, can result in early menopause (Kumar *et al*., 2012).

The effect of arsenic exposure on ovarian organ can cause damage to ovarian morphology while folliculogenesis affects follicular regression. Arsenic exposure causes a decrease in the number of follicles

including primordial, primary, secondary and *de Graaf* follicles and increases the number of atretic follicles (Chattopadhyay and Ghosh, 2010; Akram *et al.*, 2010; Mehta and Hundal, 2016; Khatun *et al.*, 2018; Yu *et al.*, 2019).

Many studies have examined the benefits of datesfruit for female's reproductive health because they are believed to have an effect on oocyte quality, sperm and ovum interactions, implantation and early embryonic development through antioxidant mechanisms. (Abdi *et al.*, 2017; Saryono *et al.*, 2018). The antioxidant properties of Ajwa dates are expected to be able to suppress free radicals through *scavenger* pathway, which have an effect to reduce disease proliferation due to the strong antioxidant content in Ajwa dates (Ahmed *et al.*, 2016; Al-Yahya *et al.*, 2016). Therefore, in this study, we are interested in conducting studies on the effects of Ajwadate extract (*Phoenix dactylifera L.*) on the female reproductive system, especially on follicle development in female Wistar rats (*Rattusnorvegicus*) and the lack of research on the effects of Ajwa date palm extract in preventing exposure arsenic metal toxicity.

MATERIALS AND METHODS

Chemical

Arsenic powder (As_2O_3) produced by LobaChemie as much as 500 mg was dissolved in 1000 mL *Normal Saline* 0.9% then stirred with a *magnetic stirrer* on heating at 50 °C for 3-4 hours to make the arsenic powder completely dissolve.

Preparation of Extract The

Types of dates used for the study were 400 grams of Ajwa dates. Selection of dates by selecting dates that are old, not rotten, separated from the seeds, then thinly sliced and the flesh of the dates is taken. The extract making refers to previous research, namely research by Vayalil (2002) and Al-Rasheed *et al.,*(2015) with slight modifications. The flesh of the ajwa dates that have been separated from the seeds, mashed with a *mortar* and *pestle*, then added 1200 ml of water (ratio 1: 3, g/ml). The thick date palm extract was centrifuged at 4°C for 15 minutes at a speed of 10,000*g*, then the supernatant was taken.

Animals and Ethics Statement

Research procedures have been accepted by the Health Research Ethics Commission, Faculty of Medicine, University of Brawijaya. In this study, there were 5 treatment groups where there were 5 repetitions for each group. Negative control was rats given *Normal Saline* and not given date palm extract and arsenic, positive control rats exposed to arsenic 3mg/kg BW/day, treatment 1, 2 and 3 rats given date extracts with 3 different doses (2ml / KgBW / day; 4ml/KgBB/day; 8ml/KgBB/day), then exposed to 3 mg/KgBB / day arsenic for 30 days. The research was conducted at the Laboratory of Bioscience, Anatomical Pathology and Biochemistry Laboratory, Faculty of Medicine, University of Brawijaya.

Vaginal Smears Collection

After 30 days of treatment, a swab was performed to determine the rat oestrous cycle. Mice in the proestrus phase were terminated. The swab samples were stained with *methylene blue* and observed the morphology of the epithelial cells under a microscope (Sezer *et al.*, 2020).

Histological Examination

Number of Follicles. Ovarian samples were stained with *Hematoxylin Eosin (H&E),* criteria for follicular classification based on Myers *et al.* (2004). The results of the staining of the sample were then viewed under a microscope *Dot slide Olympus XC 10* and the number of follicles was calculated *using Dot Slide software.*

Data Analysis

Data were analyzed using software *SPSS* 25.0

RESULTS AND DISCUSSION

Note: The mean number of ovarian follicles based on the treatment group. On the mean ± SD, if it contains different letters, it means that there is a significant difference ($p \leq 0.05$) and if it contains the same letter, it means that there is no significant difference ($p \geq 0.05$).

The number of primordial follicles showed a significant result with p-value = 0.043. There was a decrease in the number of primordial follicles in the arsenic-exposed only group (2.4 ± 2.607^{a}) compared to the healthy or negative control groups (2.6 ± 1.949^{a}), but the decrease was not statistically significant. In the group of mice given Ajwa date extract at a dose of 4 ml/kg BW, there was a significant increase in the number of primordial follicles compared to the positive control group that was given arsenic alone.

The same thing happened to the number of primary follicles, all treatment groups showed significant results statistically with a value of p = 0.029. Although in the positive control group (2.4 ± 2.607[a]) there was a decrease in the number of secondary follicles, the decrease was not significant compared to the negative control group (2.6 ± 1,949[a]) statistically. Ajwa date extract caused a protective effect on the number of primary follicles in female rats exposed to arsenic with the highest dose of date extract, 8 ml/kg BW. In treatment group 3 (3.0 ± 1,000[ab]) there was a significant increase in the number of primary follicles compared to the arsenic exposed group (1.6 ± 0.547[a]).

In the number of secondary follicles, the group of mice exposed to arsenic (1.2 ± 1.095[a]) had a decrease in number but not significantly compared to the group of rats that were either healthy or not exposed to arsenic (2.4 ± 1.140[a]). In the treatment group given date palm extract 8 ml/kg BW or Treatment 3 (2.0 ± 0,707[a]) there was an increase in the number of secondary follicles compared to the positive control group (1.2 ± 1.095[a]), the mean value of the P3 group approached the negative control group (2.4 ± 1.140[a]).). However, there was an increase in the number of secondary follicles with a mean value that exceeded the group of healthy rats that occurred in the treatment group given date extract 4 ml/kg BW (4.2 ± 3,768[a]).

Then the number of early antral follicles also decreased the number of early antral follicles in the positive control group (0.8 ± 0.836[a]) compared to the negative control group/only given Normal saline (1.2 ± 0.447[a]), but the decrease was not significant. There were interesting results in the group of mice given date palm extract at a dose of 4 ml/kg BW (1.2 ± 0.446[a]) and 8 ml/kg BW (1.2 ± 0.447[a]) where the value of the number of early antral follicles increased, and the mean value was the same as healthy mice group (1.2 ± 0.447[a]), whereas, at the smallest dose of date extract, 2 ml/kg BW, there was an increase in the number of early antral follicles (2.0 ± 1.000[a]) compared to the mice exposed to arsenic alone.

In addition to the number of follicles mentioned in this study, researchers also counted the number of antral or *Graafian follicles* that was published. The results obtained were that there was a significant decrease in the number of *Graafian follicles* in mice exposed to arsenic and date palm extracts can increase the number of *Graafian follicles* in female rats exposed to arsenic (Kusumasari *et al*., 2020).

The decrease in the number of follicles in the group of rats exposed to arsenic in this study was the same as previous research, namely Mehta and Hundal (2016) that rats exposed to arsenic caused problems in folliculogenesis. The decrease in the number of follicles occurs due to the mechanism of arsenic toxicity in various ways so that it affects the disruption of folliculogenesis. One of them is arsenic exposure causes an increase in *Reactive Oxygen Species (ROS)* which results in oxidative stress on the ovaries (Flora, 2011; Sun *et al*., 2016). The increase in ROS is usually followed by an increase in lipid peroxidase and MDA which are biomarkers of oxidative stress. Homeostatic changes in the body, such as increased levels of ROS due to arsenic exposure, also alter the activity of antioxidant enzymes, which act as a first-line defence against free radicals. Inhibition of antioxidant enzymes will cause excessive levels of superoxide anions (Banerjee *et al*., 2014), excessive accumulation of superoxide anions can cause follicular regression (Khatun *et al*., 2018). Arsenic exposure also inhibits the peroxidase enzyme in the follicular fluid so that it interferes with folliculogenesis due to high levels of free radicals (Chattopadhyay and Ghosh, 2010). Apart from going through the ROS pathway, arsenic toxicity can damage DNA and damage ovarian cells (Akram *et al*., 2009). A drastic decrease in the number of follicles caused by environmental factors such as heavy metal exposure, namely arsenic, can

Table 1. Effect of Ajwa Dates Extract on Ovarian Follicles Count of Female Rats.

Follicle	Negative Control Mean±SD	Positive Control (arsenic) Mean±SD	Treatment 1 (arsenic+date extract 2 ml/kgBB) Mean±SD	Treatment 2 (arsenic+date 4 ml/kgBB) Mean±SD	Treatment 3 arsenic+date (8 ml/kgBB) Mean±SD
Primordial	2.6±1.949[a]	2.4±2.607[a]	3.0 ±2.645[a]	7.0±3.082[ab]	13.2±12.336[a]
Primer	2.6±1.516[a]	1.6±0.547[a]	1.2 ±0.447[a]	1.8±0.447[a]	3.0±1.000[ab]
Sekunder	2.4±1.140[a]	1.2±1.095[a]	1.2 ±0.836[a]	4.2±3.768[a]	2.0±0.707[a]
Early Antral	1.2±0.447[a]	0.8±0.836[a]	2.0 ±1.000[a]	1.2±0.446[a]	1.2±0.447[a]

result in premature menopause in women (Kumar *et al.*, 2012).

The effect of arsenic on the endocrine system is also one of the causes of disturbances in follicular development. Arsenic exposure indirectly interferes with the regulation of the ovarian pituitary axis so that it affects decreasing levels of the *Follicle Stimulating Hormone* (FSH) hormone and decreasing levels of the steroid estrogen hormone where the action of this hormone is needed in the folliculogenesis process. If there is inhibition of hormones that affect follicle development, it can result in low follicle regression and ovarian mass (Khatun *et al.*, 2018). Therefore, in this study, the group of mice exposed to arsenic alone had a decreased number of follicles compared to the healthy group of mice.

The preventive effect of Ajwa date fruit extract in this study is due to the antioxidant content contained in dates, namely melatonin, carotene, phenolic compounds, flavonoids and contains vitamins C, E and vitamin A. Phenolic compounds contained in dates are routine, catechin, *caffeic acid, gallic acid, quercetin, luteolin* and *apigenin* (Chaira *et al.*, 2007; Al-Farsi and Lee, 2008; Ben Abdallah *et al.*, 2009; Ali *et al.*, 2015; Eid *et al.*, 2015; Khalid *et al.*, 2017). Apart from phenolic compounds, a study conducted by Chao (2007) proved that dates contain estradiol.

The work of antioxidants in Ajwa date extract by inhibiting lipid peroxidase so that it can act as an antioxidant that stops reactions associated with oxidative stress (Zhang *et al.*, 2013). The antioxidants contained in Ajwa dates can stop the bonding reactions associated with oxidative stress by reducing the formation of ROS (Ben Abdallah *et al.*, 2009). The phenolic content of Ajwa dates, with their redox properties, can act as a *scavenger* ROS, neutralize free radicals, and break down peroxides (Kchaou *et al.*, 2014). So that in this study proved that Ajwa date extract had a protective effect on ovarian follicles in female rats exposed to arsenic.

CONCLUSIONS

In this study, it can be concluded that arsenic exposure can affect the number of ovarian follicles and Ajwa date extract which contains high antioxidants can protect ovarian follicles from damage caused by arsenic exposure.

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