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Effect of Water Extract of *Labisia pumila* Var *Alata* on Aorta of Ovariectomized *Sprague Dawley* Rats

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Abstract: *Labisia pumila* var *alata*, locally known Kacip Fatimah, is a traditional herb used by Malay women to induce and facilitate childbirth as well as to increase a woman's libido. This study was aimed to investigate the effect of *Labisia pumila* consumption on maintaining the integrity of the aortic wall in ovariectomized rats. Thirty-five adult female *Sprague Dawley* rats, 6 months old, were used in the experiment. Rats were divided into Normal (NOR) and ovariectomized (OVXC, KF and ERT) groups. Body weight was checked and recorded monthly. After four months, rats were sacrificed by cervical dislocation and tissue samples from the aorta were collected for histological studies. Results showed that water extract of *Labisia pumila*-treatment maintained the elastic lamellae architecture of the ovariectomized rat aortae in a manner comparable to that of the normal rats. Results implied a possible role for *Labisia pumila* in modulating postmenopausal cardiovascular risks.

Key words: Ovariectomy, *labisia pumila* var *alata*, aorta

Introduction

Cardiovascular disease remains the leading cause of death by a non-communicable disease (NCD) in Malaysia. According to a 2002 publication of the World Health Organization (WHO) it persistently accounts for 30% of deaths. Cardiovascular disease also represents the leading cause of death among women in Western societies. Statistics show that annually, more than 500,000 women in the United States die of cardiovascular disease with about half of these deaths are caused by coronary artery disease (CAD) (American Heart Association, 2000). Currently, postmenopausal women account for more than 30% of the female population at risk for CAD (Ariyo and Villablanca, 2002). Some research indicates decreased cardiovascular risk with hormone replacement therapy (HRT) (Grodstein *et al*., 1997).

The aorta stiffens naturally with age in a process that begins after menopause in women. Thus, aortic stiffness is among the list of cardiovascular risks (atherosclerosis, worsened arterial function and arterial degeneration) that most women face after menopause. In a review by (Anthony, 2002), aortic stiffness was reported to be associated with age, sex, heart rate, blood pressure, carbohydrate metabolism measures, adiposity, physical activity, smoking, and plasma lipid concentrations. It is also associated with indicators of atherosclerosis such as carotid artery intima media thickness. (Boutouyrie *et al*., 2002) reported that aortic stiffness is an independent predictor of primary coronary events in hypertensive patients. Thus aortic stiffness is presented as a useful measure of cardiovascular health and predictive of cardiovascular morbidity and mortality. In recent years phytoestrogens were found to have a potential role in postmenopausal women's health and became an area of intense research interest. Animal model research has shown that soy protein can inhibit atherosclerosis (Anthony *et al*., 1997; Clarkson *et al*., 2001 and Adams *et al*., 2002). Evidence from human research indicated that soy with isoflavones can improve endothelial-dependent vascular reactivity in postmenopausal women (Teede *et al*., 2001) and isoflavone pills improve arterial elasticity in postmenopausal women (Nestel *et al*., 1997; Nestel *et al*., 1999). Phytoestrogen intake was found to be associated with lower aortic stiffness in Dutch postmenopausal women (van der Schouw *et al*., 2002), although (Van der Schouw *et al*., 2005) reported no inverse association between phytoestrogen intake and cardiovascular disease risk.

Labisia pumila var *alata* is a traditional herb used by Malay women to induce and facilitate childbirth as well as to increase a woman's libido (Fig. 1). The herb is locally known as Kacip Fatimah and was reported to have estrogenic properties (Institute for Medical Research, [IMR], 2002). Theoretically, phytoestrogens can act as anti-estrogenic agents by blocking the estrogen receptors and exerting weaker estrogenic

Fig. 1: *Labisia pumila var alata*. Institute for Medical Research Nursery, Malaysia

effect compared with the hormone (Institute of Food Science and Technology, [IFST], 2001). Water extract of *Labisia pumila* was shown to be able to displace estradiol binding to antibodies raised against estradiol, making it similar to other estrogens such as estrone and estradiol, (Husniza *et al.,* 2000 as cited in IMR, 2002), The extract was found to produce a dose-response effect on the reproductive hormones of female rats, notably on the estradiol and free testosterone levels (IMR, 2002).

Objective of the study: *Labisia pumila* is widely used among women in Malaysia but to date there is little scientific information available about its effects and mechanism of action. The primary objective of this study was to determine the effect of water extract of *Labisia pumila* var *alata* consumption (as a drink) on the properties of the aorta of ovariectomized rats (OVX-rats) and to compare these effects to those exerted by Estrogen Replacement Therapy (ERT). This work has the approval of the Animal Ethics Committee at the Institute for Medical Research – Malaysia.

Materials and Methods

Preparation of water extract of *labisia pumila* var *alata*: The preparation of water extract of *Labisia pumila* var *alata* is done by subjecting the dried plant material to water to form a water-soluble extract and then desiccating the extract. The starting plant material is fully dehydrated by drying at 40°C for three days. Water at approximately 80°C is then used for the extraction process over a period of three hours and with continues stirring. The extraction is repeated with an equal volume of fresh water, i.e. two stages process, in the ratio of one part dried plant material and six parts water. Spray-drying method is then used to desiccate the extract. The tower inlet and outlet temperatures are set at 185°C and 107°C, respectively.

Animal and tissue preparation: All procedures were carried out in accordance with the institutional guidelines for animal research of the Institute for Medical Research-Malaysia. Thirty five adult female *Sprague Dawley* rats, six months old, were used in the experiment. The rats were housed at normal room temperature with adequate ventilation and normal 12-h light-dark cycle with free access to food (commercial laboratory rat food) and water and were divided into two main groups; Normal (NOR, n = 9) and ovariectomized. The ovariectomized divided in to OVX control (OVXC, n = 8), *Labisia pumila*-treated (OVX/KF, n = 9) and ERT-treated (OVX/ERT, n = 9). Bilateral ovariectomy was performed under anesthesia using a ventral approach. Rats were anesthetized with IM injection comprised of the following: Zoletil 50 (Virbac Laboratories, France) 0.1 ml, Ketamav 0.1 ml (MAVLAB, Australia), Xylazil 0.03 ml (Troy Laboratories, Australia). After ovariectomy, rats were randomly allocated to the nominated groups. Treatment started one month after OVX in the following manner; ERT (120 µg/kg/day) and standardized water extract (patent pending) of *Labisia pumila* (17.5 mg/kg/day), were given orally in the drinking water. After three months of treatment, rats were sacrificed by cervical dislocation. Tissue samples from the aortic arch were collected and fixed immediately in 10% formalin for light microscopy. Following 24 hours fixation, tissues were processed for paraffin embedding. 5µ thick paraffin sections of aorta were then obtained, dried overnight, dewaxed in xylene, dehydrated in series of alcohol to water and then stained in a jar with Verhoeff's haematoxlin. This staining procedure was described by Verhoeff in 1908 for staining of elastic tissue (Drury and Wallington, 1980). Finally; section were dehydrated in series of alcohol, cleared in xylene and mounted in Dibutyl Phathalate in Xylene [DPX] (BDH Laboratory Supplies, England).

Results

This study demonstrated the changes that take place in the aortic wall following ovariectomy (OVX). Light microscopy revealed different features between the NOR rats and the OVX-rats (OVX/C, OVX/ERT & OVX/KF). OVX induced morphological changes in the elasticity and thickness of the aortic wall, the latter probably resulting

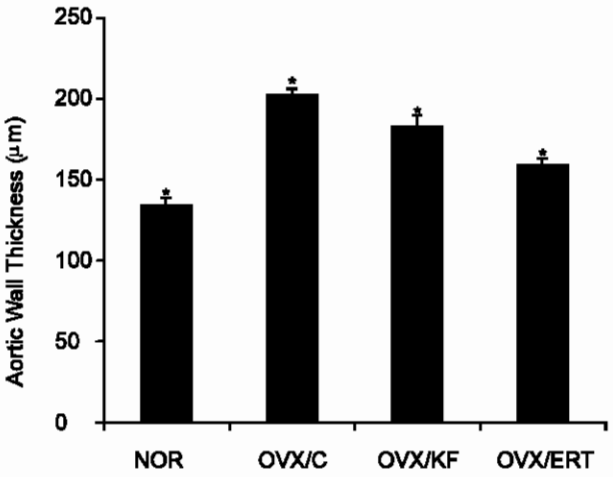

Fig. 2: Comparison between aortic wall thicknesses reported for each group. Values are mean ± standard error of the mean. The aortic wall of the ovariectomized control rats (OVX/C) as well as the treated groups (OVX/ERT & OVX/KF) were significantly ($P<0.05$) thicker that those of the normal control (NOR) rats. The aortic wall of the OVX/C was also significantly thicker than those of the treatment groups. Among the treatment groups, OVX/KF rats showed a significantly thicker aortic wall compared to OVX/ERT rats.

from the disrupted elastic lamellae architecture and thickened tunica adventitia layer. Increased aortic wall thickness of the OVX-rats compared to the NOR rats was found to be statistically significant at ($P<0.05$). The values recorded for each group were as follow: NOR (134.05 ± 3.39579 µm), OVXC (201.57 ± 4.02 µm), OVX/ERT (159.28 ± 2.85 µm) and OVX/KF (182.11 ± 5.88 µm). The increase in the aortic wall thickness of the OVX/C group was also significant when compared to the treatment groups with 20.98% and 9.65% difference compared to OVX/ERT and OVX/KF-treated groups respectively. The difference between OVX/ERT and OVX/KF-treated (14.33%) was also found significant. (Fig. 2) illustrates the values of aortic wall thicknesses reported for each group.

The tunica intima and tunica adventitia of the OVX/C rats were thicker than those seen in the other groups. Verhoeff's Haematoxylin stain revealed elastic lamellae architecture of the tunica media of the OVX/KF rats comparable to that of the NOR rats whereas that of OVX/C rats was found to be thick and the elastic lamellae architecture was disrupted. The elastic lamellae architecture of the ERT-treated rats showed some disturbance but not as great as that seen in the OVX/C rats. (Fig. 3) shows micrographs of the stained sections of the aortae of the four study groups.

Discussion

The first part of the present study demonstrated part of the cardiovascular changes that occur following OVX (surgical menopause), such as increased aortic wall thickness and deterioration of the elasticity of the aortic wall. A more elastic aorta is advantageous because it conducts blood smoothly from the heart and puts less stress on other organs. The mechanism through which menopause exerts its effect on the cardiovascular system remains unexplained. However, going through menopause has shown to negatively affect the elastic properties of the aortic root in hypertensive women (Karpanou *et al.*, 1996). The deterioration of the elastic lamellae architecture in the OVX-rats probably is related to the change in the hormonal homeostasis (Karpanou *et al.*, 1996) as a result of the removal of the ovaries. Although monitoring the blood pressure of the experimental animal was beyond the scope of the present study, it could not be ruled out that these animals developed hypertension as a consequence of OVX and weight gain. Hypertension was reported to provoke morphologic changes in the large arteries and to affect arterial distensibility in particular (McVeigh *et al.*, 1991 and Reneman *et al.*, 1992). Too, the effect of aging must be considered as aging is one of the factors that known to speed the aortic rigidity (Avolio, 1992) by inducing progressive distortion of the arrangement of elastin lamellae (O'Rourke *et al.*, 1993). Thus, ovariectomy-induced estrogen deficiency, hypertension and the aging process are all suggested to have contributed to the detrimental changes in the elasticity of the aortic wall.

This study demonstrated a possible role for *Labisia pumila* in opposing the effects produced by OVX similar to that reported for ERT. Aortae of the *Labisia pumila* and ERT-treated rats showed normal elastic lamellae architecture comparable to that of the normal rats, especially with *Labisia pumila* treatment. The results of this study are consistent with previous research that demonstrated the effect of estrogen and phytoestrogen treatments. Animal research has also shown that ERT has a vasodilatory effects as well as an effect on the structure and mechanical properties of large arteries (Magness and Rosenfeld, 1989; Williams *et al.*, 1990 and Jiang *et al.*, 1991). Phytoestrogen intake was also reported to be associated with lower aortic stiffness (van der Schouw *et al.*, 2002) thus it would be possible to apply the same concept to *Labisia pumila*, based on the estrogenic activity of the extract. As estrogen is known to have cardio-protective effects in postmenopausal women (Skafar *et al.*, 1997), it is possible to hypothesize that *Labisia pumila* may have similar cardio-protective effects.

Our results are also consistent with previous studies that investigated the regulation of endothelial and vascular smooth muscle mechanisms by estrogen. (Li,

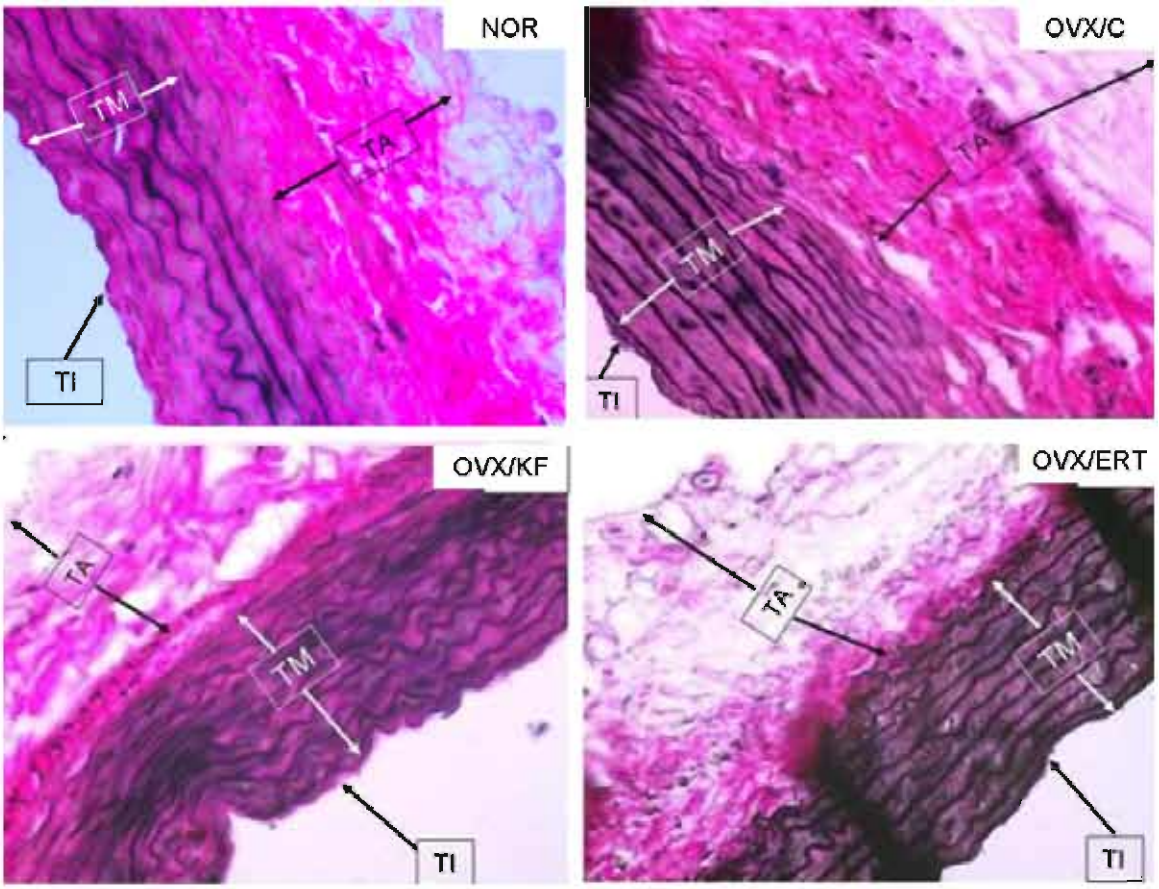

Fig. 3: Micrographs (Verhoeff's Haematoxylin & Van Gieson stain x 40) of Aortae of NOR, OVX/C, OVX/KF & OVX/ERT rats. (TI; Tunica Intima, TM; tunica Media, TA; Tunica Adventitia). Tunica media of the *Labisia pumila*-treated (OVX/KF) rats showed normal elastic lamellae architecture compared to that of the NOR rats while that of OVX/C and ERT-treated rats are thick and their elastic lamellae architecture are disrupted.

2004) reported that OVX markedly attenuated the contractile responses to vasopressin (VP) whereas three to four weeks of estrogen replacement therapy with 17β-estradiol completely restored the contractile responses to VP. It is thought that the disrupted elastic lamellae architecture observed in the aorta of the OVX/C rats explains the decrease in the contractile response to VP reported by (Li, 2004) in OVX-female rats, whereas the reverse is true for the *Labisia pumila* and ERT and 17β-estradiol treatments. Knowing that *Labisia pumila* has estrogenic activity, it is logical to relate the effect observed here to the same reason and suggest that *Labisia pumila* may potentiate the contractile responses to VP. These findings are also consistent with previous studies that demonstrated the capability of phytoestrogens to interact with the estrogen receptor and exert estrogen-like effects (Adlercreutz and Mazur, 1997; Clarkson *et al.*, 1995; Dubey *et al.*, 1999).

Studies have reported an association between aortic stiffness with indicators of atherosclerosis such as carotid artery intima media thickness (Mackey *et al.*, 2002 and van Popele *et al.*, 2001). Relating the findings of the present study to these reports suggest a possible role for *Labisia pumila* in treating menopausal-induced aortic stiffness. In a conclusion, both *Labisia pumila* and ERT were able to maintain the integrity and the morphology of the aortic wall. Results indicate a possible role for *Labisia pumila* var *alata* in modulating postmenopause cardiovascular risks in a similar manner to that know about estrogen.

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