




Original Research Report

THE EFFECTS OF PURPLE SWEET POTATO (*Ipomoea batatas* L.) ETHANOL EXTRACT ON BLADDER UROTHELIAL LAYER AND SMOOTH MUSCLE THICKNESSES IN MENOPAUSAL FEMALE WISTAR RATS**Bismantara A. Putra^{1*}, Kadek Budi Santosa², I Wayan Nirvana³ , Nyoman Golden³ , Gede Wirya Kusuma Duarsa² , Ida Bagus Made Suryawisesa⁴**

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ABSTRACT

Postmenopausal women experience estrogen hormone deficiency, which can cause thinning of the smooth muscle and urothelial layer of the bladder, leading to lower urinary tract symptoms (LUTS). Hormone replacement therapy (HRT) has been the primary choice for addressing these problems. However, long-term prescription of HRT can result in several adverse effects, including a higher risk of breast cancer and cardiovascular diseases. Phytoestrogen, an estrogen-like compound derived from plants such as purple sweet potatoes (*Ipomoea batatas* L.), contains anthocyanin that could serve as a better alternative to estrogen replacement therapy. This study aimed to demonstrate the effects of phytoestrogens in purple sweet potatoes on the histomorphology of the bladder in menopausal female Wistar rats (*Rattus norvegicus*). This experimental study used a posttest-only control group design. A total of 36 Wistar rats undergoing ovariectomy were randomly assigned into two groups, with 18 samples in each group. The experimental group received the purple sweet potato ethanol extract orally, while the control group received a placebo. The structures of the smooth muscle and urothelial layer of the bladder were observed using a microscope. Data were analyzed using an independent t-test to compare bladder smooth muscle and urothelial layer thickness between groups, with a significance of $p < 0.05$. A normality test was performed to determine the normal distribution of the data. Normally distributed data were assessed to find the mean and standard deviation (SD). Significant differences were found in the mean thickness of the smooth muscle and urothelial layer between both groups ($p = 0.00$), with both being thicker in the experimental group. In conclusion, phytoestrogens in purple sweet potatoes can influence the histomorphology of the bladder. Ovariectomized female Wistar rats that received purple sweet potato ethanol extract exhibited thicker smooth muscle and urothelial layer of the bladder.

Keywords: Purple sweet potatoes; smooth muscle thickness; postmenopause; lower urinary tract symptoms; human & health

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Highlights:

1. Purple sweet potatoes cultivated in Bali, Indonesia, were found to be rich in phytoestrogen due to the high levels of anthocyanin-type flavonoids.
2. The phytoestrogen in purple sweet potato ethanol extract demonstrated estrogenic activity and the potential to substitute hormone replacement therapy (HRT) as the treatment of lower urinary tract symptoms.

INTRODUCTION

Menopause is one of the changes that older women experience due to the inability of ovarian follicles to produce estrogen and inhibin, resulting in various changes in organ structure and function as well as an increased risk of developing chronic diseases. One of the hypoestrogenism symptoms during menopause that greatly interferes with quality of life is lower urinary tract symptoms (LUTS), which includes stress or urge urinary incontinence, nocturia, a weak urine stream, straining, urinary hesitancy, and terminal dribbling, among others (Azadzi & Siroky 2013, Varella et al. 2016). A decreasing estrogen level in postmenopausal women will cause cell apoptosis, which leads to atrophy of the smooth muscle and urothelial layer in the bladder. Atrophy of smooth muscle will reduce the contractility of the bladder and also reduce the thickness of the urothelial layer (Birder et al. 2012, Robinson et al. 2013).

Since all of the clinical symptoms are caused by a decrease in estrogen levels, the most rational treatment is estrogen-based hormone replacement therapy (HRT). Several studies have shown that HRT effectively eases lower urinary tract symptoms in menopausal women (Lobo et al. 2014). However, even though HRT provides excellent benefits, more than 80% of women who have received it do not intend to continue the treatment due to various medical reasons. Side effects of HRT include an increased risk of blood clots, liver disease, and cancer. Since HRT carries several potential complications, it is necessary to search for an estrogen substitute (Bedell et al. 2014).

A number of plants produce naturally-sourced compounds that may serve as a substitute for estrogen. One of the compounds is phytoestrogen, which has estrogen-like effects without the harmful side effects of estrogen (Desmawati & Sulastru 2019). Phytoestrogens are divided into four types: isoflavones, flavonoids, stilbenes, and lignans. Because of their similar molecular structure to estrogen, flavonoid compounds can bind to and activate estrogen receptors on target cells. Several phytoestrogens have been found to bind to both types of estrogen receptors, i.e., estrogen receptor beta (ER β) and estrogen receptor alpha (ER α). The binding of phytoestrogens to receptors can induce the expression of estrogen-responsive genes and trigger cell proliferation (Sugiritama 2020).

Purple sweet potatoes cultivated in Bali, Indonesia, are potentially useful as a source of phytoestrogens due to their high content of anthocyanin-type flavonoids. The results of a preliminary study revealed that purple sweet potato ethanol extract given to menopausal animal models was able to

increase ER α and ER β messenger ribonucleic acid (mRNA) expression at an optimal dose of 4 mL per day. Compared to the control group, female rats that underwent bilateral ovariectomy and received purple sweet potato ethanol extract had thicker vaginal epithelium and a better degree of maturation (Yuwono et al. 2018, Adnyana et al. 2019). The purpose of this study was to prove the effects of purple sweet potato ethanol extract on the bladder histomorphology of menopausal animal models. The effects were analyzed by measuring the thickness of the smooth muscle and urothelial layer of the bladder.

MATERIALS AND METHODS

This was an experimental study with a posttest-only control group design. This study was conducted in the Histology Laboratory, Faculty of Medicine, Universitas Udayana, Denpasar, Indonesia, between April and November 2022. We received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Udayana (No. 182/UN14.2.2.VII.14/LT/2022 on 2/1/2022). This study utilized healthy female Wistar rats (*Rattus norvegicus*). The number of samples was determined by the Federer formula (Dharmawati et al. 2019). According to the formula, 36 rats weighing 200–250 g, aged 10–12 weeks, and undergoing ovariectomy were used as samples.

Ovariectomy (OVX), or the removal of the ovaries, started with the induction of anesthesia. Isoflurane with a 5% concentration was used for the induction, and it was maintained at a 2.5% concentration. Coaxial nose cones were used to deliver oxygen. We performed bilateral ovariectomy using a double dorsolateral approach (Sophocleous & Idris 2019, Souza et al. 2019). The rats were anesthetized and fixed with plaster. The rats' dorsal ridges were shaved bilaterally. As the ovaries were located on both sides of the abdomen and below the kidneys, we incised the skin medially to the most prominent dorsal region. In young or thin rats, a bulge that could indicate the incision site might not be visible. Therefore, the incision site was found by placing a thumb on the uppermost proximal thigh. The base of the distal phalanx was incised medially. In order to show the dorsolateral abdominal muscles, such as the external oblique muscle, a 1.5 cm area of the skin was incised. By dissecting the muscle, the adipose tissue surrounding the ovary was revealed. As a result, it created an entrance to the peritoneal cavity (Souza et al. 2019, Setiawan et al. 2022). In order to prevent the detachment of small ovarian fragments, fat around the incision site was carefully removed. After identifying the ovary and uterine horn, the ovarian tissue was removed in a single step by ligating the distal horn. The rats' uterine horns,

muscles, and skins were sutured at the end of the procedure (Khajuria et al. 2012).

A total of 36 rats were randomly divided into two groups of 18 rats. The experimental group received 4 mL of purple sweet potato ethanol extract orally per day, while the control group received a saline solution. The purple sweet potato ethanol extract contained 119 mg/mL of anthocyanins (Setiawan et al. 2022). Rats that died or became ill were dropped out of the experiment.

Locally grown purple sweet potatoes (*Ipomoea batatas* L.) were used as the source material for the ethanol extract in this study. After peeling the skin, the sweet potatoes were transversely cut into thick slices of 2–2.5 cm. One kg of sweet potatoes was soaked in 1 L of 90% liquid before being filtered through three layers of gauze. The next step was boiling the filtered liquid (Sugiritama 2020). This material contained 119 mg/mL of anthocyanin. After successfully producing the ethanol extract, 1 mL of distilled water was added to the extract for each rat. Spectrophotometer measurements indicated anthocyanin absorption at 520 and 720 nm. The fresh purple sweet potatoes had significant quantities of anthocyanin.

All post-ovariectomy procedures lasted 30 days. The rats were terminated at the conclusion of the experiment. Histological preparations were made from a 1×1 cm segment of the rats' bladder and stained with hematoxylin and eosin. The bladder's smooth muscle and urothelial layer thicknesses were measured using an Olympus CX41 microscope (Olympus Corp., Japan) and an Optilab camera (Optilab, Indonesia) with 400X magnifying power. The measurement points were from the base to the top of the smooth muscle and urothelial layer (Zderic & Chacko 2012). Measurements were expressed in micrometers (µm).

Data obtained from the experiment were analyzed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). We conducted a normality test to determine the normal distribution of the data. Numerical data with a normal distribution were provided as mean±standard deviation (SD). We also conducted an independent t-test to compare the thickness of the bladder smooth muscle and urothelial layer between the two groups. A p-value of <0.05 was considered significant (Hazra & Gogtay 2016).

RESULTS

A normality test was performed to analyze the data. All variables showed a normal distribution, as indicated by the mean and standard deviation. The

results of the analysis are shown in Table 1.

Figure 1 shows the smooth muscles as well as the urothelial layers of both the control group and the experimental group. When compared to the control group, the smooth muscles and urothelial layers were found to be significantly thicker in the experimental group.

Table 1. Normality test results of the control and experimental groups.

Variables	Mean (µm)	SD	Normality
Urothelial layer thickness			
Control group	7.78	7.62	0.13
Experimental group	40.56	17.88	0.53
Smooth muscle thickness			
Control group	27.08	12.93	0.53
Experimental group	48.13	17.45	0.65

Notes: SD=Standard deviation

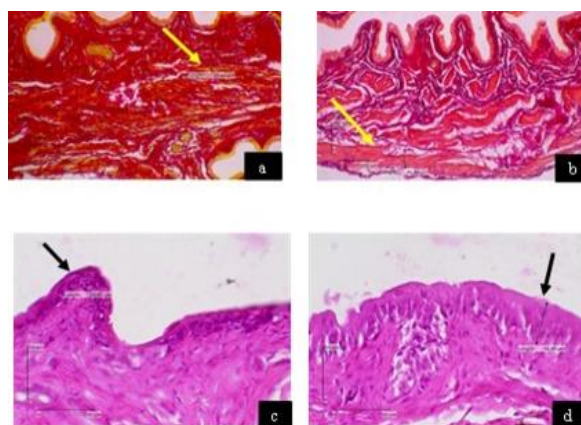


Figure 1. Histological picture of the result of the study using a microscope with 400x magnification. (a)Smooth muscle of the control group; (b)Smooth muscle of the experimental group; (c)Urothelial layer of the control group; (d)Urothelial layer of the experimental group.

An analysis using an independent t-test was performed to determine the statistical significance of the differences in smooth muscle and urothelial layer variables between the control and experimental groups. The results revealed that there was a statistically significant difference in the

bladder smooth muscle and urothelial layer thickness between the two groups ($p < 0.05$). The p-values for both variables were 0.00, indicating statistically significant differences in smooth muscle and urothelial layer thickness between the control group and the experimental group (Table 2).

Table 2. Independent t-test results between the control and experimental groups.

Variables	95% CI	p
Urothelial layer thickness		
Control group		
Experimental group	-29.67 – (-10.72)	0.00
Smooth muscle thickness		
Control group		
Experimental group	-31.45 – (-10.65)	0.00

Notes: CI=Confidence interval

The results of the analysis indicated that the experimental group had significantly thicker smooth muscle and urothelial layer compared to the control group. These findings suggested that the administration of purple sweet potato ethanol extract had effects on the thickness of these layers.

DISCUSSION

Our experiment aimed to determine the effects of phytoestrogens on the histomorphology profile of ovariectomized rats' bladders. The experiment was focused on the smooth muscle and urothelial layer of the bladder. Ovariectomized rats have been used as an established menopausal model in research (Rejeki et al. 2018). The female lower urinary tract has been reported as a target organ for the action of sex steroid hormones. The bladder urothelium and smooth muscle are sensitive to changes in circulating estrogen, so ovariectomy can induce significant urothelial and smooth muscle atrophy (Liang et al. 2013). In an observation two weeks following ovariectomy, the amount of circulating estradiol decreased by 50%. The estradiol concentration remained relatively constant for six weeks after the ovariectomy (Malone et al. 2014). In this study, the optimal time to begin intervention was determined to be two weeks after ovariectomy.

The effects of phytoestrogen on the menopausal bladder were still unclear. Our study provides valuable information on the histological measurement of smooth muscle and urothelial layer thickness after the administration of phytoestrogen (Figure 1). Yuwono et al. (2018) assessed the effects of phytoestrogen on the female genitalia of menopausal rats. They found that intervention using purple sweet potato ethanol extract enhanced epithelial maturation and differentiation and

increased epithelial thickness in rats that underwent oophorectomy. In this current study, we found significant differences in the bladder urothelial layer and smooth muscle thickness between the two groups, with the experimental group having a higher mean thickness.

In this study, the thickness of bladder layers increased despite phytoestrogen being a non-steroidal polyphenolic compound derived from plants. Phytoestrogen can carry out the same biological function as estrogen. It has been suggested that purple sweet potato ethanol extract contains phytoestrogen compounds and has potential estrogenic activity. Orally administered purple sweet potato ethanol extract undergoes intestinal metabolism to be converted into an active substance by the normal intestinal flora (Sugiritama et al. 2022). This compound resembles estrogen structurally, though its estrogenic activity is slightly lighter.

Phytoestrogen enters the bloodstream and binds to estrogen receptors in the bladder cells. In the present study, phytoestrogens were found to bind to urothelial cells and smooth muscle. The phytoestrogen content selectively modulates the estrogen receptor. After binding to the estrogen receptor, it modulates transcription and gene expression to induce changes in cell physiology. This will trigger the proliferation of cells and increase the thickness of the cell layer (Tao et al. 2022). Smooth muscle cell proliferation and migration are crucial events in the pathophysiology of vascular diseases. In ovariectomized mice, the aortic tissue sections stained with hematoxylin and eosin exhibited increased wall thickness and vascular smooth muscle hyperplasia (Yuwono et al. 2018, Sugiritama et al. 2022).

Several studies have suggested that the thickening of the bladder smooth muscle can increase its contractility. There is a strong correlation between smooth muscle contractility and urethral resistance, both of which play an essential role in the mechanism of lower urinary tract symptoms. When distention of the bladder wall occurs, the smooth muscle stimulates the spinal cord and pons for sympathetic inhibition, somatic nerve inhibition, and parasympathetic stimulation (Zderic & Chacko 2012). The voiding phase begins when the rhabdosphincter and bladder neck relax while the smooth muscle contracts and urethral resistance decreases, allowing urine to be expelled. Smooth muscle contraction is a factor that plays a vital role in the micturition mechanism. If the contraction is inadequate, the micturition process will be disrupted, resulting in lower urinary tract symptoms, especially voiding symptoms. By increasing the thickness of the smooth muscle layer, it is

anticipated that the lower urinary tract symptoms will become less severe (Aikawa et al. 2003, Valentini et al. 2016).

Urothelium consists of cells with a variety of functions. It separates the contents of the bladder from the underlying lining. It also functions as a sensory organ that conveys chemical and physical stimulation to the afferent nervous system and underlying smooth muscle (Birder et al. 2012). Changes in the urothelium can influence the growth and function of smooth muscle cells and afferent nerves, resulting in lower urinary tract symptoms. Numerous studies have demonstrated that a thin urothelial layer is a risk factor for lower urinary tract symptoms, including overactive bladder and bladder pain syndrome (interstitial cystitis) (Birder & Andersson 2013).

Reduced permeability caused by a thin urothelium can increase the likelihood of water, urea, and other harmful substances being present in the urine and infiltrating the underlying tissue, including tissues in the nerves. This will increase the incidence of bladder inflammation and cause lower urinary tract symptoms (Dalghi et al. 2020). As the urothelial layer normally responds to stimuli from the outside of the cell, the thinning of the urothelial layer diminishes sensory function. When the urothelium is stimulated, it releases molecular and cellular mechanisms that enable it to detect the stimulus. This results in the modulation of smooth muscle contraction and relaxation, which is crucial to the micturition mechanism. The urothelium releases various mediators, such as adenosine triphosphate (ATP), acetylcholine, prostaglandins, nitric oxide, and nerve growth factor (Birder et al. 2012, Keay et al. 2014, Sellers et al. 2018). It is anticipated that by increasing the thickness of the bladder smooth muscle and urothelial layer, lower urinary tract symptoms will improve. Further research is required to support the evidence that increasing the thickness of both the smooth muscle and urothelial layer can alleviate or prevent lower urinary tract symptoms in menopausal women.

Strength and limitations

This study can demonstrate the potential of purple sweet potato ethanol extract as a natural source of phytoestrogen. Therefore, it can be used as an alternative to hormone replacement therapy (HRT) for treating lower urinary tract symptoms. The phytoestrogen in purple sweet potato ethanol extract has the ability to increase smooth muscle and urothelial layer thickness. However, due to the short duration of this study, it might not be able to demonstrate the long-term effects or potential side effects associated with prolonged use of purple sweet potato ethanol extract.

CONCLUSION

This study provides strong evidence that phytoestrogens in purple sweet potatoes can influence the histomorphology of the bladder. The intervention of orally administering the purple sweet potato ethanol extract resulted in increased smooth muscle and urothelial layer thickness in the experimental group compared to the control group of menopausal female rats.

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Conflict of interest

None.

Ethical consideration

This study received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Udayana, Denpasar, Indonesia, with registration No. 182/UN14.2.2.VII.14/LT/2022 on 2/1/2022.

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None.

Author contribution

BAP contributed to the conceptualization and study design. BAP, KBS, and IWN contributed to the methodology, data curation, investigation, and original draft preparation. NG, GWKD, and IBMS contributed to the investigation, original draft preparation, and supervision.

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