# TALINUM PANICULATUM (JACQ.) GERTN: A MEDICINAL PLANT WITH POTENTIAL ESTROGENIC ACTIVITY IN OVARIECTOMIZED RATS

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## ABSTRACT

Objective: *Talinum paniculatum* (Jacq.) *Gertn* (*T. paniculatum*), is extensively used in Asian traditional medicine as a reproductive tonic. However, there is no conclusive scientific data to support this practice. This study was conducted to explore the possible estrogenic activity of *T. paniculatum* extracts compared with 17ß-estradiol (E$_2$) in adult bilaterally ovariectomized (OVX) rats for the experimental model of menopause.

Methods: OVX female Wistar rats were randomly divided into six groups. The first group was orally treated with sesame oil as a vehicle control. The other six groups were treated with different treatments consisted of the groups which were treated by a positive standard control of 17ß-estradiol (10µg. kg$^{-1}$BW), *T. paniculatum* root or leaf extract at two different doses (100 and 1,000 mg.kg$^{-1}$BW) for 42 consecutive days, respectively. Estrogenic activity was evaluated by determining the vaginal cornification, relative uterine weight (%RU), relative mammary weight (%RM), and their histoarchitecture changes.

Results: *T. paniculatum* root and leaf extracts significantly increased the vaginal cornification ($P<0.001$). Compared to OVX control, a dose dependency response of %RU, %RM and proliferative changes in vagina, uterus and mammary ductular tissue were significantly observed in OVX treated with all dosages of *T. paniculatum* from root and leaf extracts ($P<0.05$).

Conclusion: *T. paniculatum* extracts possess estrogenic activity in the OVX rats, which can be helpful in managing reproductive tissues regression during menopause in a natural way through herbal resources without any toxicity.

Keywords: *Talinum paniculatum* (Jacq.) Gertn; Estrogenic activity; Ovariectomized rat; Vaginal cornification; Uterus; Mammary gland

## INTRODUCTION

The reproductive disorder has always been the critical issue for women during menopause. Over 70% of menopausal women experienced undesirable urogenital degenerations that include uterine atrophy and vaginal dryness[1]. These complications lead them to depend on hormone replacement therapy, leading to undesirable side effects[2,3]. The substitutions of medicinal herbs or phytoestrogenic substances are not as persuasive in the estrogenic property as the classical synthetic estrogen, but they are safer in terms of undesirable side effects. *Talinum paniculatum* (Jacq.) Gertn (*T. paniculatum*) or "*Som Java*" is one of the plants in Portulacaceae family that contains notable medicinal properties[4]. *T. paniculatum* is a wild deciduous perennial herb with well-developed root system. It is naturally grown around the world, including Thailand with the local name of Wan Pak Pang. In Thailand, the locals consume the leaf as vegetable supplement and the roots as reproductive tonic. Preparation of *Talinum* spp. has long been used in ancient traditional medicine, particularly in the treatment of type-2 diabetes, inflammatory skin problems, gastrointestinal disturbance, general weakness and reproductive disorders[5,6,7]. The root has active constituents such as steroidal saponins, polyphenols and essential oil[8]. Additionally, Ramos and colleagues isolated and reported that compesterol, β-sistosterol, stigmasterol could be extracted from the leaf of *T. paniculatum*[9].

To our knowledge, the plants in *Talinum* spp. were recognized for their some constituents and biological properties and extensively used in Asian traditional medicine as a reproductive tonic, however, *T. paniculatum* still lacks of scientific data to clarify its estrogenic property. Therefore, this study was designed to evaluate the estrogenic activity of *T. paniculatum* extracts by observing the estrogen-responsiveness parameters. The observation included the relative of vaginal cornification, uterus or mammary weight to body weight, and the histological structure changes in female reproductive organs by using the rodent ovariectomy as an animal model of menopause[10].

## MATERIALS AND METHODS

### Plant material collection and identification

The plants *T. paniculatum* were collected from northeastern area of Thailand, where they grew under natural conditions in November 2010. Voucher specimen was identified and deposited at the Royal Forest Department of Thailand, Bangkok, Thailand (BKF174387).

The powder of root or leaf (10 g) was separately extracted with methanol in a Soxhlet apparatus for 12 h. The extracts were evaporated to dryness under a reduced pressure at low temperature in a rotary evaporated, dried by a freeze dryer and stored at -20°C until use. The yield of the root and leaf extract was 6.67% and 9.62%, respectively.

### Phytochemical screening

Phytochemical screening of the crude extracts were analyzed using GC-MS (A Agilent Technologies 7890A gas chromatograph, coupled with an Agilent Technologies 5975C (EI) mass spectrometer). The separation was performed on an HP-5MS column; 30 m x 0.25 mm ID x 0.25 mm film thickness. The temperature of the column was programmed from 50 °C to 300°C at 10°C /min. The injector temperature and the detector temperature were 250°C. Helium was used as the carrier gas with a constant flow rate of 1.0 µL/min. All separated compounds were identified from the recorded mass spectra by comparison with the mass spectra from the NIST and Wiley libraries.

### Animals and treatments

Animal care, environmental conditions and use followed the guidelines of Laboratory Animal Resources, National Research Council of Thailand. The procedures of the experiment were approved by the Institutional Animal Care and Use Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Ovariectomized (OVX) female Wistar rats (200-250g) were randomly divided into six groups. A period of 14 days was allowed for wound healing and acclimatization prior to treatment. OVX negative control group was orally treated with 10% (v/v) Tween 80 combined with sesame oil as a vehicle control. The other five groups were treated with different treatments consisted of the groups which were treated by a positive standard control of 17ß-estradiol (10µg.kg$^{-1}$BW), *T. paniculatum* root or leaf extract at two different doses (100 and 1,000 mg.kg$^{-1}$BW, respectively) for 42 consecutive days.

**Determination of body weight and relative uterine or mammary weight**

Rats were humanely sacrificed at the end of the experiment by $CO_2$ asphyxia. All connective tissues were removed prior to wet weight recording. Body weight, horns of uteri and inguinal mammary tissue weights were recorded. Relative uterine and mammary weights were calculated by this following formula:

Relative uterus (%RU) or mammary weight (%RM) =

$$\frac{uterus\ or\ mammary\ weight\ (g) \times 100}{body\ weight\ (g)}$$

**Vaginal cornification assay**

Vaginal smear was performed to examine cellular differentiation and to evaluate the presence of leukocytes, nucleated cells, or cornified cells. Vaginal smear samples were collected between 9:00-10:00 am daily by gently inserting the tip of dropper into the vagina, flushing normal saline (0.9% NaCl) in and out, and placing the fluid onto microscope slides and stained by Methylene blue dripping[11]. The appearance of cornified cells was used as an indicator of estrogenic activity and percentage of cornified cells was evaluated using the following formula:

Percentage of cornified cell =

$$\frac{cornified\ cells \times 100}{cornified\ cells + nucleated\ cells + leucocytes}$$

**Histological analysis**

After fixation, the horns of rat uteri, vagina and mammary tissue were cut into short segments using the paraffin technique. Sections of 5 μm thicknesses were cut using routine hematoxylin and eosin (H&E) method.

All organs were observed and measured on H&E stained slides, 3 randomly chosen areas of the section were measured per slide. Images of organ cross-sections (n=3) were taken using a Nikon Eclipse 80i Upright microscope (Hollywood International Co., Ltd., Thailand) and Cell^D imaging software (Olympus, EforL International Co., Ltd., Thailand). The number, thickness, size of the organs and epithelial lining were analyzed by using Image J v1.41 software[12].

**Statistical analysis**

All data are expressed to the mean value + standard error of the mean (SEM). Statistical analysis of difference was carried out by analysis of variance (ANOVA) by using SPSS windows program version 11 (SPSS Institute, Inc., Chicago, IL, USA). A probability level less than 5% ($P<0.05$) was considered statistically significant.

**RESULTS**

**GC/MS analysis**

The GC/MS analysis of root extract showed 5 phytosterols that had retention times (minutes) of 50.48(17.37%), 49.75(4.23%), 50.60(4.10%), 49.88(1.84%) and 49.35(1.56%), which are corresponded to β-sitosterol, stigmasterol, stigmastan-3-ol-(3β, 5α, 24S), stigmast-22-en-3-ol and campesterol, respectively. 13 other known compounds, mainly fatty acid (0.50%-11.32%), and 2 unknown compounds were detected.

The leaf extract showed 4 phytosterols that had retention times (minutes) of 50.49(10.60%), 50.61(2.76%), 49.76(0.85%) and 49.36(0.80%) which are corresponded to β-sitosterol, stigmastanol, stigmasterol and campesterol, respectively. 12 other known compounds, mainly phytol (69.31%), α-tocopherol (0.99%), fatty acid (0.43-3.41%) and 2 unknown compounds were identified (data not shown).

**Body weight and relative uterine and mammary weight changes**

42 days after ovariectomy, the means of body weight were not different among the groups. All rat groups did not show any data of acute toxicity of abnormal clinical signs or death during 42 day treatment period.

At the end of the experiment, the results showed that bilateral OVX enhanced sustained increases in final body weight, but decreased the percentages of relative uterine weight (%RU) when compared to $E_2$ positive control group ($P<0.05$).

As illustrated in Table 1, the mean %RU of OVX vehicle control group was significantly reduced by approximately 2- to 3-fold when compared with positive $E_2$ control group ($P<0.05$). Feeding with the root extracts (100 and 1,000 mg.kg⁻¹BW) and low dosage of leaf extract (100 mg.kg⁻¹BW) did not show significant effects on the %RU as compared to OVX vehicle control. The group treated by $E_2$ and 1,000 mg.kg⁻¹BW of leaf extract showed significant increases in %RU ($P<0.05$, n=5).

The effect of the plant extracts on mammary tissue was also evaluated by observing the percentage of relative mammary wet weight (% RM) in OVX rats. Atrophic mammary mass was present in OVX-received vehicle control rats whereas, the administration of $E_2$ intensely increased %RM ($P<0.05$). Experimental data showed that the treatment of OVX rats for 42-d with 1,000 mg.kg⁻¹BW of root and leaf extracts prevented mammary tissues regression. They exhibited the significantly greater %RM than those in the OVX negative control group ($P<0.05$). Additionally, rats fed by 100 mg.kg⁻¹BW of leaf and root extract showed slightly increases in %RM compared with OVX rats fed with vehicle control (Table 1).

**Table 1: Effect of *T. paniculatum* extracts on body weight and relative uterine and mammary weight changes.**

| Group | Treatment | Dosage | Initial body weight (g) | Final body weight (g) | Relative uterine weight (%RU) | Relative mammary weight (%RM) |
|---|---|---|---|---|---|---|
| 1. | Vehicle control | 1 ml/rat/day | 205.00+3.61ᵃ | 266.00+2.17ᶜ | 0.17+0.02ᵃ | 0.22+0.01ᵃ |
| 2. | 17ß-estradiol | 10 µg.kg⁻¹BW | 201.00+2.86ᵃ | 233.00+2.79ᵃ | 0.41+0.01ᵇ | 0.86+0.01ᶜ |
| 3. | *T. paniculatum* root extract | 100 mg.kg⁻¹BW | 204.00+3.63ᵃ | 258.00+0.94ᵇᶜ | 0.15+0.01ᵃ | 0.21+0.01ᵃ |
| 4. | *T. paniculatum* root extract | 1,000 mg.kg⁻¹BW | 206.00+3.11ᵃ | 255.60+1.25ᵇᶜ | 0.14+0.01ᵃ | 0.30+0.01ᵇ |
| 5. | *T. paniculatum* leaf extract | 100 mg.kg⁻¹BW | 200.00+2.55ᵃ | 258.00+1.14ᵇᶜ | 0.16+0.01ᵃ | 0.28+0.01ᵃᵇ |
| 6. | *T. paniculatum* leaf extract | 1,000 mg.kg⁻¹BW | 204.00+2.28ᵃ | 244.60+2.86ᵃᵇ | 0.33+0.00ᵇ | 0.35+0.01ᵇ |

All values represent as mean + SEM.; n is the number of animals. Means with different superscripted letters in the same column indicate statistical significance ($P<0.05$).

**Vaginal cornification**

Estrogenic activity of *T. paniculatum* extracts were evaluated through the vaginal cytology by comparing to standardized $E_2$ administration as the positive control. Cornified cells could not be observed in the vaginal smear obtained from all OVX rats on day 14 after the operation. This result confirmed the menopausal pattern with atrophic vaginal epithelium as characterized by vaginal smear consisting of parabasal cells, leukocytes and nucleated epithelial cells.

All vaginal smears obtained from OVX rats are shown in Fig. 1. During the experimental period, the vaginal smear of OVX control group did not show any cornified cell. The persistent estrous stage was detected in the group-treated with $E_2$ and all doses of *T. paniculatum* root and leaf extracts. The mean percentage of vaginal cornification obtained from the plant extract treated groups significantly increased as a dose dependent manner, but they were lesser than those of positive $E_2$ control groups ($P<0.05$). The high dose of root and leaf extracts (1,000 mg.kg⁻¹BW) provoked a

significant differentiation of vaginal epithelial cells to the exfoliated cornified cells in the smear compared to the OVX control (*P<0.05*). The oral administration of *T. paniculatum*'s root and leaf extracts at the dose of 100 mg.kg$^{-1}$BW for 42 days could slightly induce the cornification in OVX rats as shown in Table 2.

Fig. 1 demonstrates changes of vaginal cytology on day 21 after the operation. The OVX group treated with vehicle control showed only parabasal cells and lymphocytes. All groups received *T.paniculatum* root and leaf extract illustrated the cornified cells with lesser than those of E$_2$ positive control group. Furthermore, E$_2$ treated group illustrated the cornified cells within 3 days after E$_2$ administration and showed the persistent feature of estrous condition until the end of the experiment. In all plant extracts treated groups, the cornified cells firstly presented on day 4 and 5 after treatment at the dose of 100 and 1,000 mg.kg$^{-1}$BW, respectively (data not shown).
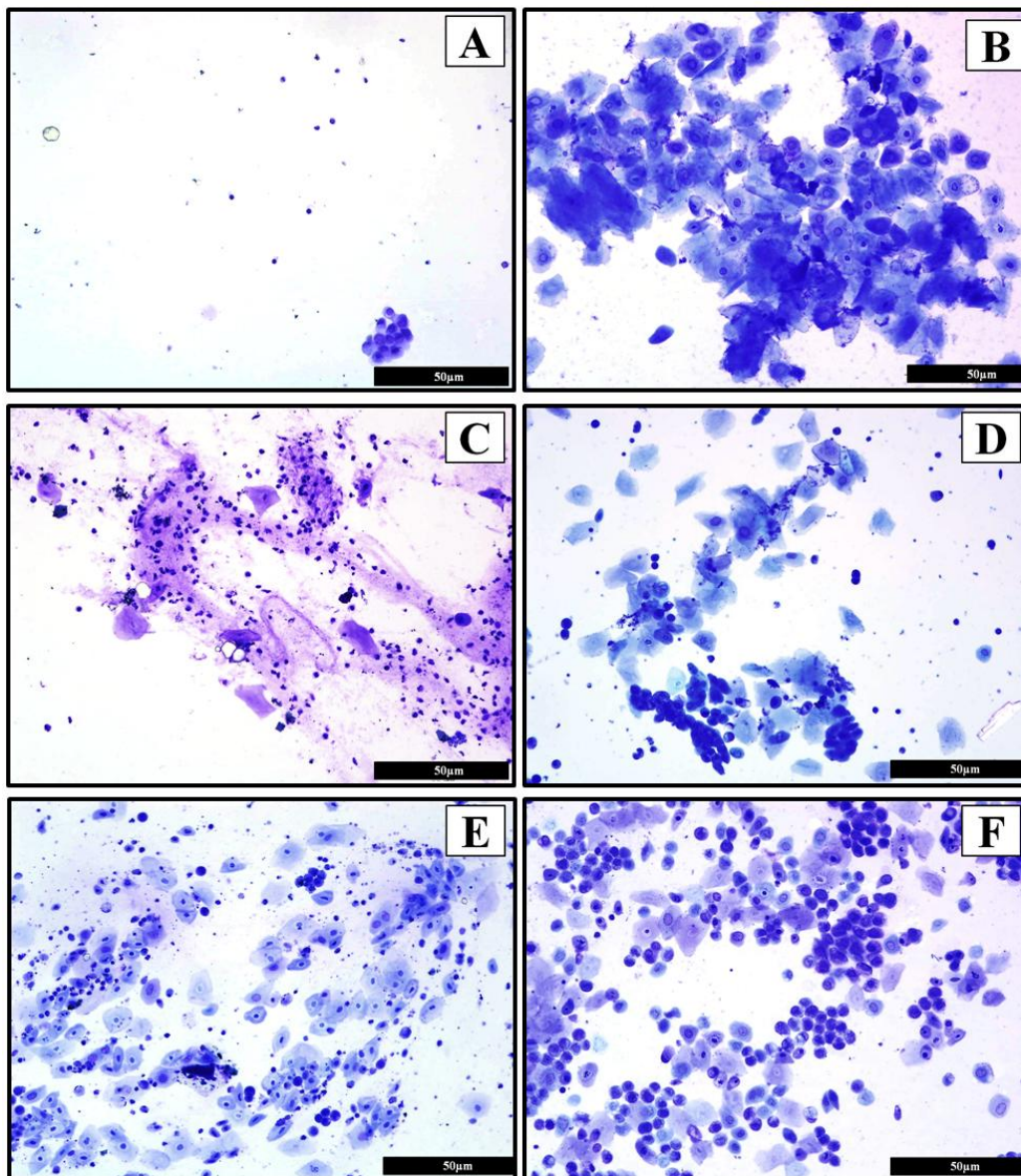


**Fig. 1: Vaginal smear photographic of methylene blue staining from the rats on day 21 after ovariectomy.**

A represents OVX control; B represents standard drug control (17β-estradiol 10μg/kg$^{-1}$BW); C represents *T. paniculatum* root extract (100 mg.kg$^{-1}$BW); D represents *T. paniculatum* root extract (1,000 mg.kg$^{-1}$BW); E represents *T. paniculicata* leaf extract (100 mg.kg$^{-1}$BW); F represents *T. paniculatum* leaf extract (1,000 mg.kg$^{-1}$BW). (Bars represent 50 μm, 200x)

**Table 2: Effect of *T. paniculatum* extracts on vaginal cornification in OVX rats, 42 day treatment period.**

| Group | Treatment | Dosage | Cornified cell (%) | | | n |
|---|---|---|---|---|---|---|
| | | | Day 1 | Day 7 | Day 42 | |
| 1. | Vehicle | 1 ml/rat/day | 00.00+0.00$^b$ | 00.00+0.00$^a$ | 00.00+0.00$^a$ | 5 |
| 2. | 17ß-estradiol | 10 μg.kg$^{-1}$BW | 00.00+0.00$^b$ | 34.15+1.46$^d$ | 56.04+1.46$^d$ | 5 |
| 3. | *T. paniculatum* root extract | 100 mg.kg$^{-1}$BW | 00.00+0.00$^b$ | 15.86+1.73$^b$ | 26.29+0.77$^c$ | 5 |
| 4. | *T. paniculatum* root extract | 1,000 mg.kg$^{-1}$BW | 00.00+0.00$^b$ | 26.27+0.64$^{cd}$ | 39.45+0.63$^c$ | 5 |
| 5. | *T. paniculatum* leaf extract | 100 mg.kg$^{-1}$BW | 00.00+0.00$^b$ | 16.30+0.84$^b$ | 21.50+0.54$^b$ | 5 |
| 6. | *T. paniculatum* leaf extract | 1,000 mg.kg$^{-1}$BW | 00.00+0.00$^b$ | 18.94+0.60$^{bc}$ | 39.35+1.76$^b$ | 5 |

All values represent as mean + SEM; n is the number of animals. Means with different superscripted letters in the same column indicate statistical significance (*P<0.05*).
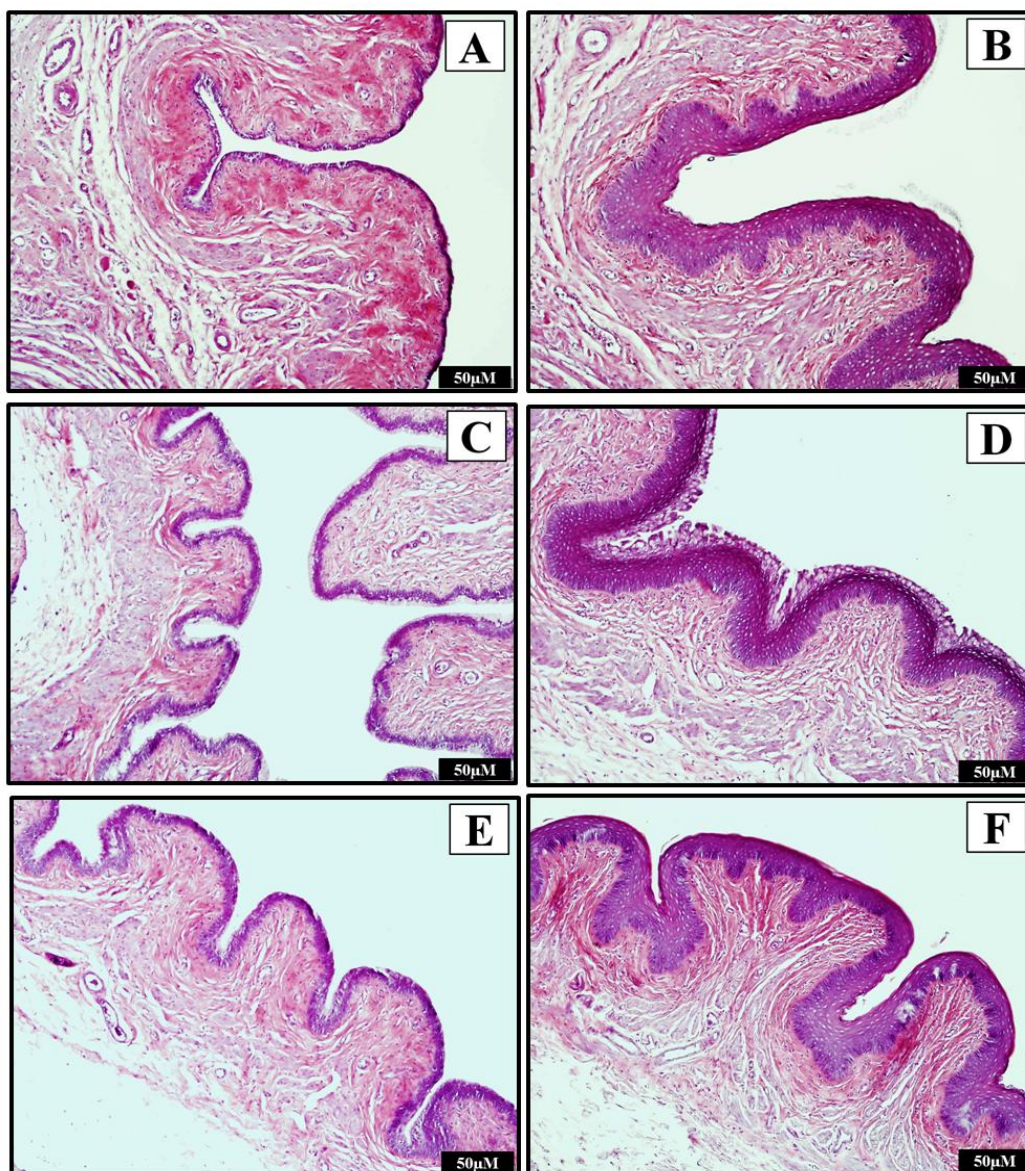
**Fig. 2: Representative images of H&E staining of vaginal tissue from the rats on day 42 after ovariectomy.**

A represents the atrophic pattern of vaginal epithelial lining in OVX rats received vehicle control showing undeveloped vaginal epithelial surface comprised by atrophic cuboidal or undefined flattened cells; B represents the keratinization and thickening of the vaginal epithelial layer in represents standard drug control (17β-estradiol 10μg.kg$^{-1}$BW); C represents *T. paniculatum* root extract (100 mg.kg$^{-1}$BW); D represents *T. paniculatum* root extract (1,000 mg.kg$^{-1}$BW); E represents *T. paniculatum* leaf extract (100 mg.kg$^{-1}$BW); F represents *T. paniculatum* leaf extract (1,000 mg.kg$^{-1}$BW) (Bars represent 50 μm; 100x).

**Histological observation of female reproductive organs**

**Vaginal histologic observation**

The representative vaginal histology is demonstrated in Fig. 2. In OVX control group, the eradication of ovarian hormones stimulation caused an atrophy of the vaginal epithelium which characterized by poorly inactive epithelial lining. This layer consisted of one to two shrivel cuboidal or flattened squamous cell type with a diminutive mucous cells (Fig. 2A). The groups treated by $E_2$ and all dosages of the plant extracts showed proliferative changes of the vaginal epithelial layers. The responsiveness of the vaginal epithelial thickness to *T. paniculatum* extracts depended on the quantity of the plant extracts which were fed to OVX rats. The results exhibited that oral administration of root and leaf extracts at the dose of 1,000 mg.kg$^{-1}$BW generated more response than the dose of 100 mg.kg$^{-1}$BW.

The typical estrogenic pattern, a keratinized stratified squamous epithelium was outstanding illustrated in positive $E_2$ treated rats

(Fig. 2E). This area was covered by high amount of mucous cells. In OVX rats treated with 1,000 mg.kg$^{-1}$BW of root and leaf extract (Fig. 2D and 2F), the histological feature of the vaginal sections demonstrated a thickening keratinized stratified squamous epithelium that was almost comparable to $E_2$ treated rats. The basal layer (stratum basale) of *T. paniculatum* extract-treated groups was established by pseudo-stratified low columnar mucous cells. Thus they were more developed than that of OVX control group, which composed only one or two of undefined cuboidal epithelial cells.

The vaginal epithelial layer in each section was also measured. OVX rats markedly showed a decrease in the thickness of the vaginal epithelial layer (8.26 + 0.57μm) compared with the positive $E_2$ control group (25.00 + 0.62μm) (*P<0.05*). All rats which were fed by plant extracts showed the evidence of vaginal epithelial expansion. A dose-dependent increase of the epithelial layer thickness was observed in rats supplemented with elevating dose of *T. paniculatum* extracts.

The results also showed that, oral feeding by 1,000 mg.kg$^{-1}$BW of the leaf extract, the epithelial thickness was restored (26.49 + 0.33μm) compared to the positive E$_2$ treated rats (25.53 + 0.62μm) (*P=0.47*). Treatment with 100 mg.kg$^{-1}$BW of the leaf extract showed lesser response (16.72 + 0.33μm) compared to 1,000 mg.kg$^{-1}$BW of the leaf extract. The groups that were fed by the root extracts (100 and 1,000 mg.kg$^{-1}$BW) exhibited less effective than the leaf extract treated groups at the same dose (13.49 + 0.88μm and 20.97 + 0.45μm, respectively).
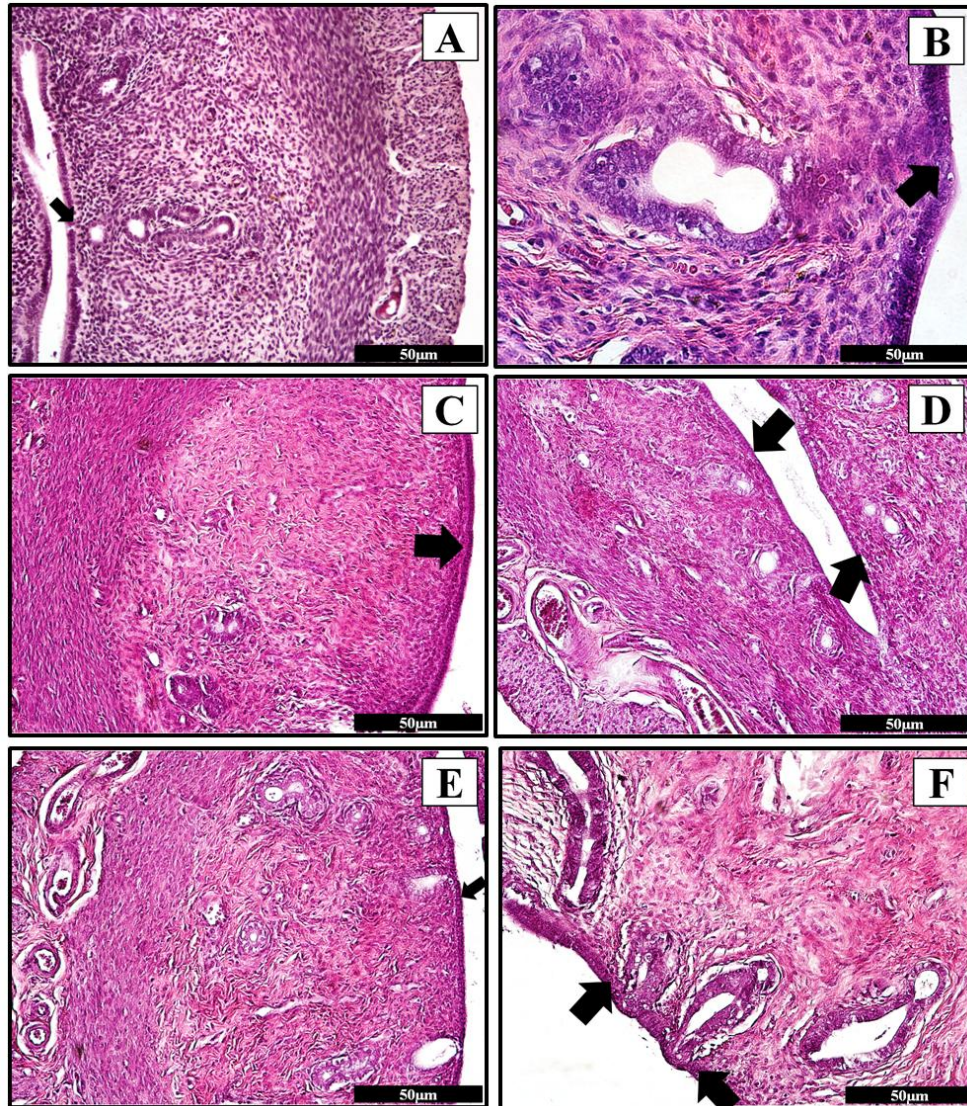


**Fig. 3: Representative images of H&E staining of endometrial gland and surface epithelium (arrow) histomorphology of the OVX rats treated by various treatments on day 42.**

A represents the atrophic pattern of uterus in OVX rat received vehicle control; B represents the representative of estrogenic effect demonstrating the tall columnar surface epithelium in positive E$_2$ control rat (17β-estradiol 10μg/kg); C represents *T. paniculatum* root extract (100 mg.kg$^{-1}$BW); D represents *T. paniculatum* root extract (1,000 mg.kg$^{-1}$BW); E represents *T. paniculatum* leaf extract (100 mg.kg$^{-1}$BW); F represents *T. paniculatum* leaf extract (1,000 mg.kg$^{-1}$BW) (Bars represent 50 μm; 200x).

### Uterine histological observation

#### Endometrium

The histological transverse sections of OVX control uterus showed a narrow atrophic endometrium with the evidence of endometrial hypoplasia. In this study, as can be seen in Fig. 3A, the uterine sections of OVX control are dense and thin. The histological feature demonstrated typical atrophic feature with the thinning of endometrial layer. This layer contained atrophied uterine glands and poor vascularity which covered by low cuboidal epithelial. The surface epithelium was covered with shorten simple columnar and inactive cuboidal types.

Oral administration of E$_2$ remarkably stimulated the size and all structures of the uterus as illustrated by an increasing in endometrial thickness, well developed uterine gland and more vascularity. The bulky epithelial layer was well developed which indicated by the columnar cell type. Furthermore, the histological findings of the uterus in this group demonstrated varying extent of endometrial thickening, which was dependable to the dosage of the extracts used, i. e. the higher the dose the greater the degree of thickening and proliferation of the endometrial layer (Fig. 3C, 3D, 3E and 3F). There was no significant change of endometrial proliferation in the groups treated by both dosages of the root (100 and 1,000 mg.kg$^{-1}$BW) and 100 mg.kg$^{-1}$BW of leaf extract. The treatment with 1,000 mg.kg$^{-1}$BW of leaf extract was potentially stimulated the histological architecture of the uterus as illustrated by well-developed glands and thickening of endometrial layer.

#### Endometrial gland

In E$_2$ treated uteri, the histological section showed glandular profiles covered with height of the simple columnar epithelium. Some glandular sections were lined with pseudo-stratified epithelium. The

light microscopic observations depicted numerous branching endometrial glands in this group. On the other hand, the small, closed, and non-branching endometrial glands were found in OVX-receive vehicle uterine section. The general morphology of cell structure did not notably differ between the positive $E_2$ controls vs. OVX negative control. With including its sizes, the number and distribution of glands were greatly condensed in OVX uteri. The treatment with $E_2$, all

structures were hypertrophic and hyperplastic; the sizes, number and distribution of uterine glands were intensively observed. In addition, the treatment by all *T. paniculatum* extracts showed evidence of the uterotrophic properties as illustrated by increasing the numbers of uterine glands compared to OVX negative control group. The oral feeding by 1,000 mg.kg$^{-1}$BW of the leaf extract revealed the most effective to prevent the regression of the uterus.
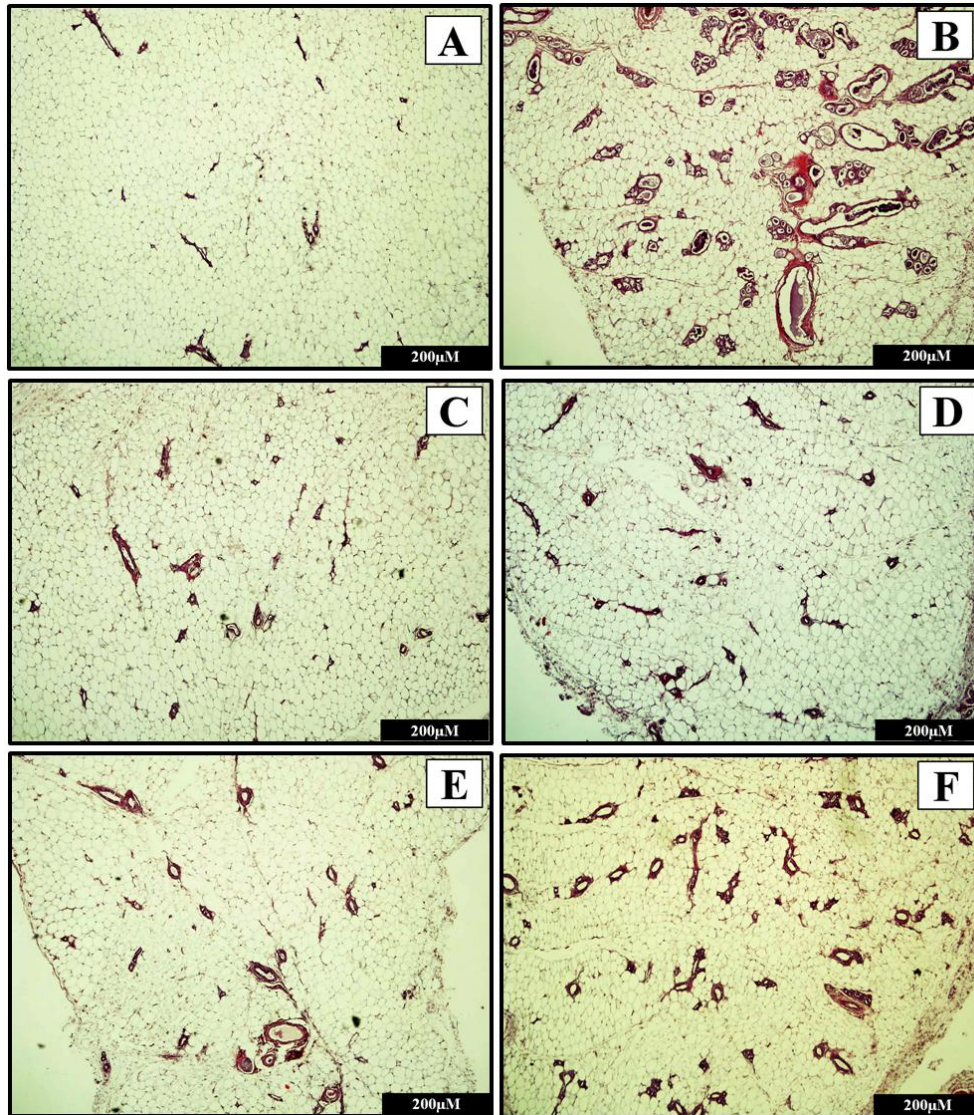


**Fig. 4: Photographic of H&E staining on mammary tissue preparations from the OVX rats administered by the different treatments on day 42.**

A represents OVX control; B represents standard drug control (17β-estradiol 10μg/kg); C represents *T. paniculatum* root extract (100 mg.kg$^{-1}$BW); D represents *T. paniculatum* root extract (1,000 mg.kg$^{-1}$BW); E represents *T. paniculatum* leaf extract (100 mg.kg$^{-1}$BW); F represents *T. paniculatum* leaf extract (1,000 mg.kg$^{-1}$BW) (Bars represent 200 μm; 40x).

### *Mammary tissue histological observation*

Fig. 4 shows histological observation of representative mammary tissue from one animal per treatment group. The revolutionized of overall mammary development and epithelial duct proliferation were considerably different in the microscopic features as indicated by the number and the mammary duct organization. Proliferation of mammary mass was decreased around 74.42% in OVX rats compared to positive $E_2$ control rats (0.22 + 0.01 vs. 0.86 + 0.01%) (*P<0.05*) and corresponded to a decrease in total mammary duct per section (15.33 + 0.69). In positive $E_2$ control group, the histological section showed more complex branching mammary epithelial duct and the section from OVX negative control rat was almost completely absent in the number. In addition, the mammary tissues obtained from OVX control consisted of one or two major ducts with limited branching. The degree of ductular formation related to the plenty of parenchymal

tissue presented which leading to a decrease in the total %RM. OVX rats received $E_2$ had substantially much more parenchymal tissues and large mammary gland containing the secretory fluid. The mammary ductular structure without the secretory fluid was present in all rats which were treated by the plant extracts. The partial extended of ductular formation was observed in OVX rats administered by 100 mg.kg$^{-1}$BW of root and leaf extract.

### DISCUSSION

Female reproductive organs undergo numerous physiological and biochemical changes that depend on the ovarian steroid hormones. The physiological level of these hormones are associated not only the initiation, but also in the developmental process of reproductive system[13,14]. The gonadal ablation or bilateral ovariectomy causes a decrease in these hormones; hence directly affect their target

organs. Genital atrophy and mammary tissue regression are the common clinical presentations that occur after the cessation of ovarian activity. These symptoms are the serious problems in postmenopausal women which lead to decrease their life quality. Exogenous hormone supplementation induces the intrinsic hormonal equilibrium and affects the normal physiology through gross morphological, histological and biochemical modifications.

The underlying mechanisms by which phytoestrogens exert their effects on body composition and obesity after menopause are still unclear; they are acknowledged to have the beneficial effects on body fat distribution and lipid metabolism[15]. The current data exhibited the elevation of final body weight of OVX rats received vehicle control. This might conceivably be due to overiectomy-induced hyperphagia along with a decrease in energy expenditure. The treatment of $E_2$ prospectively reversed these effects. According to mild rising of the final body weight in the groups which treated by *T. paniculatum* extracts, the results suggested that these compounds did not have any statistically effect on the body weight.

It is well known that the estrous cycle undergoes physiological and biochemical changes under the influence of reproductive hormones that leads to dynamic changes of the uterus and vagina. Estrogen is the key hormone to enhance uterine growth and vaginal cornification response by genomic or non-genomic pathways. Subsequently, it increases uterine weight and the keratinization of the vagina. Due to the lack of estrogen level from the removal of ovaries, the uterus and vagina become atrophy[16]. The rat vaginal wall provides an excellent model to determine the estrogenic activity of the estrogenic substances as it is a simple, sensitive, and inexpensive method[11]. Vaginal cytology assay is a practical technique which firstly conducted by Cook *et al.* in 1933[17]. The estrogenic-like compounds have been clarified to have an effect on vaginal epithelium differentiation[18,19]. They affect the vaginal epithelium by changing it into squamous cell then shading into lumen. The current experiment confirmed menopausal stage in OVX rats by monitoring the cellular differentiation in vaginal smears for 14 consecutive days, and none of these rats were cycling. The cornification and keratinization were observable in the rat vaginal smears following the treatment of *T. paniculatum* extracts within the first week after experimenting. This indicated that the estrogenic effects of *T. paniculatum* extracts occurred by short-term consumption. Additionally, the dose dependent increases in percentage of vaginal cornification induced by the extracts of *T. paniculatum* revealed its estrogenic activity.

Estrogen can induce vaginal cornification indicating the estrous stage, and the full cornification requires the higher surging of circulating estrogen level[20]. Buchanan and colleagues suggested that the proliferation of vaginal epithelium was interceded indirectly through estrogen receptor-α (ERα) which mediated by estrogen-induced cornification and stratification[21]. It is also documented to induce uterine growth response by non-genomic action which associates with increases in vascular permeability, water imbibitions, and cellular infiltration[22]. Additionally, mammary tissues are profoundly endocrine-sensitive organs that rely on ovarian steroids and other hormonal signals for their proper growth and differentiation[23,24]. Therefore, there were subjected to estrogenic activity evaluation of this plant. In the present study, primary source of estrogen was terminated due to the removal of ovaries. The appearance of vaginal cornification, the increasing of the parameters in uterotrophic, and mammogenic effect attributed to describe the estrogenic effect of *T. paniculatum* extracts; which confirmed by their histological features.

In this study, the GC/MS analysis of *T. panicaulatum* crude extracts showed the non-steroidal phytoestrogens such as campesterol, β-sitosterol, stigmasterol, stigmastan-3-ol, stigmast-22-en-3-ol and stigmastanol. These phytosterols have been claimed to possess estrogenic activity due to their affinity to estrogen receptors[25,26,27,28]. Taken together, this could be supported that the possible mechanism by which *T. paniculatum* extracts produced estrogenic activity in OVX rats may be due to their phytosterols.

## CONCLUSION

Treatment with the extracts exhibited a dose-dependent estrogenic activity by inducing vaginal cornification and effectively restored the histological structure of female reproductive tissues in OVX rats. In conclusion, *T. paniculatum* root and leaf extracts possess the beneficial effects which can be helpful in managing reproductive tissues regression during menopause in a natural way through herbal resources without any toxicity.

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