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Effects of the Mixture of *Erigeron floribundus* (*Asteraceae*) and *Tragia benthamii* (*Euphorbiaceae*) on the Growth and Architecture of Estrogen-Sensitive Sexual Organs

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Abstract

The mixture of *Erigeron floribundus* and *Tragia benthamii* (AEMEFTB) is traditionally used against pelvic pain, dysmenorrhea and female sexual dysfunctions. In a recent study, we showed that the aqueous extract of the mixture of AEMEFTB suppresses the endometrium growth in rats with experimental endometriosis. The present study was aimed at investigating the effects of AEMEFTB on estrogen's sensitive sexual organs growth and architecture. Immature gonado-intact female rats were randomly distributed into 7 groups of 5 animals each and daily treated during one week with either distilled water (10 ml/kg), refined palm oil (1 ml/kg) or 17β-estradiol (1 µg/day). Plant extract groups received aqueous extract of AEMEFTB at 130 or 260 mg/kg. The remaining groups were co-administered with 17β-estradiol (1 µg) plus 130 or 260 mg/kg of the plant mixture. Moreover, thirty-five immature female rats were bilaterally ovariectomized, then left and treated as before. Five other females, considered as sham animals, orally received distilled water (10 ml/kg). The body weight of each animal was recorded daily and at the end of the treatment (day 8), animals were sacrificed under anesthesia, and the vaginas, uteruses and ovaries (if any) were collected for analysis. Treatment with AEMEFTB did not affect the ovarian weight and architecture in gonado-intact immature female rats. However, a moderate increase of the uterine weight was recorded in animals treated with plant mixture at the high dose (260 mg/kg). On the contrary, a drop in the uterine growth index and total plasmatic proteins was observed in immature females co-administered with the extract and estradiol. Results from this work showed that the mixture of *Erigeron floribundus* and *Tragia benthamii* possesses a weak but observable estrogen-mimetic potential.

Keywords

Estrogen, *Erigeron floribundus*, Rat, Sexual Organs, *Tragia benthamii*

1. Introduction

Nature has been a great source for thousands of therapeutic molecules and continues to play a key role in primary health care worldwide, and especially in developing countries for a long time [1]. Medicinal plants have increasingly gained public and professional acceptance due to their variety of active principles on one hand, but also to progress in the understanding of the mechanisms by which they influence the biological processes and improve health quality on the other hand [2].

Many of these medicinal plants contain phytoestrogens which could act either as estrogenic or anti-estrogenic agents depending on the dose, level of endogenous estrogen in the body and types of estrogen receptor [3] [4]. These biological properties of medicinal plants are exploited in the treatment of some estrogen-dependent ailments. Thus, Doyle *et al.* [5] and Watcho *et al.* [6] have respectively reported that *Pimenta dioica* and *Bambusa vulgaris* alleviate menopause-induced bone and metabolic damages. *Erigeron floribundus* (*Asteraceae*) is an herbaceous plant commonly found in Cameroon as a weed along roadsides. It is used in folk medicine to treat angina, female infertility, dental pain, headache and various diseases of microbial and non-microbial origin [7]. *Tragia benthamii* is a climbing herb of the Euphorbiaceae family, widely spread in west, central and South Africa. It is traditionally used in Cameroon as an abortifacient, antimicrobial and infertility in women [8]. The mixture of these two species is traditionally used against pelvic pain, dysmenorrhea and female sexual dysfunctions in the West-Cameroon Region.

In a recent study, we showed that the aqueous extract of the mixture of *Erigeron floribundus* and *Tragia benthamii* (AEMEFTB) suppresses the endometrium growth in rats with experimental endometriosis, possesses antioxidant, anti-inflammatory activities and regulates plasmatic sexual hormone concentrations [9]. These preliminary findings indicate that the AEMEFTB could interfere with the synthesis/activity of endogenous estrogens.

Since estrogens are involved in the growth and functions of female sexual organs including the vaginas, uteruses and ovaries, the present study was therefore aimed at investigating the effects of AEMEFTB on the growth (Trophy City) and architecture of some estrogen's sensitive sexual organs. We used a worldwide animal model for the assessment of estrogenic/antiestrogenic activity of compounds: the immature female rats.

2. Materials and Methods

2.1. Animal Care

Immature female Wistar rats aged 4 to 5 weeks old were reared in the animal

house of the Research Unit of Animal Physiology and Phytopharmacology (URPAP) of the Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon. They were maintained at room temperature with a natural light/dark cycle and standard laboratory rat diet without soybean to avoid interference with phytoestrogens. All animals were given tap water *ad libitum*. The research proposal was approved by the scientific committee of the Department of Animal Biology, University of Dschang, which follows the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European community guidelines; EEC Council Direction 2010/63/EU of 22 September 2010 [10].

2.2. Plant Material

The plant material was obtained as described by Djuidje *et al.* [9]. Briefly, fresh plants of *T. benthamii* and *E. floribundus* were harvested in the West Region of Cameroon and authenticated at the Cameroon National Herbarium, by comparison with the specimen registered under Vaucher numbers 9747/SRF/Cam (*T. benthamii*) and 33115/HNC (*E. floribundus*) respectively.

Collected plants were reduced into small pieces, shade-dried and powdered. To obtain the aqueous extract, 72 g of powder mixture (ratio 1:1) was boiled in 4.5 L of distilled water for 15 min. The resulting solution was filtered at room temperature and the filtrate was oven-dried at 45˚C for two days to give 16.39 g of aqueous extract, corresponding to an extraction yield of 22.76%.

3. Experimental Design

3.1. Effects of AEMEFTB on Estrogen-Sensitive Organs in Immature Gonado-Intact Female Rats

Immature gonado-intact female rats (aged 4 to 5 weeks) were randomly distributed into 7 groups of 5 animals each and daily treated for one week. Control groups received either distilled water (10 ml/kg, per os), refined palm oil (1 ml/kg, subcutaneously) or 17β-estradiol (1 µg/day, subcutaneously). Two other groups received aqueous extract at 130 or 260 mg/kg per os. The remaining groups were co-administered with 17β-estradiol (1 µg) plus 130 or 260 mg/kg of the plant extract. AEMEFTB was dissolved in distilled water while 17β-estradiol was prepared in warm refined palm oil. During testing, the body weight of each animal was recorded daily and the weight gain determined.

At the end of the treatment (day 8), animals were sacrificed under anesthesia and vagina, uterus and ovaries were collected for analysis.

3.2. Effects of AEMEFTB on Estrogen-Sensitive Organs in Immature Ovariectomized Rats

Thirty-five immature female rats aged 4 to 5 weeks were bilaterally ovariectomized (OVX) as described by Cariton [11]. Five other females underwent the surgical process without ovary ablation and constituted the sham group. After 7

days of post-surgery recovery, OVX rats were divided into 7 groups of 5 females each and treated during one week with either distilled water (10 ml/kg, per os), refined palm oil (1 ml/kg, subcutaneously), 17β-estradiol (1 µg/day, subcutaneously) or AEMEFTB (130 or 260 mg/kg, per os). As previously mentioned, the remaining groups were co-administered with 17β-estradiol (1 µg) plus 130 or 260 mg/kg of AEMEFTB. Sham animals orally received distilled water (10 ml/kg). At the end of the treatment, animals were sacrificed under anesthesia and, vagina and uterus were collected for assessments.

3.2.1. Sacrifice and Sample Collection

Twenty-four hours after the last treatment, animals were weighed and sacrificed under anesthesia. The vagina, ovaries (if any) and uterus were collected, cleaned of connective tissues, weighed and fixed (half section of the uterus) in 10% formalin for histological analysis. The remaining part of the uterus was homogenized in sterile saline solution (NaCl 0.9%) at 5%, centrifuged at 3000 rpm for 15 minutes and the supernatant was collected for total protein content measurement. The relative sexual organ weights were calculated and used as an index of organ growth. Uterine total proteins were quantified using Biuret colorimetric methods, according to the instructions of the commercial kit (Chronolab, Chronolab Systems, Spain).

3.2.2. Histological Analysis

Histological procedure as described by Cannet [12] was followed. In short, fixed tissues in 10% formalin were dehydrated in ethanol and embedded with paraffin. The preparation was then cut into pieces of 5 µm thickness before slides hematoxylin-eosin staining procedure. Slides were examined under light microscope (Olympus) and the uterine and vaginal epithelial heights were measured using Olympus DP 21 software. The structural characteristics of the ovaries were also described.

3.2.3. Statistical Analysis

Data were expressed as mean ± Standard Error of Mean (SEM). One-way Analysis of Variance (ANOVA) followed by Fisher LSD post-hoc was performed to estimate statistical differences between data. Analysis was done using STATISTICA software Version 8.0 (StatSoft, Inc., Tulsa. USA). A probability of $p < 0.05$ was considered significant.

4. Results

4.1. Effects of AEMEFTB in Normal Immature Female Rats

4.1.1. Effects of AEMEFTB on Body Weight Gain

Figure 1 shows the effects of treatments on the body weight gain. A dose-dependent increase in the body weight gain was recorded when compared with various controls (untreated and oil). The effect was more expressed in females co-treated with AEMEFTB and estradiol with a significant increase ($P < 0.05$) observed in group receiving the higher dose of the plant (260 mg/kg + E2).

4.1.2. Effects of AEMEFTB on Ovarian Weight and Architecture

Estradiol treatment was followed by an increase in the ovary weights when compared with the untreated group. AEMEFTB-treated animals showed no change. However, when AEMEFTB was co-administered with estradiol, an increase was clearly observed in the ovary weights (Figure 2).

Microphotographic evaluation of the ovaries (Figure 3) showed normal follicles at different stages in the control and plant treated animals. Estradiol-treated group presented an increase number of antral follicles whereas animals in the sequential treatments exhibited an increased number of corpus luteum.

4.1.3. Effects of AEMEFTB on Uterine Wet Weight, Total Proteins and Architecture

When administered alone, AEMEFTB (260 mg/kg) improved the uterine weight compared with the untreated group, but did not affect its protein contents. AEMEFTB significantly ($P < 0.05$) decreased the uterine epithelial height compared with distilled water treated rats. As expected, estradiol induced an increase in the uterine weight, total proteins and epithelial height (Figure 4). However, these increases due to estradiol were reduced in the presence of AEMEFTB.

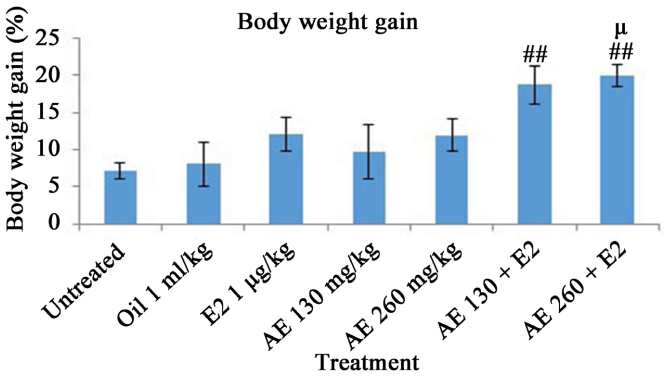

Figure 1. Effects of AEMEFTB on body weight in immature female rats. µ: $P < 0.05$, significantly different compared to estradiol group.

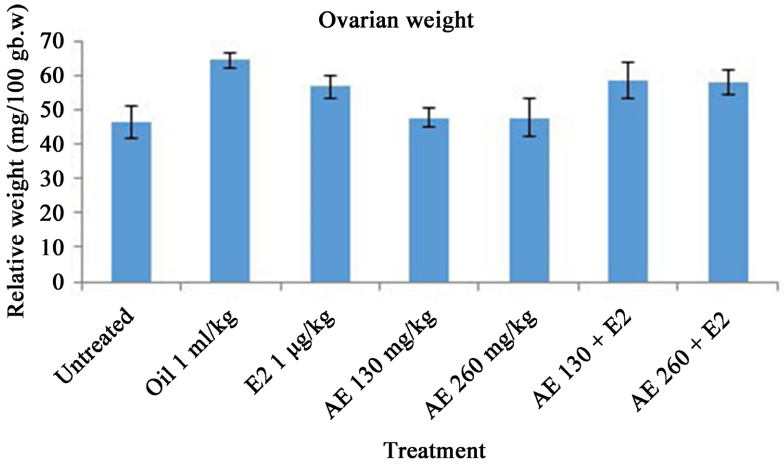

Figure 2. Effects of AEMEFTB on ovarian wet weight in immature female. E2: estradiol; AE: aqueous extract.

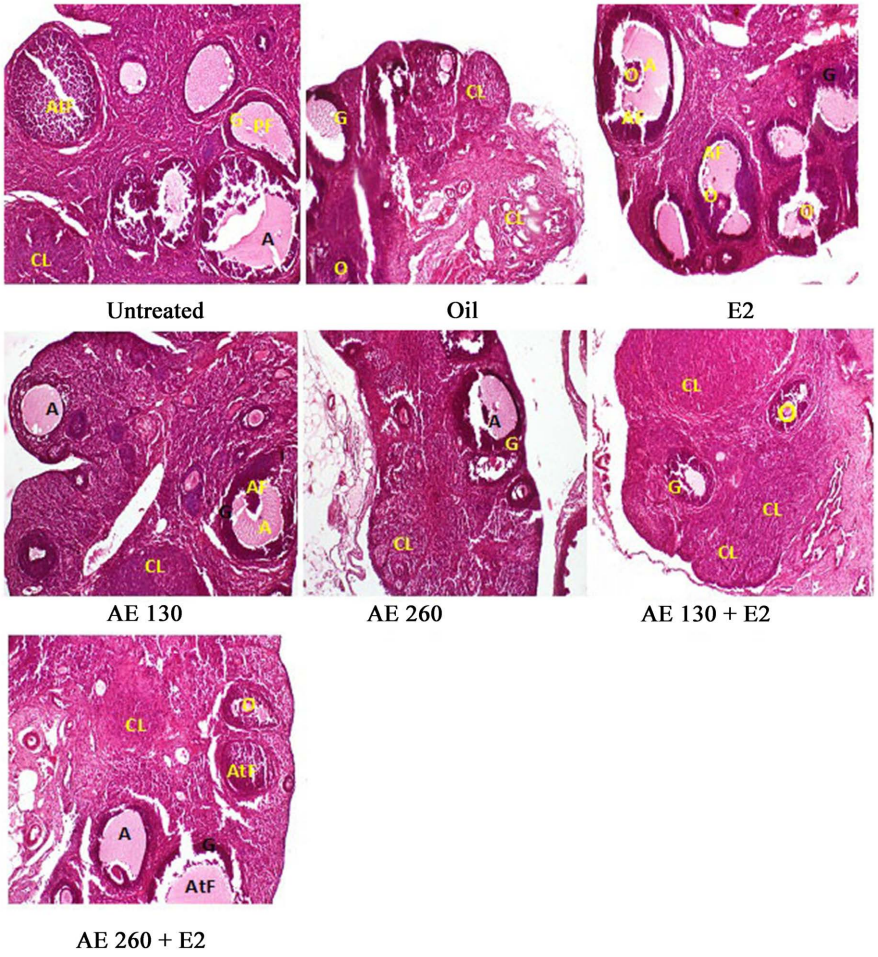

Figure 3. Microphotographs (×200, hematoxylin and eosin staining) presenting the effects of treatments on the ovary's architecture. E2: estradiol; AE: aqueous extract; A: antrum; O: oocyte; CL: corpus luteum; PF: preantral follicle; G: granulose cells, AF: antral follicle.

Figure 5 shows the uterus with an increase stromal lamination, a flattened cuboidal endometrial epithelium in a disturbed pattern and, a reduced lumen in the untreated group. Plant extracts together with estradiol group reduced stromal lamination, large cuboidal tissues, large lumen and an organized pattern similar to the untreated group.

4.2. Effects of AEMEFTB in Ovariectomized Rats

4.2.1. Effects of AEMEFTB on Body Weight Gain

Treatment of OVX rats with estradiol significantly increased ($P < 0.01$) the body weight gain when compared with OVX + Oil-treated group. On the contrary, a non significant reduction was observed in plant-treated groups. In addition, plant and estradiol co-treatment resulted in a significant ($P < 0.05$ - 0.001) reduction of the body weight gain compared with estradiol-treated group (**Figure 6**).

4.2.2. Effects of AEMEFTB on Uterine Weight, Epithelial Height and Architecture

Estradiol treatment resulted in an increase in uterine weight as compared with

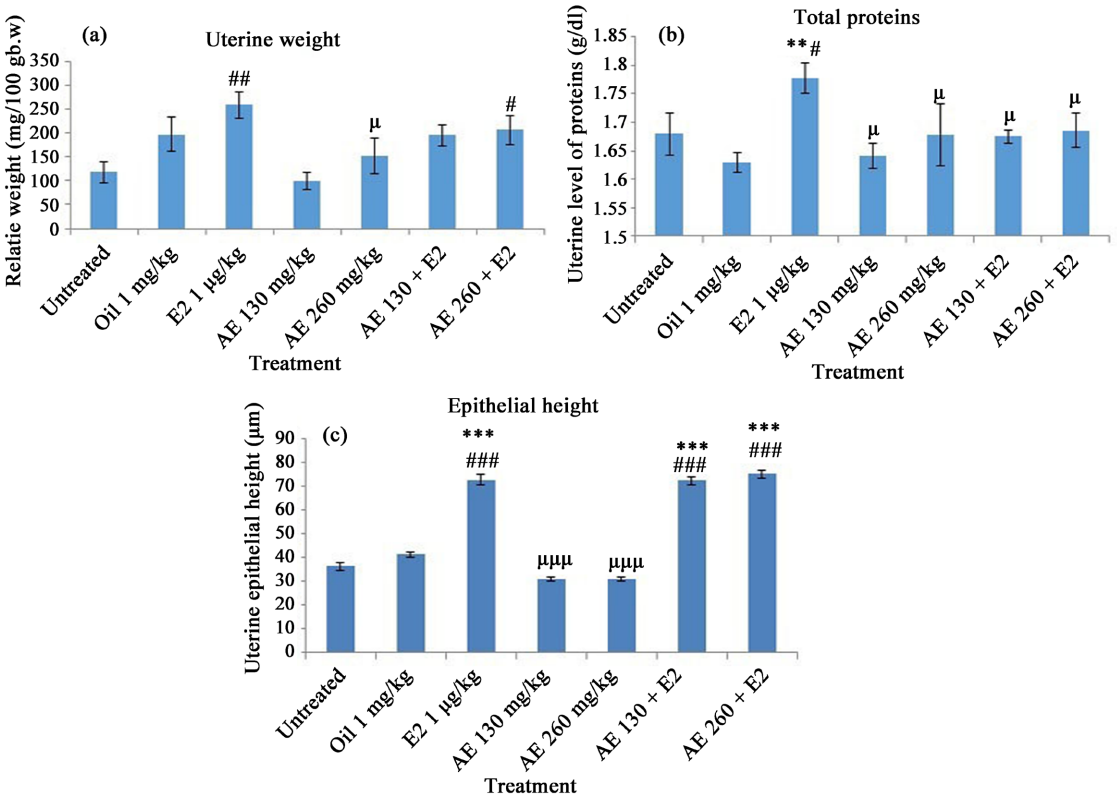

Figure 4. Effects of 7 days treatment on the uterine weight (a), uterine total proteins (b) and epithelial height (c). **P < 0.01 and ***P < 0.001: significantly different compared to oil group; µ: P < 0.05 and µµµ: P < 0.001, significantly different compared to estradiol group; #: P < 0.05, significantly different compared to untreated group.

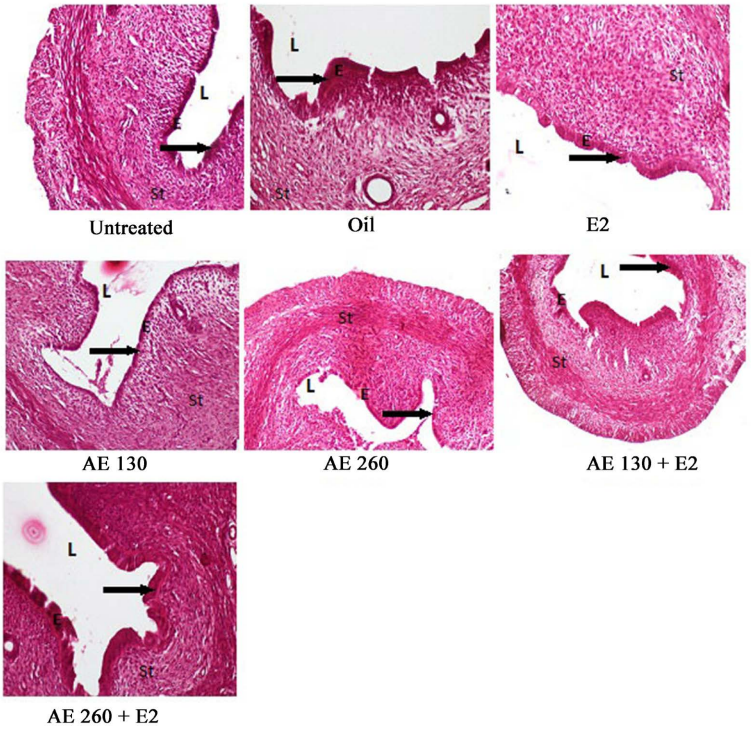

Figure 5. Microphotographs (×200, hematoxylin and eosin staining) presenting the effects of treatments on the uterus architecture. E2: estradiol; AE: aqueous extract; L: lumen; E: epithelium; St: stroma; black arrow = cubic cell.

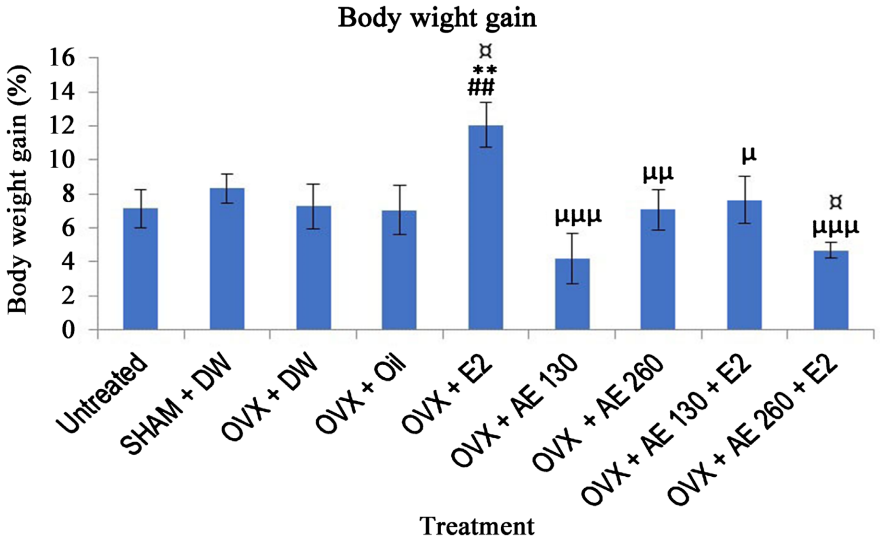

Figure 6. Effects of AEMEFTB on body weight in ovariectomized immature female. **P < 0.01: significantly different compared to oil group; μ: P < 0.05, μμ: P < 0.01 and μμμ: P < 0.001, significantly different compared to estradiol group.

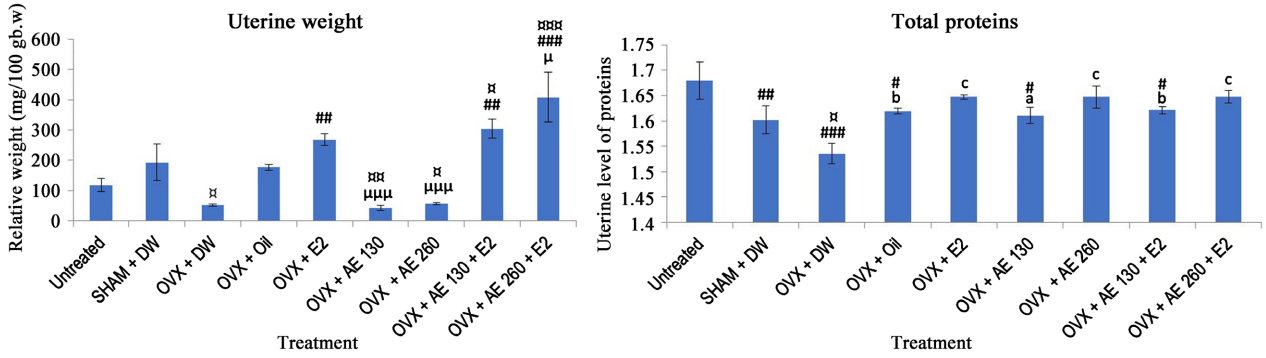

Figure 7. Effects of AEMEFTB on uterine weight (a), total proteins (b) of ovariectomized rats. μ: P < 0.05 and μμμ: P < 0.001, significantly different compared to estradiol group. #: P < 0.05, significantly different compared to untreated group; a: P < 0.05 and c: P < 0.001, significantly different compared to OVX + DW group.

control. Plant extract had no effect on the uterine weight as compared with OVX + DW. However, co-administration of plant extract with estradiol induced an increase in this parameter with a significant effect (P < 0.05) observed in AE260 + E2-treated group compared to estradiol group (**Figure 7(a)**).

Similar to estradiol, plant extract significantly (p < 0.01) increased the uterine total proteins as compared with OVX + DW. The association between estradiol and plant extract did not produce any change compared with OVX + E2.

As shown on **Figure 8**, estradiol induced a significant (p < 0.001) increase in uterine epithelial height as compared with control. Plant extract-treated groups showed no effect on uterine epithelial height. Concerning uterine histology, **Figure 9** shows an atrophic uterus with thin cuboidal endometrial epithelium and loose connective tissue composed of round nuclei in the OVX group. Plant extract as well as estradiol induced a proliferation of uterine cells and an increase in epithelial layer.

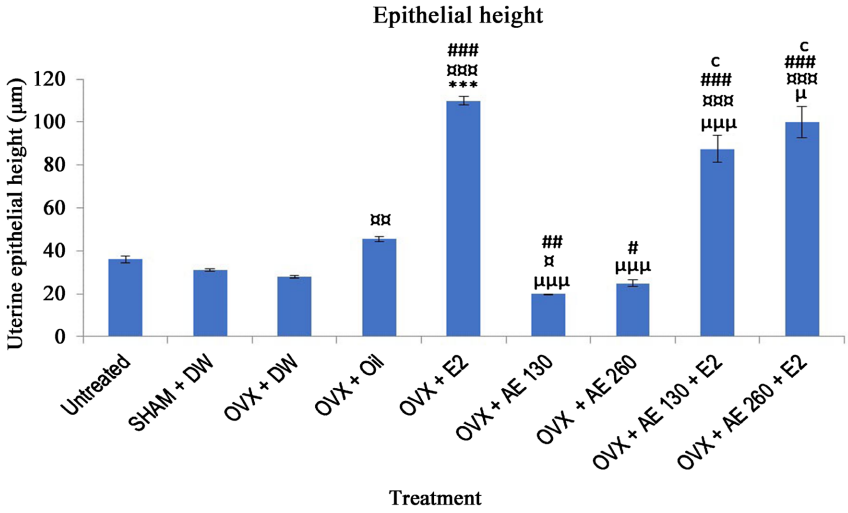

Figure 8. Effects of AEMEFTB on uterine epithelial height of ovariectomized rats. µ: P < 0.05 and µµµ: P < 0.001, significantly different compared to estradiol group; ***P < 0.001: significantly different compared to oil group.

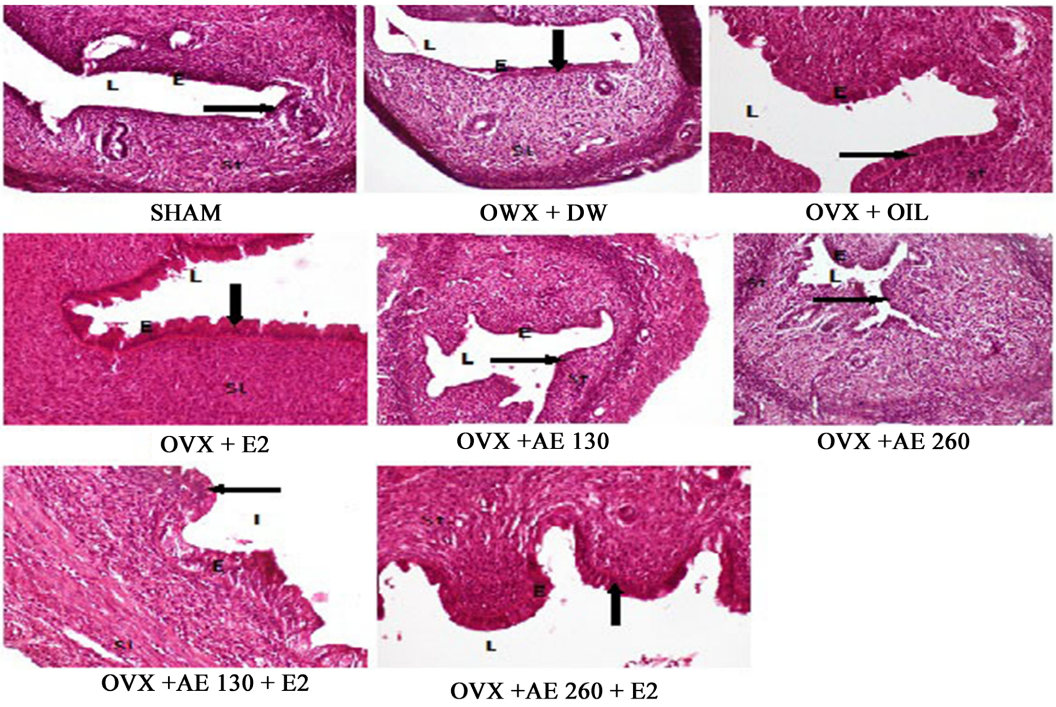

Figure 9. Microphotographs (×200, hematoxylin and eosin staining) of the uterus. E2: estradiol; AE: aqueous extract; L: lumen; E: epithelium; St: stroma; black arrow = cubic cells.

4.2.3. Effects of AEMEFTB on Vaginal Weight and Architecture

Figure 10 shows that estradiol increased ($p < 0.001$) the vaginal epithelial height as compared with controls. No change was observed upon treatment with plant extracts compared with distilled water group. Moreover, no difference was also noticed in animal receiving both plant and estradiol.

Vagina histology (**Figure 11**) examination revealed a normal architecture in control groups; the OVX group consisted of the germinative layer (stratum

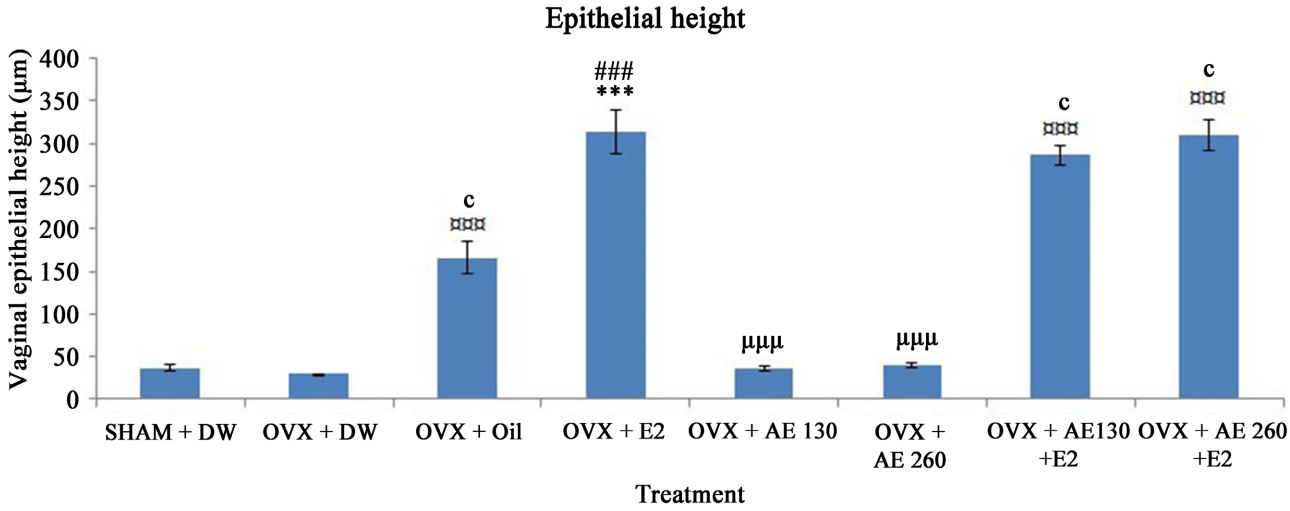

Figure 10. Effects of AEMEFTB on the vaginal epithelial height of ovariectomized rats. ***P < 0.001: significantly different compared to oil group; µµµ: P < 0.001, significantly different compared to estradiol group.

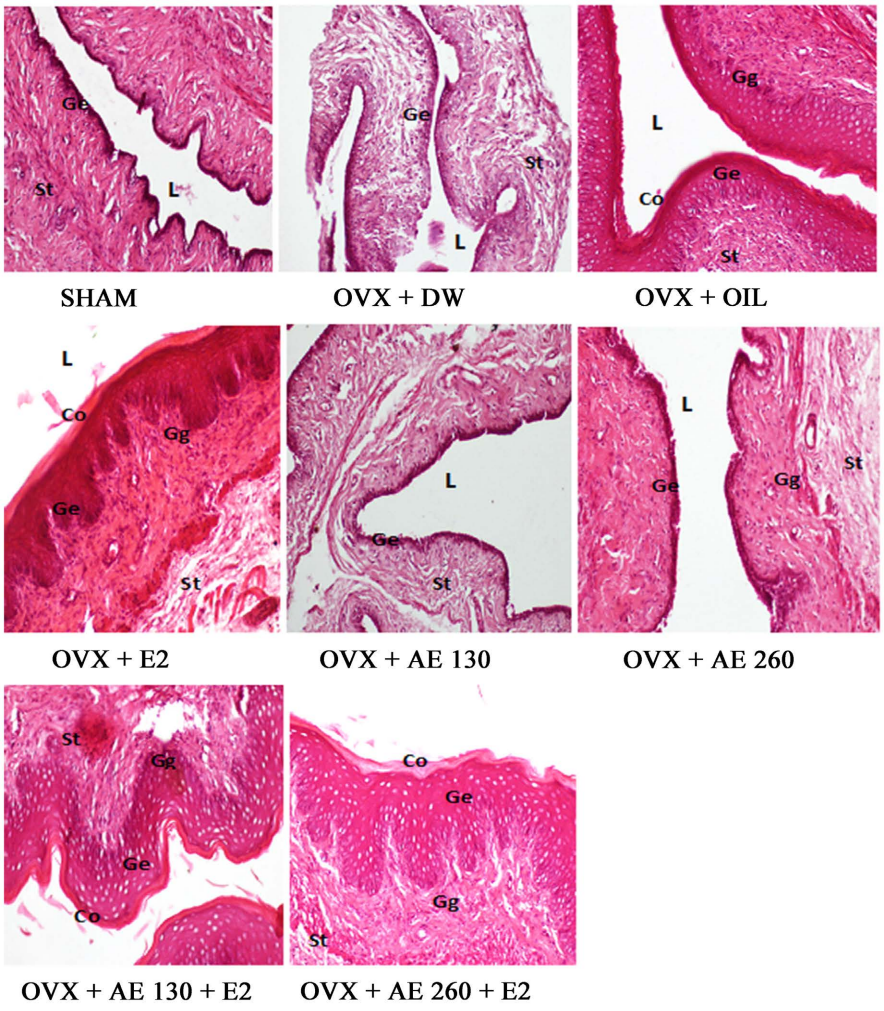

Figure 11. Microphotographs (×200, hematoxylin and eosin staining) of the vaginal epithelia. E2: estradiol; AE: aqueous extract, L: vaginal lumen, Co: stratum corneum, Ge: stratum germinativum, Gg: stratum granulosum, St: stroma.

germinativum). After treatment with estradiol and co-administration, the vaginal epithelium was stratified giving place to three cell layers namely the germinative layer, the granular layer (stratum granulosum) and the cornea layer (stratum corneum). No remarkable difference was noticed upon treatment with plants extract.

5. Discussion

This study focused on the effects of the mixture of aqueous extract of *E. floribundus* and *T. benthamii* on estrogen-sensitive organs. The immature rat uterotrophic assay [13] [14], one of the most widely used methods to detect estrogenicity, was carried out. Treatment with AEMEFTB did not affect the ovarian weight and architecture in gonado-intact immature female rats. However, a moderate increase in the uterine weight in animals treated with plant mixture at high dose (260 mg/kg) was recorded. On the contrary, a drop in the uterine growth index and total plasmatic proteins was observed in immature females co-administered with the extract and estradiol. These findings denote that AEMEFTB possesses an uterotrophic activity in gonado-intact rat and which is further lowered in the presence of 17β-estrogen, a strong estrogen receptor activator used in the present study. It is well-known that estrogens and estrogen-like compounds (phytoestrogens) are key regulators of growth and differenciation in a number of tissues. They exert their biological effect following fixation to estrogen receptors in the target organs including the ovaries and uterus [15] [16].

The global effect of a plant extract is a result of complex interactions of different phytochemicals leading to antagonistic/synergistic and/or additive mechanisms. Many studies suggest that phytoestrogens may act as estrogen-like or as antiestrogenic compounds depending on the circulating levels of estradiol [17]. Therefore, it was necessary to evaluate the effects of AEMEFTB in ovariectomized rats.

Ovariectomy is generally followed by a drop in plasmatic estrogens leading to a slower growth. In the present study, no effect was observed on uterine weight after treatment with plant extract. Also, no difference was noticed between estradiol treated ovariectomized rats and those receiving the sequential treatment with estradiol and AEMEFTB. Plants extract improved epithelial cells growth in vagina of ovariectomized rats. These results corroborate those of Tchoupang *et al.* [17] who reported that the extract from "Nkui" spices increased vaginal epithelial heights, and the lumen and diameter of alveoli in the mammary glands and, altered the estradiol-induced increase of uterine wet weight.

The aqueous extract of the mixture of *Erigeron floribundus* and *Tragia benthamii* did not induce any uterine epithelium growth. This could be justified by more than one view including a dose-dependent effect or a desensitization/down-regulation of the estrogen receptor involved in the uterotrophic process. Indeed, the physiological response of a given tissue to the binding of a ligand to its receptor depends on the dose of the said ligand. Furthermore, when the concen-

tration of the ligand increases, the number of receptor sites may decrease or the receiver can lose its ability to induce a physiological response following ligand binding [16] [18].

The histological changes of the vaginal epithelium during the menstrual cycle result in the proliferation and stratification of the epithelium. The vagina has a cyclic activity and is mediated by sex steroids like estrogens [8]. Substances with estrogenic properties are capable of increasing the size of the vaginal epithelium by stimulating proliferation, stratification and cornification of vaginal epithelial cells [19]. In this study, none of the two doses of AEMEFTB induced vaginal epithelium change and therefore suggesting a week estrogenic activity of AEMEFTB on this tissue.

6. Conclusion

In summary, the results of this work showed that the mixture of *Erigeron floribundus* and *Tragia benthamii* possesses a weak but observable estrogen-mimetic potential.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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