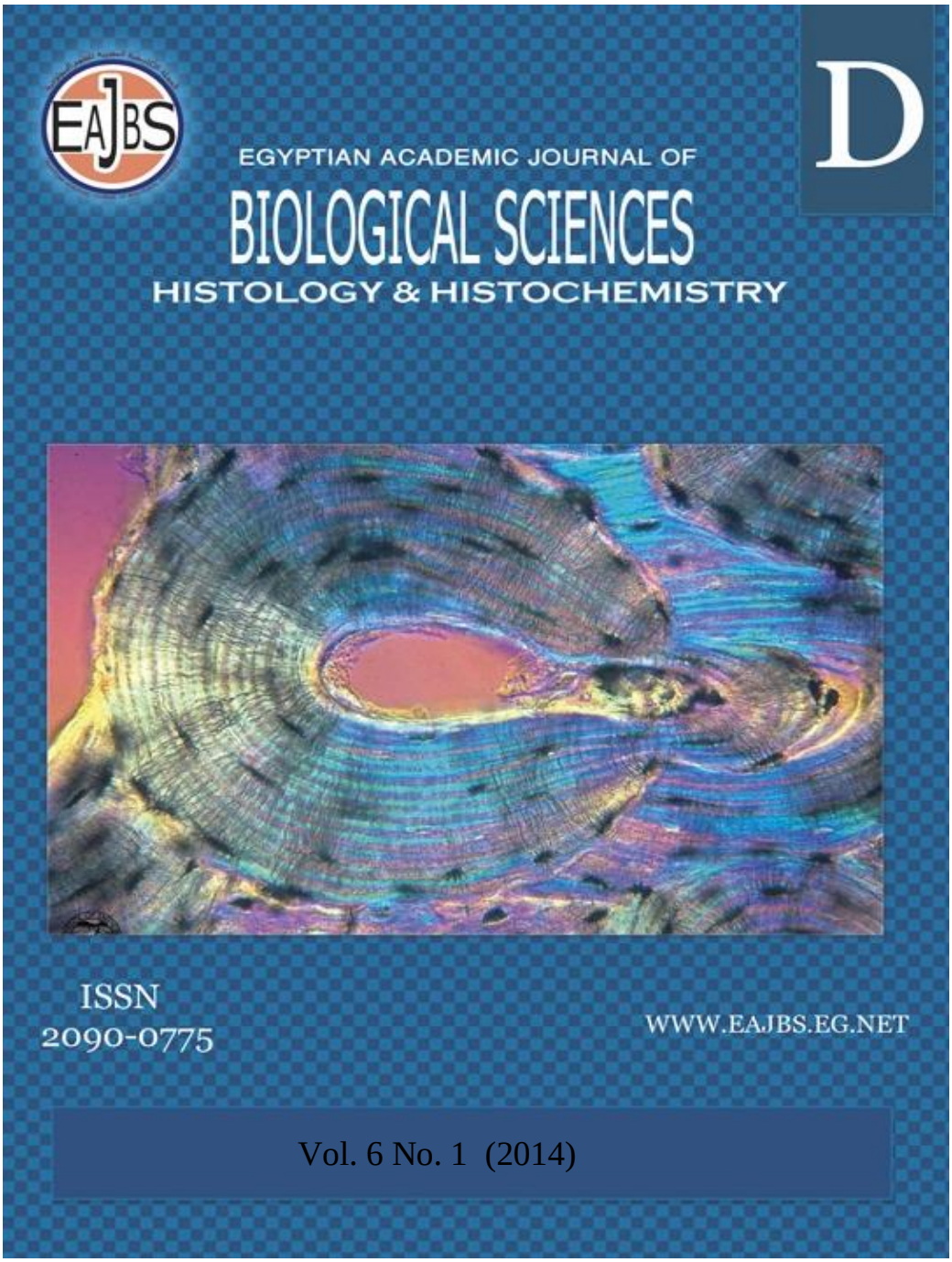

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Effects of Klimadynon® (*Cimicifuga racemosa* BNO 1055 extract) and 17β-estradiol Tratment on Uterus of Maternal Rats and their Offspring. (Histological and Morphological Studies)

Sayed A. S. Hassan[1], Dorreia A. M. Zaghloul[1], Hemely A. Hassan[2] and Zeinab k. Sayed[2]

1- Department of Anatomy & Embryology, Faculty of Medicine, Assiut University.
2- Department of Zoology, Faculty of Science, South Valley University

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ABSTRACT

This work aimed to study the effects of klimadynon® and 17β-estradiol administration on maternal rats and their postnatal day 21 young females in order to compare hormonal stimulation from each substance. Klimadynon® is a rhizome dry extract of *Cimicifuga racemosa* (CR BNO 1055 extract), the common name of this plant is black cohosh. The present study was carried out on eighteen pregnant rats weighing 170-250 gm and aged 16 weeks. The pregnant rats were arranged into three groups: control group, klimadynon group and 17β-estradiol. The klimadynon group was given daily oral dose of 33mg/rat/day,17β-estradiol group was given daily oral dose of 12.5 µg/rat/day, for an experimental period from 7 day of gestation and during lactation to postnatal day 21. The uteri of control and the two treatment groups of both mothers and their postnatal day 21 young were processed for histological examination, as well as morphometric analysis. The uterus sections of klimadynon group of mothers and their offspring showed no histological or morphometric changes. More severe lesions were apparent in uterus sections of rats treated with 17β-estradiol in the form of increase of uterine layers thickness, extremely hypertrophy and hyperplasia of luminal epithelium cells, cellular anomalies, squamous metaplasia, cystic dilatation of endometrial glands, increase the thickness of uterine layers, increase in the number of endometrial glands and formation of papillary Endometrioid adenocarcinoma.

INTRODUCTION

Klimadynon® is a flim coated tablets of dry extract of *Cimicifuga racemosa* rhizome. *Cimicifuga racemosa* rhizome dry extract (CR BNO 1055) used for manufacturing the commercially available products Klimadynon®, (Wuttke *et al.*, 2003). *Cimicifuga racemosa* a member of the buttercup family, is a plant native to the forests of North America, the Common Name is black cohosh.

Estrogen is considered to play a significant role in women's mental health, with links suggested between the hormone level, mood and well-being.

Sudden drops or fluctuations in, or long periods of sustained low levels of estrogen may be correlated with significant mood-lowering. Clinical recovery from depression postpartum, perimenopause, and postmenopause was shown to be effective after levels of estrogen were stabilized and/or restored (Lasiuk and Hegadoren, 2007).

Primary health concerns resulting from menopause include hot flashes (sudden temporary increases in body temperature due to hormone fluctuations. The external body temperature peaks extremely rapidly, and then slowly returns to normal), night sweats, difficulty sleeping, vaginal dryness or atrophy, reductions in cardiovascular health and enhanced risk for developing osteoporosis and Alzheimer's disease (Prior, 2013).

Hormone replacement therapy (HRT), estrogens in combination with progestins, helps to prevent the development of these pathologies in postmenopausal women. However, because a greater incidence of breast and endometrial cancer has been linked to some forms of HRT (Colditz *et al.*, 1995), increased attention has been placed on finding viable and safe alternatives (natural estrogenic alternatives) (Joanna *et al.*, 2002). Phytoestrogens are plant chemicals that resemble steroidal estrogens in structure and function. Although the term 'estrogen' was once restricted primarily to those compounds that bound to the estrogen receptor or stimulated growth of the female reproductive tract (Kurzer and Xu,1997).

In a recent double-blind randomized study that compared placebo, conjugated estrogens, and black cohosh (preparation BNO 1055), the therapeutic effects of black cohosh were equally potent to conjugated estrogens. Treatment with black cohosh had no effect on endometrial thickness or endometrial hyperplasia (Wuttke *et al.*, 2003). Recent data from randomized controlled studies have shown that CR consumption alleviates "hot flushes" and due to the lack of uterotropic effects can be a safe alternative to estrogen replacement therapy (Dominik *et al.*, 2008).

The fact that compounds in the CR extract BNO 1055 are able to compete with estradiol for a yet not identified estrogen receptor and the many positive estrogenic effects in animal experiments is suggestive that the extracts contain one or more not yet identified substances with SERM (Selective Estrogen Receptors Modulator) activity. If further studies confirm these data, CR BNO 1055 (Klimadynon® or Menofem®) would appear as an ideal SERM. Therefore, CR BNO 1055 may be an alternative to classical HRT, which has serious risks (Wolfgang *et al.*, 2003).

The present work aimed to study the effects of klimadynon® (CR BNO 1055 extract) and 17β-estradiol administration on maternal rats and their postnatal day 21 young females, (PND21), in order to compare hormonal stimulation from each substance, taking in consideration histological investigation of uterus of mothers and postnatal day 21 young. Also morphometric analysis of uterus of mothers and their offspring.

MATERIALS AND METHODS

In this study, eighteen pregnant albino rats initially weighing between 170 - 250 g body weight, with age 16 weeks and their 48 immature female offspring were used. Animals were obtained from the animal house of Assiute University. Animals were maintained under normal conditions, with free access of standard diet, water was allowed ad-libitum in the normal daily light and darkness cycle. Animals were caged in pairs in clear plastic cages

containing wood chips for bedding (Warren *et al.*, 2004). Female were made pregnant by keeping them with healthy fertile male rat overnight.

The dams were assigned to three groups:

1-control group, composed of six mothers rats and twenty one postnatal day 21 young females (PND21).

2- klimadynon group, composed of six mothers rats and nineteen PND21, dams were dosed by gavage from GD7 to GD21 and from pup day 2 to PD 21 (Karen *et al.*,2013) with 33 mg/rat/day of Klimadynon® (*Cimicifuga racemosa* extract BNO 1055) (Seidlov´a-Wuttke *et al.*, 2005). The dry extract was suspended in water (Hilke *et al.*, 2003*).*

3-17β-estradiol group, composed of six mothers rats and eight PND21, dams were dosed by gavage from GD7 to GD21 and from pup day 2 to PND 21 and with 12.5 µg/rat/day of 17β-estradiol (Emrah and Suzan, 2011 and Karen *et al.*, 2013).

On postnatal day 21 the dams and their offspring were scarified. Uterus specimens of mothers and their offspring of both control and different experimental groups were separated. Also at necropsy the uteri (uterus with vagina including cervix, Minerva *et al.*, 2012) of mothers and their young's were removed, trimmed of fat and connective tissue, and weighed (Emrah and Suzan 2011 and Karen *et al.*, 2013)

For histological examination, the specimens were fixed in 10% formal saline, dehydrate in ascending grade of ethyl alcohol, cleared in xylol and mounted in molten paraplast at (58-62°C) and processed for microtome at 5µm thick. Sections were routinely stained in Harris Haematoxylin and eosin.

For morphometric analysis, each uterine horn cut into three equal pieces. By image analysis system (Leica ICC50), the following parameters were determined for the control and tested groups of animals. The thickness of myometrium, endometrium and the uterine luminal epithelium height (µm), also the number of endometrial glands were counted from these different segments (Guillermo *et al.*, 2007 & Emrah and Suzan, 2011).

Statistical analysis of the data was performed using windows SPSS (ver. 17.0 with one way ANOVA followed by Scheffe s post hoc test to compare the different between the control and the treatment groups. Significance of differences is marked with an asterisk.

RESULTS

A-HISTOLOGICAL RESULTS:

Histological Observation of Maternal Uterus:

In control mothers rats the uterus wall (Fig. 1) was composed of endometrium, myometrium, and perimetrium. The myometrium was composed of inner longitudinal, middle circular and highly vascular and outer longitudinal muscle layer. The endometrium, or mucosal lining of the uterus (Fig. 2), is composed of a simple columnar epithelium, or superficial epithelium, and a lamina propria. The lumina propria was composed of a dense irregular connective tissue highly cellular with abundance of reticular fibers and houses endometrial glands. The endometrial glands appear to be oval, round, or elongated shape with simple cuboidal epithelium.

In klimadynon-treated mothers rat (33mg/rat/day), given orally, showed that treatment with klimadynon dose not exert any uterotropic effects at the cellular level. Also, no case of endometrial hyperplasia, hypertrophy, or more serious adverse endometrial outcomes occurred (Fig. 3& 4).

In 17β-estradiol-treated mothers rats, (12.5µg/rat/day), given orally, showed change as compared with that of control, in the myometrium, endometrium, endometrial gland, and luminal epithelium. The area of

endometrium (Fig. 5), appears to be clearly thicker in estradiol rats with respect to control rats. The endometrium glands (Fig. 7, 8, 9, & 10), showed squamous metaplasia (two or three layers of cells, constituting a stratified epithelium), with epithelial bridging crossing the lumen. Glands with cellular anomalies were cylindrical epithelium, low nuclei/cytoplasm ratio,undefined cytoplasm borders, and cystic dilatation (usually large size, enlarged lumen and flat epithelium). The luminal epithelium cell height (Fig. 6, 7 & 8), showed increased indicates extremely hypertrophy and hyperplasia of the cells. In particular the cell displayed a columnar pseudostratified-like aspect with randomly located nuclei, vaculor degeneration and presence of intracellular cysts. Also formation of papillary Endometrioid adenocarcinoma (Fig. 7 & 8), that in which the tumor elements are arranged as a solid spherical nodule projecting from the epithelial surface. Endometrioid adenocarcinoma is the most common form of endometrial carcinoma, containing tumor cells differentiated into glandular tissue with little or no stroma.

Histological Observation of Young's (postnatal day 21) Uterus:

In PND21 young's of control mothers rats the uterus was composed of endometrium, myometrium, and perimetrium. The myometrium is composed of inner longitudinal layer, middle circular layer and highly vascular and outer longitudinal layer. The endometrium (Fig. 11), was composed of a simple columnar epithelium and a lamina propria. The endometrial glands appear to be oval, round, or elongated shape with simple cuboidal epithelium.

In PND21 young's of klimadynon-treated mothers rats (Fig. 12), the uterus sections showed that the treatment with klimadynon dose not exerts any uterotropic effects at the cellular level. Also, no case of endometrial hyperplasia or more serious adverse endometrial outcomes occurred.

In PND21 young's of 17β-estradiol treated mother's rats, there were changes as compared with that of control and klimadynon groups. The thickness of uterine wall (Fig. 13), appears to be clearly thicker. The endometrium glands showed increase in number and cystic dilatation. The luminal epithelium cell height increased indicates hypertrophy of the cells.

B-MORPHOMETRIC ANALYSIS

Morphometric Analysis of Mothers Uterus.

Uterine weights.

The weight of uterus (uterus with vagina including cervix) of animals treated with klimadynon showed increase, but this increase was not statistically significant P >0.05. Uterine weights in rats treated with 17β-estradiol were increased, and this increase was very high statistically significant P< 0.01 compared to the control (Table 1 & Fig. 14).

Table 1: Uterus with vagina weights (mg) of mothers rats of control and treated groups.

	Control	Klimadynon (33mg/rat/day)	17β-estradiol (12.5µg/rat/day)
Mean	238.22	290.88	667.74
SD.	69.73	103.45	102.65
SE.	31.19	46.28	45.91
Significance		0.68 NS	0.000 ***

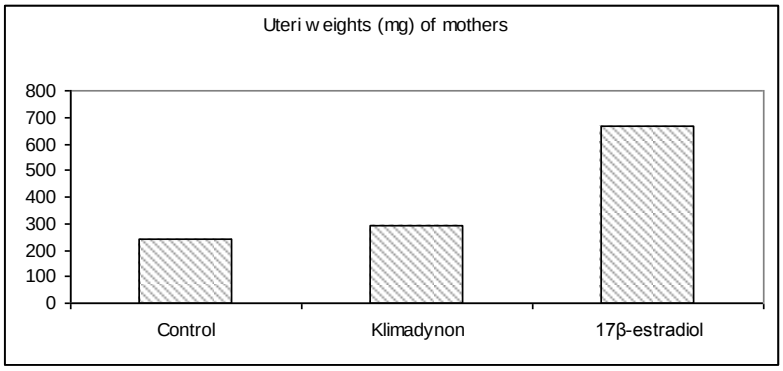

Fig. 14: uteri weights (mg) of mothers rats of control and treated groups.

The Myometrium layer of the uterus.

The morphometric analysis showed that the myometrium layer thickness of the uterus of klimadynon treated group was not different than that of control group. The thickness of myometrium layer of the uterus of 17β-estradiol treated group was high compared to the control and this increase was very high statistically significant P< 0.01 (Table 2 & Fig. 15).

Table 2: Thickness of myometrium, endometrium,luminal epithelial hight, total area (µm) of uterine layers and number of endometrial glands of mothers rats of control and treated groups.

		Myometrium (µm)	Endometrium (µm)	Luminal epithelial cell heights (µm)	Total area (µm)	No. of endometrial glands
Control	Mean	263.39	262.9737	16.19	542.56	11.8
	SD.	127.45	161.21	5.85	247.78	9.58
	SE.	24.53	31.02	11.27	47.68	3.03
	Sig.					
Klimadynon 33mg/rat/day	Mean	259.69	292.89	19.22	571.8	16.1
	SD.	71.88	137.14	7.80	189.94	8.77
	SE.	13.83	26.39	1.50	36.55	2.78
	Sig.	0.997 NS	0.881 NS	0.885 NS	0.94 NS	0.628 NS
17β-estradiol 12.5µg/rat/day	Mean	477.45	476.40	41.33	995.18	24
	SD.	243.69	313.06	37.65	440.13	11.14
	SE.	46.89	60.25	7.25	84.70	3.52
	Sig.	0.000***	0.003**	0.000***	0.000**	0.035*

P > 0.05 (NS) → No Significant. P > 0.01 (*) → Significant difference.
p < 0.05 (**) → High significant difference p < 0.01 (***) → Very high significant difference.

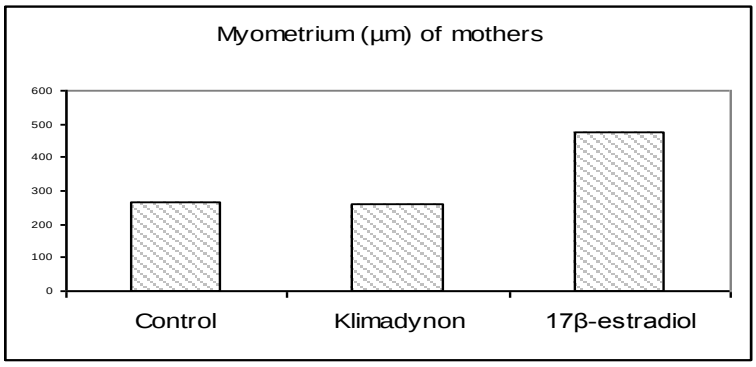

Fig. 15: Thickness of myometrium (µm) of uterine layers of control mothers rats and treated groups.

The Endometrium layer of the uterus.

The area of endometrium of uterus layers slightly increase in thickness in klimadynon group, but this increase was not statistically significant P > 0.05, while at the 17β-estradiol treated group

was very high significantly increase P< 0.01 compared to the control (Table 2 & Fig. 16).

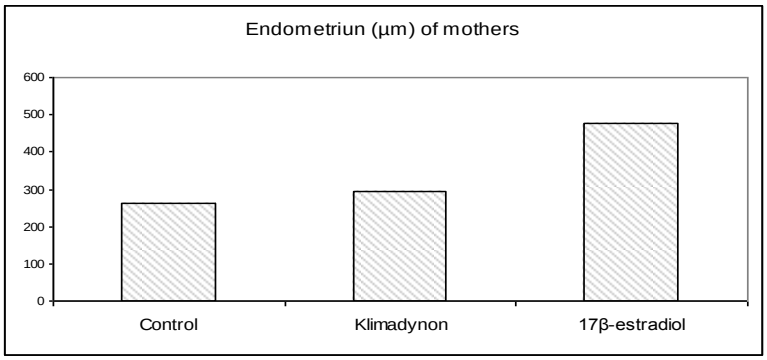

Fig. 16: Thickness of endometrium (µm) of uterine layers of control mothers rats and treated groups.

The Luminal Epithelial cell Heights.

The Luminal Epithelial cell Heights slightly increase in klimadynon group, but this increase was not statistically significant P>0.05, while at the 17β-estradiol treated group was very high significantly increase P< 0.01 compared to the control (Table 2 & Fig. 17).

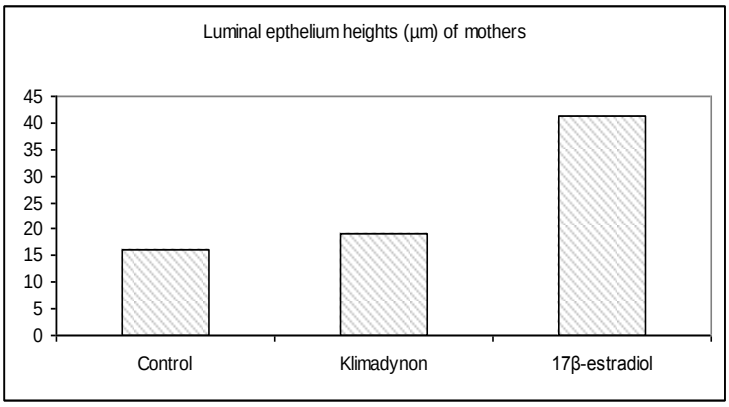

Fig. 17: Luminal epithelium heights (µm) of uterine layers of control mothers rats and treated groups.

The Total Area of uterus layers.

The total area of uterus layers (myometrium, endometrium plus luminal epithelium) showed an increase in treated group klimadynon, but not statistically significant P > 0.05 while at 17β-estradiol treated group was very high significantly increase P< 0.01 than that of control (Table 2 & Fig. 18).

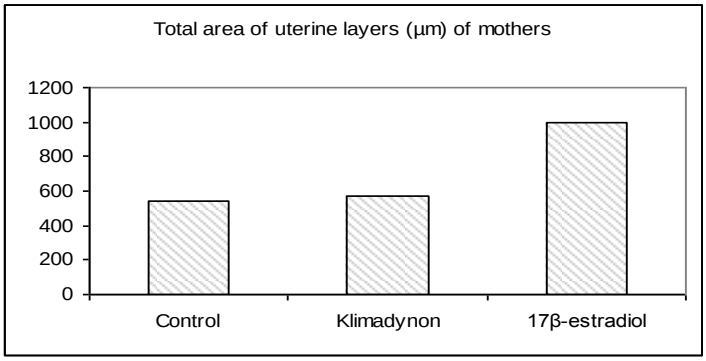

Fig. 18: Total area (µm) of uterine layers of control mothers rats and treated groups.

Number of Endometrial Glands.

Morphometric analysis showed that the number of endometrium glands of klimadynon group did not differ from the control, while at 17β-estradiol treated group the endometrial glands was high statistically significant increase, $P < 0.05$ compared to the control (Table 2 & Fig. 19).

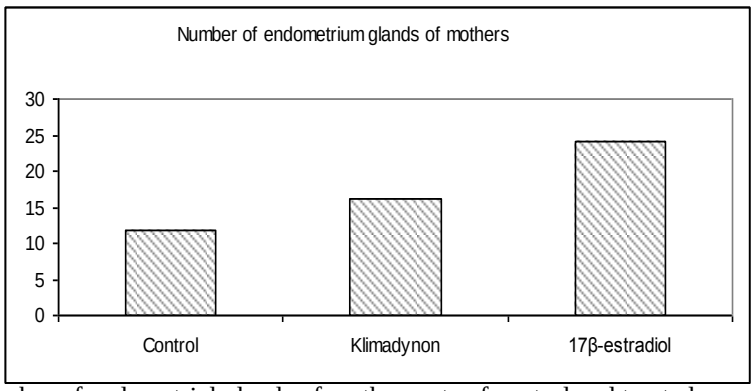

Fig. 19: Number of endometrial glands of mothers rats of control and treated groups.

2-Morphometric analysis of uterus of postnatal youns day 21:

Uteri weights.

The uteri weights (uterus with vagina including cervix) in animals treated with klimadynon increase, but this increase was not statistically significant $P > 0.05$, while at treatment with 17β-estradiol caused high significant increase in uteri weights $P< 0.05$ compared to the control (Table 3 & Fig. 20).

Table 3: Uterus with vagina weight (mg) of postnatal youngs (21day) of control and treated goups.

	Control	Klimadynon	17β-estradiol
Mean	19.3	21.7	31.6
SD.	4.3	5.17	11.5
SE.	1.6	2.15	4.33
Significance		0.85 NS	0.029 **

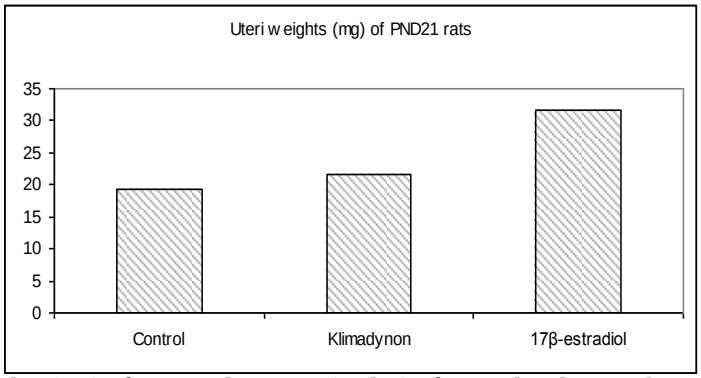

Fig. 20: Uteri weights (mg) of postnatal young's (21day) of control and treated groups.

Myometrium and Endometrium layers of the uterus.

The morphometric analysis of postnatal youngs day 21 showed that the myometrium and endometrium layers of the uterus of klimadynon treated group was not different than that of control group, while at the 17β-estradiol treated group were high significantly increase $P< 0.05$ compared to the control (Table 4, Fig. 21 & Fig. 22, respectively).

Table 4: Thickness of myometrium, endometrium, luminal epithelial hight, total area (µm) of uterine layers and number of endometrial glands of mothers rats of control and treated groups.

		Myometrium (µm)	Endometrium (µm)	Luminal epithelial cell heights (µm)	Total area (µm)	No. of endometrial glands
Control)	Mean	62.86	88.64	9.56	161.06	2.23
	SD.	13.23	48.27	2.52	50.2	1.54
	SE.	2.55	9.29	0.48	9.66	0.42
	Sig.					
Klimadynon	Mean	65.98	89.11	8.85	163.94	1.77
	SD.	17.62	47.36	2.79	52.30	0.93
	SE.	3.39	9.11	0.54	10.07	0.26
	Sig.	0.0922 NS	0.999 NS	0.779 NS	0.986 NS	0.73 NS
17β-estradiol	Mean	90.05	131.42	12.21	233.68	2.46
	SD.	43.86	58.94	5.12	83.2	1.80
	SE.	8.44	11.34	1	16.01	0.50
	Sig.	0.003**	0.013**	0.037**	.000***	0.92 NS

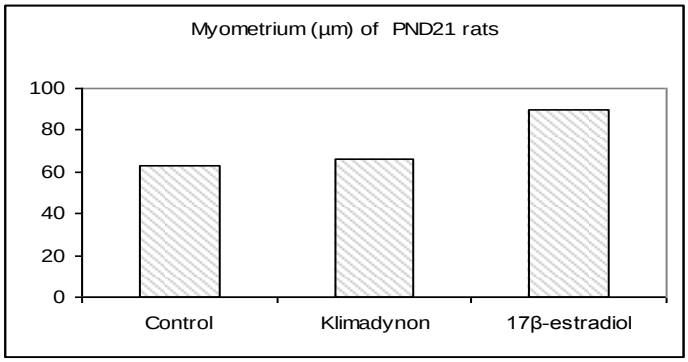

Fig. 21: Thickness of myometrium (µm) of uterine layers of postnatal young's (21day) of control and treated groups.

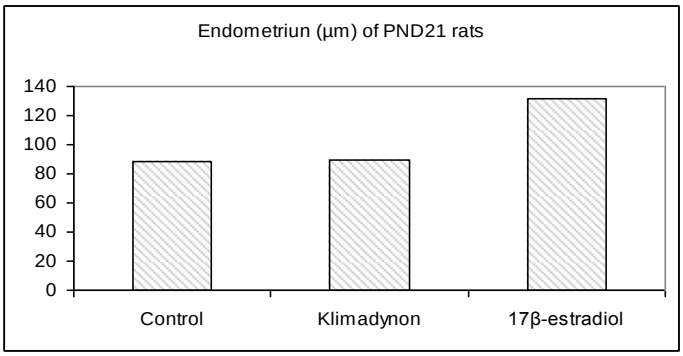

Fig. 22: Thickness of endometrium (µm) of uterine layers of postnatal young's (21day) of control and treated groups.

The luminal epithelial cell height.

The luminal epithelial cell heights was not differ in klimadynon group than that of control group, while at the 17β-estradiol treated group was high significantly increase P< 0.05 compared to the control (Table 3 Fig. 23).

The total area of uterus layers.

The myometrium, endometrium plus luminal epithelium was not differ in treated group of klimadynon than that of control group, while at 17β-estradiol treated group was very high significant increase P< 0.01 than that of control (Table 3 Fig. 24).

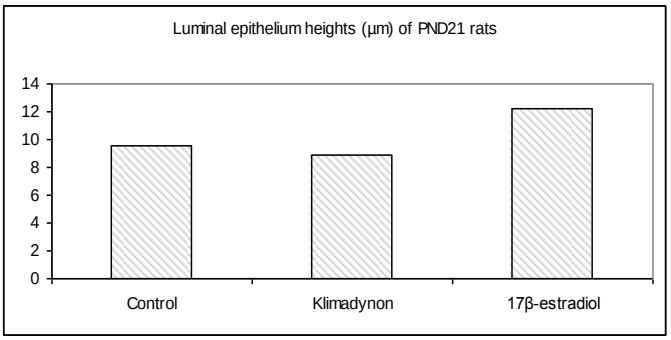

Fig. 23: Luminal epithelium heights (µm) of uterine layers of postnatal youngs (21day) of control and treated groups.

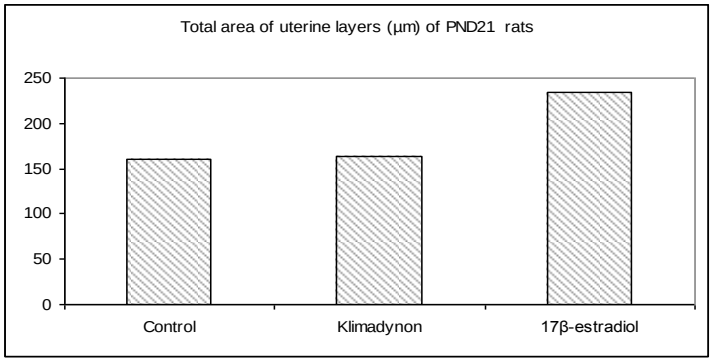

Fig. 24: The total area (µm) of uterine layers postnatal youngs (21day) of control and treated groups.

The number of endometrial glands.

The number of endometrial glands of klimadynon group showed reduction of endometrial glands but this reduction was not statistically significant $P > 0.05$, while at 17β-estradiol treated group the endometrial glands increase, but this increase was not statistically significant $P > 0.05$ (Table 3 Fig. 25).

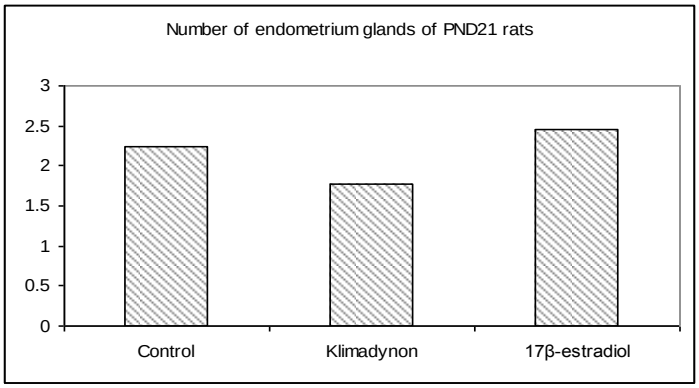

Fig. 25: Number of endometrium glands of postnatal youngs (21day) of control and treated groups.

DISCUSSION

Phytoestrogen activity has been demonstrated in animal models (Diel *et al*., 2000). Also maternal transfer of CR BNO 1055 to offspring has been demonstrated in animal and human studies (Warren *et al.*, 2004). It is well-known that phytoestrogens are available in standardized doses and they are used by women having different weight and present distinct metabolic conditions,

which may alter the final effect in various body tissues (Décio *et al.,* 2008).

The experimental model used here, involving immature rat on day 21 of life ~7 days prior to puberty (Imala *et al.,* 2011), as animal model that might be to comparable to postmenopausal women, which in line with the Organisation for Economic Co-operation and Development recommendation to test for estrogenicity either in immature or ovx rats (Guillermo *et al.,* 2007), and in line with Seidlov´a-Wuttke *et al*. (2006) who utilized a modified Hershberger assay using instead of orx immature rats the 24-day-old rats. Another experimental model used here, involving maternal rats with age 16 weeks and weighing between 170 and 250 g for 5 weeks in studying the effect of klimadynon (CR BNO 1055) and 17β-estradiol. Michael McClain *et al.* (2006) used the female rats in studying the effect of genistein (the main estrogenic component in soy). And in line with Hassanein *et al.* (1994) who study the effect of estrogen on lactating albino rats.

In this study, Klimadynon® (CR BNO 1055 extract) was administrated in a dose of 33 mg/rat/day from GD 7 to GD 21 and from PD day 2 to PD 21 (Karen *et al.,* 2013). Seidlov´a-Wuttke *et al*. (2005) On the other hand, 17β-estradiol administrated in a dose of 12.5µg /rat /day. This dose was based on other studies (Emrah and Suzan, 2011 and Karen *et al.,* 2013).

Hormonal Therapy has been considered essential in relieving menopause symptoms and for the prevention of osteoporosis and cardiovascular diseases. Meanwhile, it has been linked to increased incidence of hormone-dependent cancers (Décio *et al.,* 2008). These data have led some researchers to assess other forms of treatment that may present fewer potentially harmful side effects. Currently, phytoestrogens are some of the most studied alternatives for hot flushes and their therapeutic potential for improving menopause symptoms have been intensively evaluated in recent years (Brett and Keenan, 2007). An alternative with no uterine effects (Du¨ker *et al.,* 1991) may be Black cohosh (*Cimicifuga racemosa* or CR) preparations which were shown to reduce climacteric complaints as efficiently as conjugated estrogens (Seidlova´-Wuttke *et al.,* 2003; Wuttke *et al.,* 2003; Raus *et al.,* (2006) and Wuttke *et al.,* 2006). The uteri of rats as well as of women are estrogen receptive and both estrogen receptor types (ERα and ERβ) have been demonstrated in endo- and myometrial tissues (Seidlova -Wuttke *et al.,* 2003).

The results of this study revealed that, Klimadynon (CR BNO 1055 extract) did not stimulate endometrial growth and had no case of endometrial hyperplasia or hypertrophy. This coincides with the results of the study performed by Seidlova-Wuttke *et al*. (2003) and Décio *et al.* 2008) whom showed absence of endometrial stimulation after 12 weeks of supplementation with derivatives of *C. racemosa* in rats. Raus *et al.* (2006) observed improved climacteric symptoms without endometrial growth. These results in agreement with those of Wolfgang *et al.* (2012) who observed that the black cohosh (*Cimicifuga racemosa*) has no estrogenic effects in the mammary gland and in the uterus. Wolfgang *et al.* (2003) found that in double-blind, placebo- and conjugated estrogens controlled study on postmenopausal women, no increase of thickness of endometrium has been observed under CR BNO 1055. Seidlova-Wuttke *et al.* (2003) also reported that CR extract BNO 1055 (which is used for the production of Klimadynon® and Menofem®) had no significant uterotrophic effect, while the uteri of E2-fed animals were significantly heavier.

In the present study, treatment with klimadynon (CR BNO 1055) observed

that the thickness of uterine layers was not significantly different than that of the control group. This coincides with the results of the study performed by Wuttke *et al*. (2003) who also add that the therapeutic effects of black cohosh were equally potent to conjugated estrogens and the treatment with black cohosh had no effect on endometrial thickness or endometrial hyperplasia. These results are in hormony with Dominik *et al.* (2008) who also noticed that CR consumption alleviates "hot flushes" and due to the lack of uterotropic effects can be a safe alternative to estrogen replacement therapy.

In the present study, treatment with klimadynon showed no increase of uterine weight. These results confirm the previous studies done by Jarry and Harnischfeger (1985) who studied the effect of an ethanolic CR extract in ovariectomized rats. These effects was attributed to unknown phytoestrogens substances in CR BNO 1055 extract bind to cytosolic estrogen receptors. In accordance with this assumption were the results confirmed in vitro investigations by Seidlova-Wuttke *et al.* (2003). In addition Jarry *et al.* (1995) suggesting that they exert their effects via these receptors. These results suggest that CR extract BNO 1055 contains one or more yet unidentified substances with organ-selective SERM activities. It is, therefore, concluded that the remedy of CR BNO 1055 (Klimadynon/Menofem) may be an alternative to classical HRT in women who should not or who do not wish to practice classical HRT.

On the other hand, the current study showed increase of uterine weights of rats treated with 17β-estradiol. This was confirmed by the morphometric study in which there was a highly statistically significant increase $P< 0.01$. These results confirm previous studies done by Emrah and Suzan (2011) who studied the evaluation of the estrogenic effects of dietary perinatal phytoestrogen on the rat uterus by dosed the animals with 12.5µg/rat/day of 17β-estradiol. In accordance with the present results, Wolfgang *et al.* (2003) noticed that the treatment of ovariectomized rats, which were subcutaneously treated for 7 days, either with E2 (3.5 mg per day per animal) or with the CR extract BNO 1055 (62 mg per day per animal), E2 strongly increased uterine weight, and added that E2 stimulated progesterone receptor gene expression, estrogen receptor-b gene expression was inhibited under E2. None of these estrogenic effects were seen in animals treated with CR extract BNO 1055.

In the current study, histological analysis, showed significantly increased in the number and size of endometrial glands of 17β-estradiol group. This was confirm by the morphometric study in which there was significant increase in the number of endometrial glands in comparison to control. The same findings were reported by Emrah and Suzan (2011) whom studied the potential estrogenic effects of perinatal dietary phytoestrogen and 17β-estradiol (with dose 12.5 µg/rat/day) on the rat uterus. These results are in agreement with those of Décio *et al.* (2008) who studied castrated female wistar rats received a dose of 0.029 mg/kg of estradiol valerate and observed an increase in the size and number of endometrial glands, in addition featuring a proliferative endometrium.

In the present study. The endometrium glands showed squamous metaplasia with epithelial bridging crossing the lumen of 17β-estradiol group.These lesions described by El-Sheikh *et al.* (2011). In the present study, histological observation showed the endometrium glands with cellular anomalies, and cystic glands. Theses results are in agreement with those of Verónica *et al.* (2013). Some endometrial glands forming cysts with stratified epithelium. These results in hormony

with those of Isabel *et al.* (2014). In this study, the endometrial epithelialum of uterus of 17β-estradiol group showed extremely hyperplastic cells in a very chaotic pseudostratified organization that was riddled with cavities containing apoptotic cells. These results confirm previous studies done by Imala *et al.* (2011).

In the present study, histological examination of uterus sections of rats treated with 17β-estradiol (12.5µg/rat/day), showed formation of papillary Endometrioid adenocarcinoma (uterine papillary serous carcinoma (UPSC)). In the UPSC, the tumor elements are arranged as a solid spherical nodule projecting from the epithelial surface. In accordance with the present results was Hendrickson *et al*. (1982), who established the uterine papillary serous carcinoma (UPSC) as a distinct type of endometrial carcinoma. In addition, Sunni (2010) reported that uterine papillary serous carcinoma (UPSC) was comprises 5%-10% of newly diagnosed endometrial cancers. And added that Some UPSC tumors, when found early, will appear to be confined to a small uterine polyp, with no invasion into the wall of the uterus.

Sreeja *et al.* (2011) observed that estradiol has been tied to the development and progression of cancers such as breast cancer, ovarian cancer and endometrial cancer. And attributed this to that estradiol effects target tissues by interacting with two nuclear hormone receptors called estrogen receptor α (ERα) and estrogen receptor β (ERβ). One of the functions of these estrogen receptors is gene expression. Once the hormone binds to the estrogen receptors, the hormone-receptor complexes then bind to specific DNA sequences, possibly causing damage to the DNA and an increase in cell division and DNA replication. Eukaryotic cells respond to damaged DNA by stimulating or impairing G1, S, or G2 phases of the cell cycle to initiate DNA repair. As a result, cellular transformation and cancer cell proliferation occurs (Thomas *et al.,* 2010).

In the present study, observed an increase in the thickness of myometrium layer of uterus of rats treated with estradiol. This increase was very high statistically significant $P< 0.01$. Similar findings were reported by Emrah and Suzan (2011). The increase of myometrium thickness attributed to that the number of the myometrial muscle cells are related to estrogen levels. The muscle cells are largest and most numerous when the estrogen levels are high (Leslie and James, 2007). Also added that most of the increase in the uterine size is related to hypertrophy or hyperplasia of the smooth cells. On the other hand, current study observed no increase myometrium thickness layer of uterus of rats treated with klimadynon. These results were described in a study by Seidlova-Wuttke *et al.* (2003) who showed that extracts of *C. racemosa* contain substances not yet fully identified having estrogen activity in the hypothalamus–pituitary axis that do not stimulate myometrial cells.

In this study, it was found that the area of endometrium and myometrium showed an increase in estradiol groups. Also the total area (myometrium plus endometrium) of the uterus showed high significantly increase $P< 0.01$ than that of the control group. Furthermore, the luminal epithelial cell height increased in 17β-estradiol treated group, this increase was very high significantly increase $P<0.01$. Similar findings were reported by Emrah and Suzan (2011) but in their study the total area of estradiol rats was not significantly different than that of the control.

Emrah and Suzan (2011) attributed this increase to hypertrophy of the cells. They also attributed this increase to the Water imbibition, mainly in the endometrium, may be responsible for

uterine weight increase even though histological observation indicated no evidence of edema in the uterine wall. It is also possible that increased cell proliferation in epithelial as well as stromal cells may be responsible for the increased thickness of the endometrium. Also added that the increase of epithelial height indicates hypertrophy and hyperplasia of the cells.

The present results revealed that consumption of klimadynon via the oral maternal route had no estrogenic effects on the genital organs of immature 21 day rats. The uterine mass in uterine of Klimadynon® treated animals was not affected. In accordance with the present results, Minerva *et al.* (2012) noticed that black cohosh extract treatment of juvenile mice PND 17 for 3 days by subcutaneous injection did not increase uterus size at dose up to 100 mg/kg. Also added that, a higher dose of 500 mg/kg was acutely toxic, causing lethargy and decreased motor activity. Also added that the Co-treatment with 50µg/kg/day 17β-estradiol and BCE did not modify the uterotrophic effect of 17β-estradiol.

In the present study, morphometric analysis of uterus observed that exposure to 17β-estradiol throughout pregnancy and during lactation untile day 21 increased uterine weights of immature rat. This increase was high significant increase $P< 0.05$ compared to control. Also showed that the number of uterine glands found in the endometruium were increased, but this increase was not statistically significant $P > 0.05$. In addition the area of endometrium showed an increase in estradiol group. Furthermore, the luminal epithelial cell height increased in estradiol group. In accordance with results described here, 12.5µg/rat/day was found to increase the weight of immature rat uteri (Emrah and Suzan, 2011).

The evolution of molecular biology and new laboratory research will assure safety in the use of phytoestrogens in climacteric women (Décio *et al*., 2008). Nasr and Nafeh (2009) concluded that use of C. racemosa for 1 year by healthy postmenopausal women without evidence of liver disease does not seem to influence the liver.

Accordingly, a significant increase in the number of scientific papers published in this matter can be observed, showing the relevance and importance of the present work.

Further studies are needed in the near future to document the agonistic/antagonistic effects of *C. racimosa* extract on different oestrogenic receptors in different body systems, and to confirm the direct and indirect effects of *C. racimosa* extract on these receptors. Also there is a need to study the optimum duration of use of *C. racimosa* extract, especially in older patients and pre- and postmenopausal Women.

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EXPLANATION OF FIGURES

Fig. (1): A photomicrograph of section of uterus of control mother rat showing: myometrium (*M*), endometrium (*E*), superficial epithelium (*SE*), endometrial gland (*EG*), lumen (*L*). (HX. & E. X100)

Fig. (2): A high power magnification of endometrial section of control mother rat showing: endometrium (*E*) with simple columnar superficial epithelium (*SE*), lumen (*L*), and normal endometrial gland (*EG*). (HX. & E. X400)

Fig. (3): A photomicrograph of section of uterus of mother rat treated with klimadynon showing: myometrium (*M*), endometrium (*E*), superficial epithelium (*SE*), endometrial gland (*EG*), lumen (*L*) and blood vessel (*bv*). (HX. & E. X100)

Fig. (4): A photomicrograph of section of uterus of mother rat treated with klimadynon showing: myometrium (*M*), endometrium (*E*), superficial epithelium (*SE*), endometrial gland with cubical cells lining (*EG*), lumen (*L*) and blood vessel (*bv*). (HX. & E. X400)

Fig. (5): A photomicrograph of section of uterus of mother rat treated with 17-βestradiol showing: myometrium (*M*), increase of endometrium thickness (*E*), and increase of superficial epithelium height (*SE*). (HX. & E. X100)

Fig. (6): A photomicrograph of endometrial section of mother rat treated with 17-βestradiol showing: extremely hypertrophic superifial eopithelium (*SE*) riddled with intracellular cyst (arrow) and cytoplasmatic vaculization, and lumen (*L*). (HX. & E. X400)

Fig. (7): A photomicrograph of endometrial section of uterus of mother rat treated with 17-βestradiol showing: the endometrium (*E*), cystic glands (*cg*), lumen (*L*) and papillary formation (*P*) (endometrioid adenocarcinoma). (HX. & E. X100)

Fig. (8): A higher power magnification of the previous section showing: hyperplasial superifial epithelium (*SE*), papillary formation, a solid spherical mass projecting from the epithelial surface (*P*), and lumen (*L*). (HX. & E. X400)

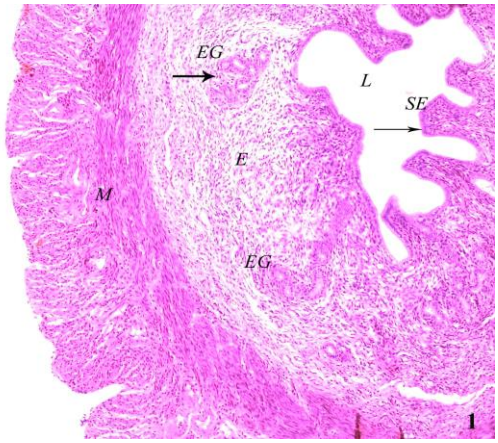
Fig. (9): A photomicrograph of endometrial section of mother rat treated with 17-βestradiol showing: hyperplasia of endometrial glands (*EG*) with epithelial bridging crossing the lumen (*), and cellular anomalies with intracellular cyst (arrow). (HX & E. X400)

Fig. (10): A photomicrograph of endometrial section of mother rat treated with 17-βestradiol showing: endometrium (*E*), hyperplasia and atrophy of the endometrial glands (*EG*). (HX. & E. X400)

Fig. (11): A photomicrograph of section of uterus of PND 21 rat of control mother rat showing: myometrium (*M*), endometrium (*E*), simple columnar superficial epithelium (*SE*), simple cubical endometrial gland lining (*EG*), lumen (*L*). (HX. & E. X400)

Fig. (12): A photomicrograph of section of uterus of PND 21 rat of mother rat treated with klimadynon showing: myometrium (*M*), endometrium (*E*), simple columnar superficial epithelium (*SE*), simple cubical endometrial gland lining (*EG*), lumen (*L*). (HX. & E. X400)

Fig. (13): A photomicrograph of endometrial section of PND 21 rat of mother rat treated with 17-βestradiol showing: thicker endometrium (*E*), hypertrophy of superficial epithelium (*SE*) and endometrial glands (*EG*). (HX. & E. X400)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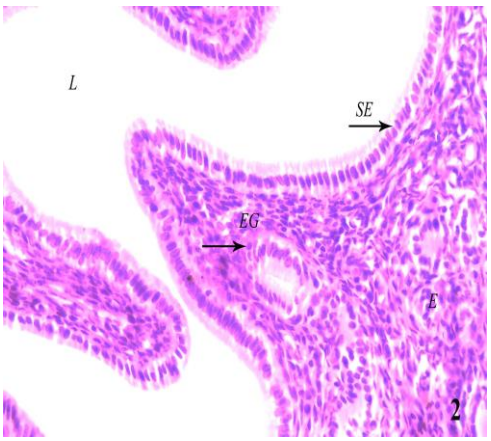
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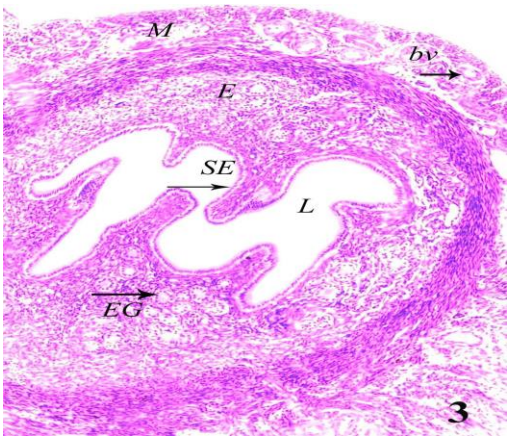
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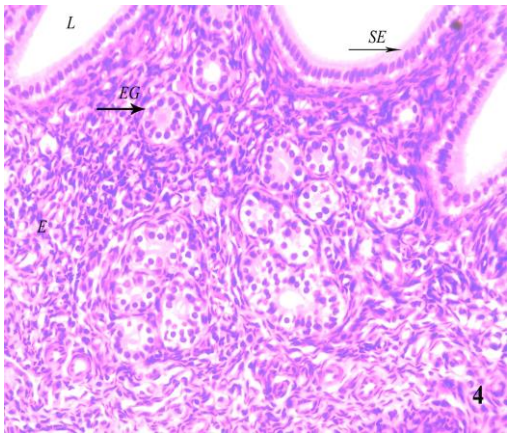
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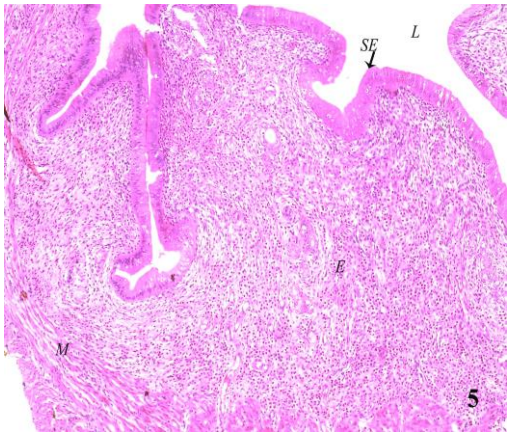
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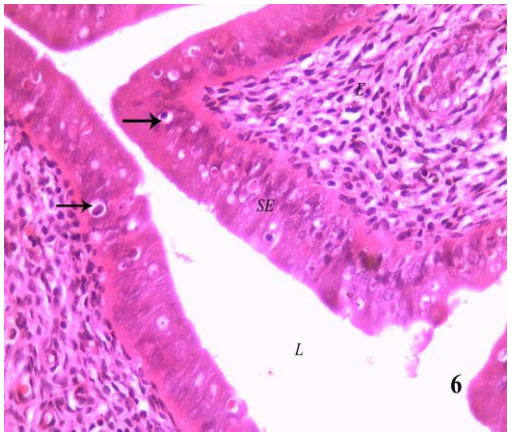
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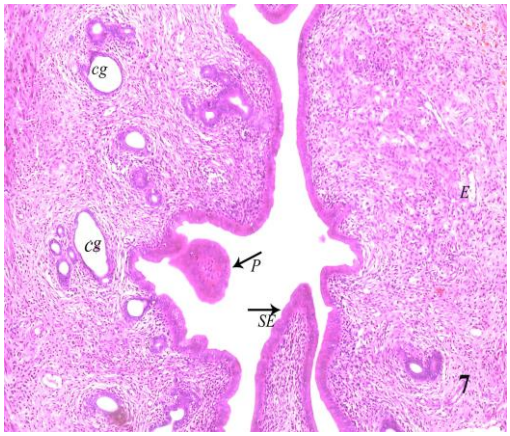
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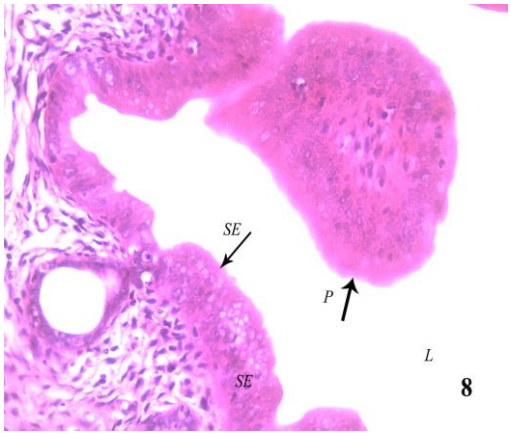
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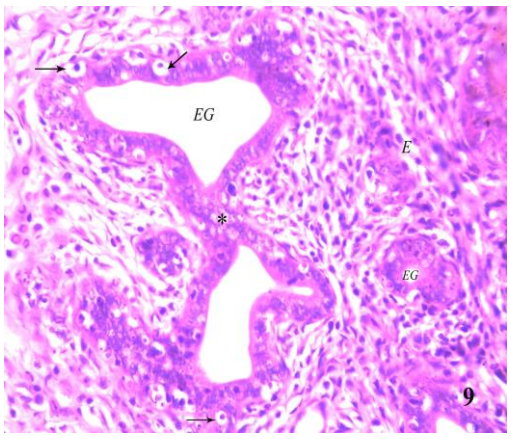
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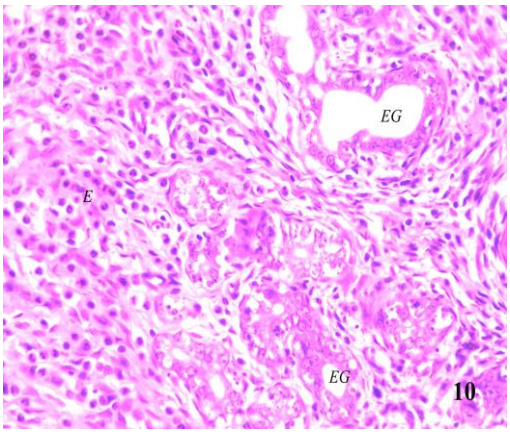
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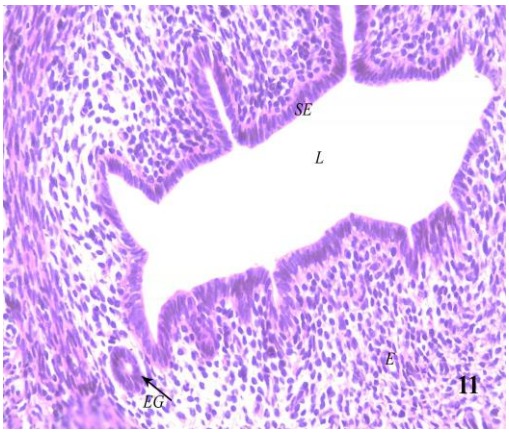
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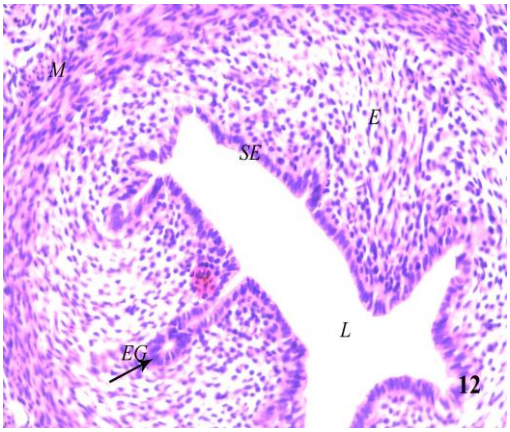
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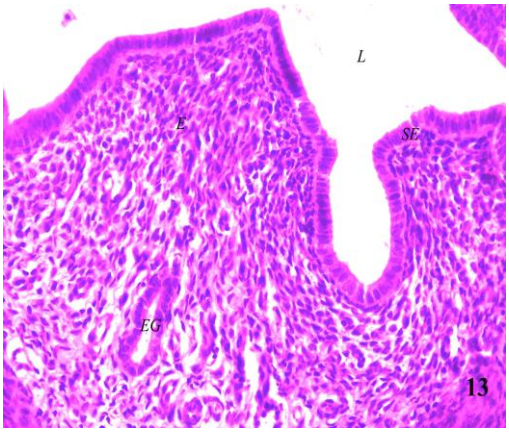
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ARABIC SUMMARY

تأثير المعالجة بالكلامدينون و١٧- بيتا استراديول علي رحم امهات الجرذان وولائدهم (دراسات هستولوجية ومورفولوجية)

سيد انور سيد حسان[١]– درية عبدالله محمد زغلول[١] – حميلي عبد الشافي حسن[٢] - زينب كمال سيد محمد[٢]
١- قسم التشريح والأجنة – كلية الطب – جامعة اسيوط
٢- قسم علم الحيوان – كلية العلوم – جامعة جنوب الوادي

يهدف هذا البحث الي دراسة تأثير تعاطي كلا من الكلاميدينون و١٧- بيتا استراديول علي أمهات الجرذان وولائدهم الأناث عمر ٢١ يوم بعد الولادة في ضوء مقارنة التحفيز الهرموني لكل منهم. الكلامدينون عبارة عن مستخلص جاف لجذور نبات السيمسفيوجا والاسم الشائع لهذا النبات هو الكوهوش الأسود. وقد استخدم في هذه الدراسة عدد ١٨ من الفئران الحوامل عمر ١٦ اسبوع وتتروح اوزانهم بين ١٧٠- ٢٥٠ وعدد ٤٨ من ولائدهم الأناث. وقد قسمت الفئران الحوامل الي ثلاثة مجموعات: مجموعة ضابطة، مجموعة الكلامدينون ومجموعة الاستراديول. تم اعطاء الكلامدينون عن طريق الفم بجرعة مقداره ٣٣ ملي جرام /فأر/يوم وتم اعطاء ١٧- بيتا استرديول عن طريق الفم بجرعة مقدارها ١٢،٥ ميكرو جرام/فأر/يوم للأمهات الحوامل من اليوم السابع للحمل واثناء الرضاعة حتي اليوم ٢١ بعد الولادة. وقد اوضحت الدراسات الهستولوجية والمورفولجية علي رحم الامهات لكلا من المجموعة الضابطة والمعاملة انه لا يوجد تغيرات هستولوجية او مورفولوجية في رحم الامهات المعاملة بالكلامدينون وايضا ولائدهم. في حين توجد تغيرات هستولوجية ومورفولجية في رحم الامهات المعاملة بالاستراديول تتمثل في زيادة كبيرة في سمك طبقات الرحم وحجم وعدد الخلايا الطلائيه المبطنة لتجويف الرحم ، تشوهات خلوية ، تمدد الغدد الرحمية بالاضافة الي سرطان بطانة الرحم.