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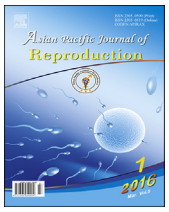

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Cytoarchitectural variations in selected rat tissues following the administration of estradiol valerate in aged female rats

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ABSTRACT

Objective: To study the histomorphological changes with reference to estradiol valerate administration in aged female albino rats.
Methods: The observations showed in young, aged and aged administered with estradiol valerate (Progynova tablets) at the dose of 2 mg/day through oral gavages for one week. The histomorphological changes were observed in ovary, uterus, vagina and liver.
Results: Estradiol velerate administration improves the ovarian stroma and germinal epithelium. The vascular hyaline degeneration reduces. Vascular protective effects of estrogen are well established. The administration may be associated with endometrial hyperplasia and neoplasia. The stroma was comparatively less edematous with less active endometrial glands. The uteri exhibited endometrial epithelial cell pseudo stratification and hypertrophy. The vaginal histoarchitecture showed increased epithelial proliferation of the vaginal wall and improving thickening. Estradiol velerate does not showed any effect on liver architecture.
Conclusions: The administration of estradiol valerate showed its vascular protective effects. It does not showed any effect on liver architecture.

1. Introduction

Estrogens are important in the growth, differentiation and function of female reproductive tissues. Estrogen is recognized as a prime hormone in regulation of female reproductive physiology [1]. Menopause, a normal state of ovarian hormone deficiency, dramatically affects older women, often producing disabling consequences. The incidence of metabolic syndrome also called insulin resistance syndrome, increases substantially during menopause [2] and advancing age. The free levels of sex hormones were decreased during aging, which may indicate an important role of sex hormones in metabolic homeostasis. Estrogen replacement is frequently the treatment of choice for maintaining reproductive function and bone mineral density in post-menopausal women and amenorrheic adolescents [3]. While estrogen's effect on the reproductive system and bone are well established, less is known about how it affects other tissues. The present study aimed to study the structural changes in reproductive tissues and liver for toxicity evaluation following the administration of estradiol valerate to aged female rats.

2. Materials and methods

In the present study healthy female albino rats were used. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India and divided in to three groups, each group containing 6 rats. First group are young rats (4 months), second group are aged rats (20 months) and third group are aged rats administered with estradiol valerate (Progynova tablets, Bayer Zydus Pharma Pvt. Ltd) (2 mg/day) [4] orally for one week with gastric gavages method. Animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean air conditioned room, with photoperiod of 12 h light and 12 h dark cycle at (25 ± 2) °C, with a relative humidity of (50 ± 5)%. The rats were fed with standard laboratory feed supplied by Hindustan Lever Ltd, Mumbai and water *ad libitum*. The usage of animals was approved by the institutional animal ethical committee, in its resolution no: 13/2012-2013(i)/a/CPCSEA/IAEC/SVU/CC – AL dt.01-07-2012. Twenty four hour after the last dose, the animals were autopsied and tissues like ovary, uterus, vagina

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and liver were fixed immediately after isolation in Bouin's fluid for 24 h. The tissues were dehydrated in various grades of alcohol, cleaned in xylene and after embedding in paraffin, blocks were prepared. Paraffin sections of 5 µm thickness were cut with rotary microtome and processed for staining. Sections were stained with hematoxylin and the water staining was made with eosin. 10 sections were taken for each tissue. The light microscope with digital camera was used for photography.

3. Results

The histological changes were observed in young, aged and estradiol valerate administered to aged female rat tissues like ovary, uterus, vagina and liver.

3.1. Histological observations in ovary

The young rat ovarian histoarchitecture showed the normal ovoid shape with normal structure of follicles in different stages of development and densely cellular stroma. The developing follicles were well placed and embedded in ovarian stroma together with graafian follicles, corpus lutea and atretic follicles (Figure 1A and D).

The histology of aged rat ovary showed an obvious reduction in the number of follicles and corpora lutea. The cortex reduces its dimensions, the follicular apparatus is progressively diminished and the primary follicles completely disappear. Vascular hyaline degeneration is present both in the cortical and medullary stroma (Figure 1B and E).

In histology of EV administered aged ovary improved ovarian stroma and germinal epithelium were observed. The vascular hyaline degeneration reduces (Figure 1C and F).

3.2. Histological observations in uterus

The histology of young uterus, exhibited a well differentiated serosa, muscularis and endometrial layers. Many well developed glands were also present and the stroma was densely cellular. The endometrium made up of a single layer of columnar epithelium. In myometrium the lamina propria connected with the compactly arranged smooth muscle layer, surrounded by the serosa and deep to it a rich network of blood vessels (Figure 2A andD).

In aged uterus the uterine lumen was slit-like and lined with small atrophic cuboidal cells. The stroma appeared dense and compact having few, small, inactive uterine glands. The luminal epithelium was low cuboidal and appeared in irregular, the uterine stroma was thickened. The myometrium and endometrial layers were reduced in thickness (Figure 2B and E).

In EV administration the uterine lumen was enlarged. The luminal epithelial cell height was reduced. The stroma was comparatively less edematous with less active endometrial glands (Figure 2C and F).

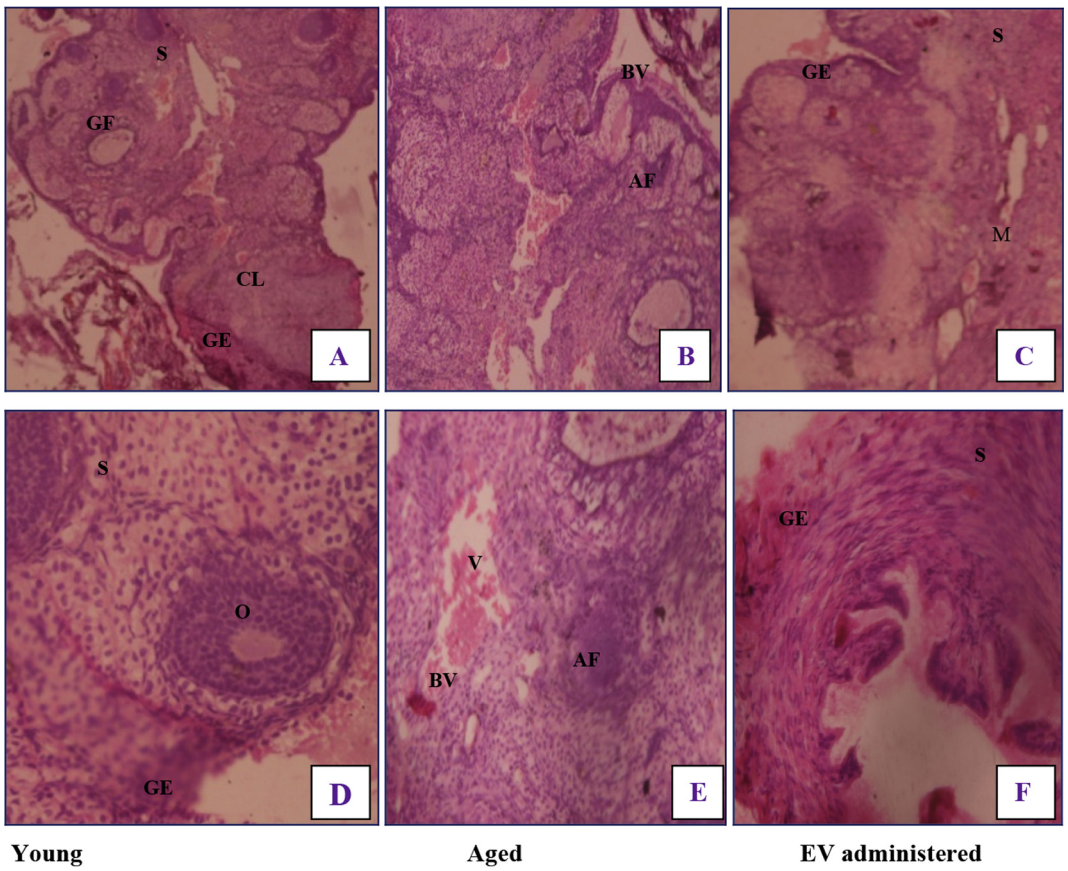

Figure 1. Histological observations of ovary (Hematoxylin and eosin; A,B,C in Mag. ×100; D, E &F are in Mag. ×400).

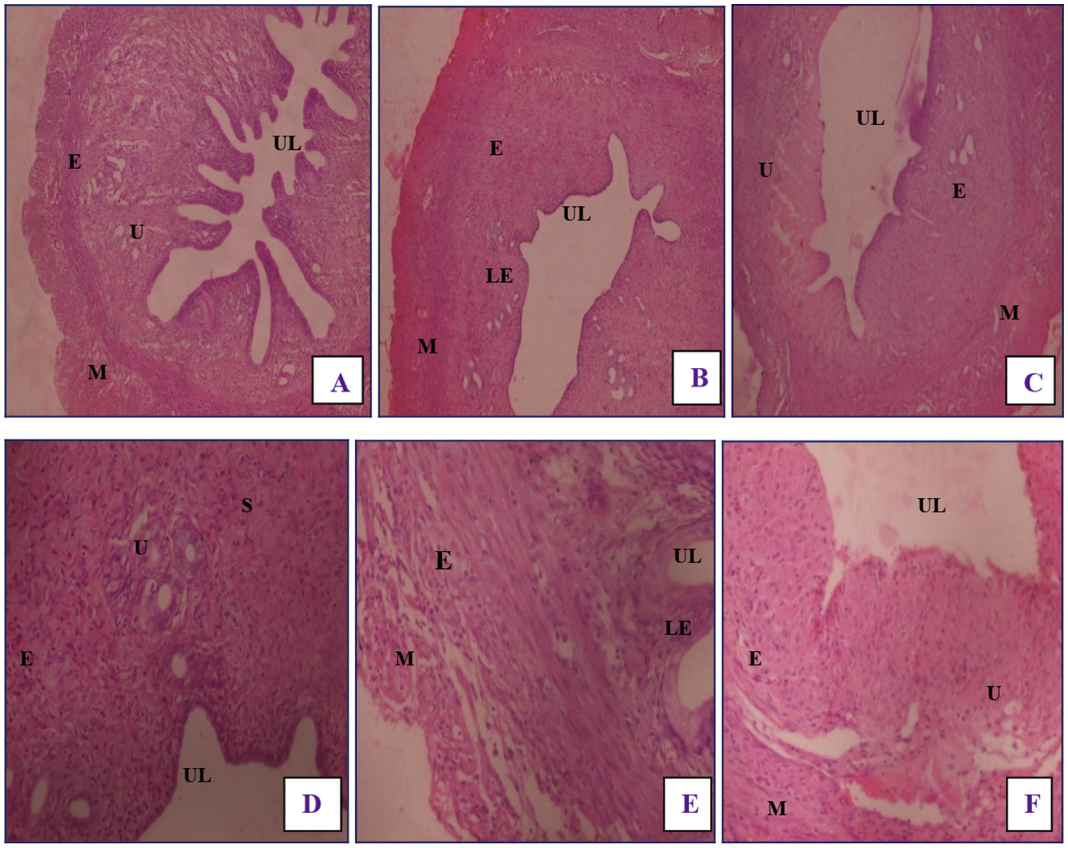

Figure 2. Histological observations of ovary (Hematoxylin and eosin; A,B,C in Mag. ×100; D, E &F are in Mag. ×400).

3.3. Histological observations in vagina

Histological section of vagina in young rat showed well developed stratum mucification, composed of plump epithelial cells containing small mucin-filled vacuoles. The luminal cells were flattened and showed large intercellular spaces. The cytoplasm of these cells was full of tonofilaments. The cells from lower layers were round with wide intercellular spaces.

Histological section of vagina in aged rat, showed atropic changes, decline in vaginal epithelial thickness, a loss of vaginal epithelial rugae, erythema and petechia, and the epithelium is thinner. The stroma showed degenerative changes and appeared flat.

The EV administered vaginal histoarchitecture showed increase epithelial proliferation of the vaginal wall, improving thickening. Superficial cells increased. In addition, there was an increase in epithelial infoldings (pegs) into the lamina propria.

3.4. Histological observations in liver

Liver in young rat showed the central vein, the initial branch of hepatic vein. Hepatocytes radiate like spokes of a wheel, from the central vein, forming anatomizing, fenestrated plates of liver cells, separated from each other by large vascular spaces known as hepatic sinusoids. No changes observed in aged rat liver architecture. Estradiol velerate does not showed any effect on liver architecture.

4. Discussion

4.1. Histological observations of ovary

Figure 1 represents the transverse section of ovary in young, aged and aged administered with estradiol valerate female albino rats in Mag. ×100 & ×400. The young rat ovarian histoarchitecture showed the normal ovoid shape with normal structure of follicles in different stages of development and densely cellular stroma [5]. The developing follicles were well placed and embedded in ovarian stroma together with graafian follicles, corpus lutea and atretic follicles. Numerous ovarian follicles in various stages of development located in the stroma of the cortex. Primordial follicles are more numerous and located in the periphery of the cortex. These follicles are smallest and most simple in structure. Few mature follicles and many atretic follicles are also seen [6].

Figure 1B and E represents histology of aged rat ovary, showed an obvious reduction in the number of follicles and corpora lutea. This characterizes ovarian atrophy in aged female rats [7]. The ovaries diminish in weight, the cortex reduces its dimensions, the follicular apparatus is progressively diminished and the primary follicles completely disappear [8]. The abundant connective tissue invades the parenchyma and the ovarian medulla becomes atrophic. With aging, both atretic follicles and corpora lutea decrease in number [9,10].

No follicles in the cortex, therefore the ovarian cortex are comprised only by the dense stroma and few blood vessels; some of these vessels underwent hyaline degeneration. Inside the ovarian parenchyma are located corpora albicantia – hyaline structures that result from the involution corpora lutea. Vascular hyaline degeneration is present both in the cortical and medullary stroma [11]. The germinal epithelium showed abnormal structure including the appearance of many degrees of invaginations along its surface. The ovarian stroma contained large number of vacuoles, atretic follicles of different sizes and congested blood vessels [6].

Figure 1C and F shows histology of EV administered aged ovary. EV administration improves the ovarian stroma and germinal epithelium. The vascular hyaline degeneration reduces, vascular protective effects of estrogen are well established [11].

4.2. Histological observations of uterus

Figure 2 represents transverse section of uterus in young, aged and aged administered with estradiol valerate female albino rats.

Figure 2A and D represents histology of young uterus, exhibited a well differentiated serosa, muscularis and endometrial layers. Many well developed glands were also present and the stroma was densely cellular [5,12]. The endometrium made up of a single layer of columnar epithelium. In myometrium the lamina propria connected with the compactly arranged smooth muscle layer, surrounded by the serosa and deep to it a rich network of blood vessels [13,14].

Figure 2B and E showed histology of aged uterus. After menopause, the endometrium normally undergoes a gradual atrophy, starting with an inactive phase in which neither proliferation nor secretion is present and ending up as a thin layer, often riddled with cystic cavities lined by a cuboidal or flat epithelium, in which the organelles are pushed to random locations and the stroma becomes fibrotic [15]. Marked changes observed in uterine morphology in aging rats, an age related decrease in estrogen receptor content was detected in the uteri of the aging rats [16].

In aged uterus the uterine lumen was slit-like and lined with small atrophic cuboidal cells. The stroma appeared dense and compact having few, small, inactive uterine glands. The luminal epithelium was low cuboidal and appeared in irregular, the uterine stroma was thickened. In some parts of the stroma, there were clusters of endometrial glands. These glands probably formed down growths of the luminal epithelium and consisted of low cuboidal epithelial cells. The myometrium and endometrial layers were reduced in thickness.

Figure 2C and F represents effect of estradiol valerate on aged uterine architecture. The endometrial tissue is a sensitive

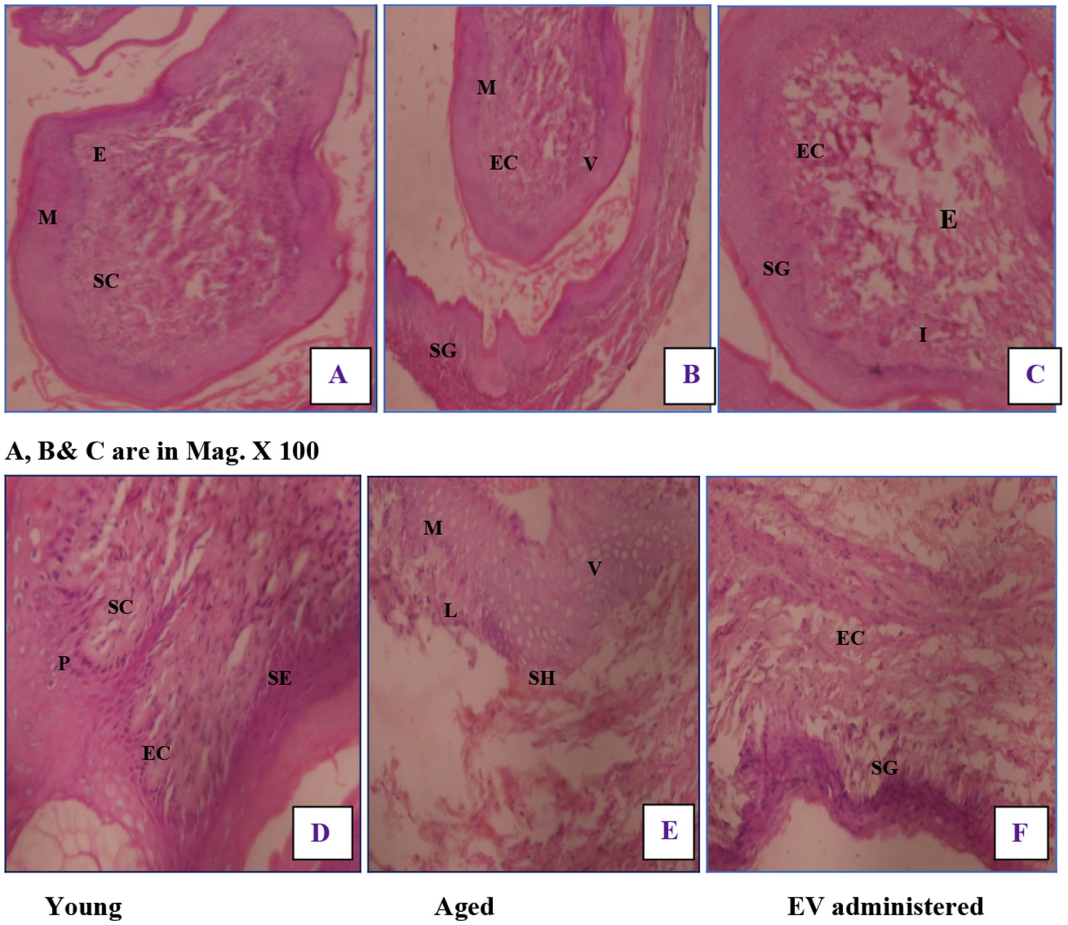

Figure 3. Histological observations of ovary (Hematoxylin and eosin; A,B,C in Mag. ×100; D, E &F are in Mag. ×400).

target for steroid sex hormones and is able to modify its structural characteristics with promptness and versatility. Hormone replacement therapy with estrogen alone may result in continuous endometrial proliferation, hyperplasia, and neoplasia [17].

The EV administration may be associated with endometrial hyperplasia and neoplasia [18]. The uterine lumen was enlarged. The luminal epithelial cell height was reduced. The stroma was comparatively less edematous with less active endometrial glands [12]. The uteri exhibited endometrial epithelial cell pseudo stratification and hypertrophy.

4.3. Histological observations of vagina

Figure 3 represents transverse section of vagina in young, aged and aged administered with estradiol valerate female albino rats.

As shown in Figure 3A and D, histological section of vagina in young rat showed well developed stratum mucification, composed of plump epithelial cells containing small mucin-filled vacuoles. The vagina is a fibromuscular tube with a wall. The luminal cells were flattened and showed large intercellular spaces. The cytoplasm of these cells was full of tonofilaments. The cells from lower layers were round with wide intercellular spaces [19].

Figure 3B and E shows histological section of vagina in aged rat, decline in vaginal epithelial thickness, a loss of vaginal epithelial rugae, erythema and petechia, and the epithelium is thinner [20].

The term vaginal atrophy describes vaginal walls that are thin, pale, dry and sometimes inflamed (i.e., atrophic vaginitis). When normal premenopausal circulating estrogen levels decrease during perimenopause or after induced menopause, the vagina shortens and narrows. The vaginal walls may exhibit small petechiae (i.e., pinpoint, nonraised, round, purple-red spots caused by intradermal or submucous hemorrhage) and become thinner (often only a few cell layers thick), less elastic and progressively smoother as rugal folds decrease. Vaginal blood flow diminishes. Although the sebaceous glands remain prominent, their secretions diminish [21]. In aged vagina the stroma showed degenerative changes and appeared flat [22]. Reduced number of polymorphonuclear cells, reduced keratinization, vaginal wall thickness decreases.

Figure 3C and F shows that EV administered vaginal histoarchitecture showed increase epithelial proliferation of the vaginal wall [22], improving thickening. Superficial cells increased [20]. In addition, there was an increase in epithelial infoldings (pegs) into the lamina propria and estradiol induced keratinization.

4.4. Histological observations of liver

Figure 4 shows the histological observations of liver in young, old and estradiol velerate administered female rats in Mag. ×100 & ×400.

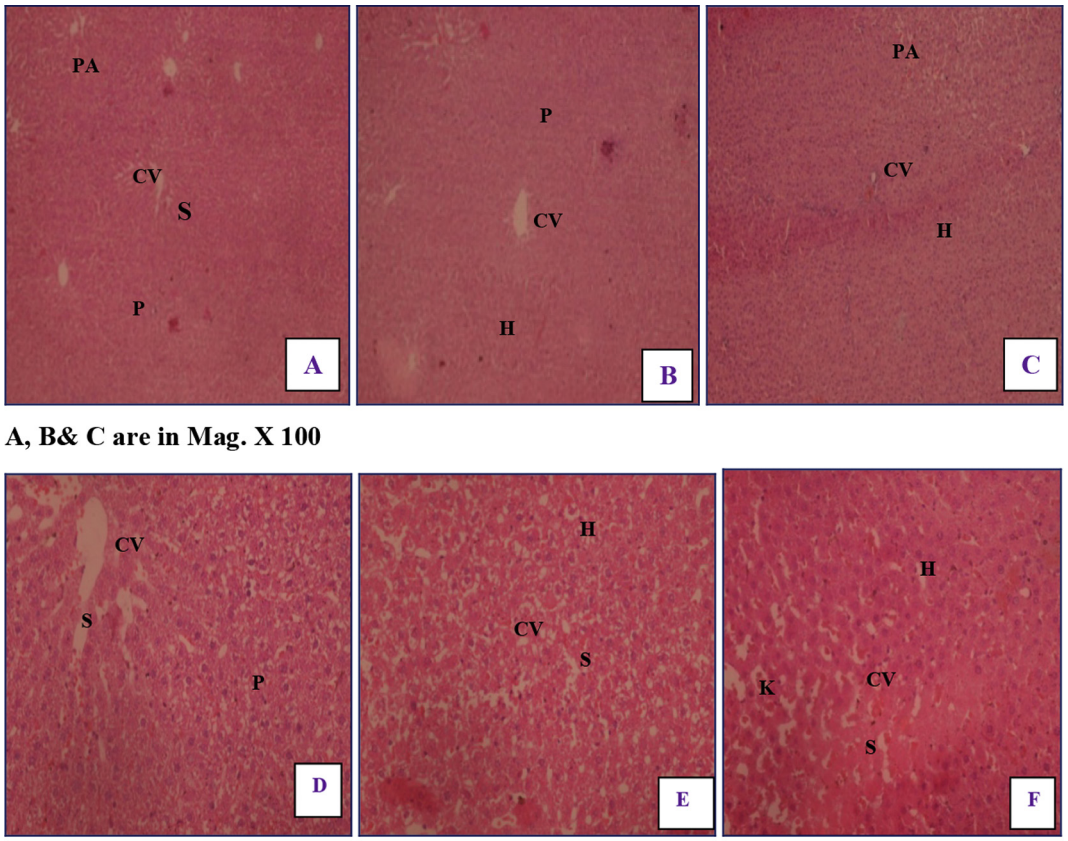

Figure 4. Histological observations of ovary (Hematoxylin and eosin; A,B,C in Mag. ×100; D, E &F are in Mag. ×400).

Figure 4A and D is the liver architecture in young female rat. Liver in young rat showed the central vein, the initial branch of hepatic vein. Hepatocytes radiate like spokes of a wheel, from the central vein, forming anatomizing, fenestrated plates of liver cells, separated from each other by large vascular spaces known as hepatic sinusoids. The portal areas were isolated from the liver parenchyma. [23] Central vein and portal triad present [24].

Figure 4B and E shows histological observations in liver of aged female rat. No changes observed in aged rat liver. The postmenopausal women are more prone to liver damage and exhibit altered liver function, as the age advances. Any form of hepatic cell damage can result in an elevation in the serum glutamate pyruvate transaminase [25]. In earlier reports serum glutamate pyruvate transaminase levels were reduced in aged liver [26]. This supports the normal architecture of liver in aged rats.

Figure 4C and F shows histological observations in liver of aged female rat administered with estradiol velerate. Estradiol velerate does not showed any effect on liver architecture.

The EV administration improves the ovarian stroma and germinal epithelium and the vascular hyaline degeneration reduces, may be associated with endometrial hyperplasia and neoplasia. The vaginal histoarchitecture showed increased epithelial proliferation of the vaginal wall, improving thickening. Superficial cells increased. Estradiol velerate does not showed any effect on liver architecture.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgment

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