



Estradiol Valerate (Progynova) On Uterine & Vaginal Metabolites of Aged Female Albino Rats

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Accepted on: 19-07-2013; Finalized on: 30-09-2013.

ABSTRACT

The female reproductive system includes the vagina, uterus, ovaries and external genitalia. The ovarian-uterine interrelationship forms an essential prerequisite for normal operation of sexual cycles in mammals. The treatment of estradiol valerate to old aged female albino rats shows some influence on the structural composition of uterus and vagina. The decreased uterine & vaginal proteins by treatment confirm the increased protease activity and slower protein biosynthetic machinery in uterus and vagina. The estradiol has direct influence on sex accessories by reduced carbohydrates. The reduced uterine cholesterol in old rats suggests the reduced endometrial proliferation. EV treatment enhances the uterine endometrial proliferation. The alterations in uterine & vaginal phospholipids, free fatty acids and triglycerides indicates the treatment reduces the uterine and vaginal prolapse.

Keywords: Aging, Estradiol valerate, Lipids, Proteins, Uterus, Vagina.

INTRODUCTION

Reproductive aging in female mammals is characterized by alterations in the levels and release pattern of the sex steroid hormone, estrogen. In women, estrogen concentrations undergo a precipitous decline at menopause, and the risks and benefits of estrogen replacement therapy on the reproductive tract, bone, cardiovascular system and brain are quite controversial.¹⁻³ Metabolic syndrome (MES) is a cluster of cardiovascular risk factors that is characterized by obesity, atherogenic dyslipidemia, and hypertension.⁴⁻⁶ Changes occur in the intricate relationship between the ovarian hormones and those produced by the pituitary gland (in the brain).⁷⁻⁹ The causes of these changes are not well understood, majorly due to the decreased levels of estrogens produced by the ovaries. It is the primary circulating estrogen before menopause.¹⁰ The ovaries become less responsive to stimulation by follicle-stimulating hormone (FSH) and luteinizing hormone (LH). To try to compensate for the decreased response, the body produces more of these ovary-stimulating hormones for a time.¹¹ Osteoporosis risk is greater in older women. This is caused, in part, by decreased estrogen levels.¹² The pubic muscles lose tone, and the vagina, uterus, or urinary bladder can fall out of position. This is called vaginal prolapsed, bladder prolapsed, or uterine prolapsed, depending on which structure drops. Estradiol is the most potent naturally occurring ovarian estrogen in mammals.¹³ Estradiol is effective for replacement therapy in woman and used to treat symptoms associated with menopause and in which hormone levels are low and to prevent osteoporosis. Estradiol is used to treat symptoms of menopause such as hot flashes, and vaginal dryness, burning, and irritation. Estrogens are used as part of some oral contraceptives, in estrogen replacement therapy for postmenopausal women, and in hormone replacement therapy for trans

women.¹⁴ The ovarian-uterine interrelationship forms an essential prerequisite for normal operation of sexual cycles in mammals.¹⁵ Hence, the present study was undertaken in order to find out the effect of estradiol valerate on old female rat uterus and vagina.

MATERIALS AND METHODS

Healthy young (4 months old) and old age (20 months old) female Wistar strain albino rats were taken and divided in to three batches. First batch young rats, second batch are old age rats and third batch are old age rats administered with Estradiol valerate (progynova tablets) (2mg/animal/day) orally for one week. All animals were maintained in standard air conditioned animal house at a temperature of 25±2°C, exposed to 12-14h day & light and fed on standard rat feed obtained from Hindustan Lever Ltd., Bombay, India. The usage of animals was approved by the Institutional Animal Ethics committee (Regd.No. 438/01/a/CPCSEA/dt.17/02/2001) in its resolution number 9/IAEC/SVU/Zoo/dt.4-3-2002. Twenty four h after the last dose, the animals were autopsied, the tissues like uterus & vagina were isolated chilled immediately and used for biochemical analysis. The TSI (Tissue Somatic Index), dry matter and water content were analyzed gravimetrically. The total proteins¹⁶, total carbohydrates¹⁷, total lipids¹⁸, total cholesterol¹⁹, Phospholipids²⁰, Triglycerides²¹ and free fatty acids²² were estimated in young, old age and old age +estradiol valerate administered rat tissues

RESULTS AND DISCUSSION

The data represented in table 1-3 indicates the gravimetric analysis, proximate analysis and lipid profiles of uterus in young, old and old treated with estradiol valerate (EV) female albino rats. The data represented in table 4-6 indicates the gravimetric analysis, proximate



analysis and lipid profiles of vagina in young, old and old treated with estradiol valerate (EV) female albino rats.

The body weight significantly increased (+24.81%) by age and the treatment does not shows any significant changes on the body weight. The weight of the uterus and TSI

were enhanced (+27.38%; +104.2%) by age and reduced (-16.5%; -17.15%) by treatment. The decreased dry matter (-75.18%) with increased (+50.1%) water content by age while two fold increment of dry matter by treatment with reduced water content (-22.2%) were noticed.

Table 1: Gravimetric analysis in **Uterus** of young, old and old + estradiol valerate treated rats

Parameter	Young (1)	% Change Significance (1&2)	Old (2)	% Change significance (2&3)	Old +Treatment (3)
Body weight (g)	200.50 ±18.43	+24.81*	250.25 ±17.89	+0.59 NS	251.75 ±24.23
Total organ weight (g)	0.471 ± 0.02	+27.38*	0.600 ± 0.04	-16.5*	0.501 ±0.003
T S I	0.117 ± 0.01	+104.2*	0.239 ± 0.02	-17.15**	0.198 ± 0.02
Dry matter (mg/g wet wt.)	400.4 ± 30.1	-75.18*	100.4 ± 9.3	+200*	300.1± 25.2
Water content (%)	60.5 ± 5.9	+50.1*	90.2 ± 7.2	-22.2*	70.1± 5.9

Mean± SD of six individual observations; + and – indicates percent increase and decrease respectively over control; *indicates P<0.001; **indicates P<0.01 the level of significance.

Table 2: proximate analysis in **Uterus** of young, old and old + estradiol valerate treated rats

Parameter	Young (1)	% Change Significance (1&2)	Old (2)	% Change significance (2&3)	Old + Treatment (3)
Total proteins (mg/g wet wt.)	50.00 ± 3.99	+ 15.58**	57.72± 3.79	-14.27*	39.54 ± 2.96
Total carbohydrates (mg/g wet wt.)	2.224± 0.123	+12.41**	2.501 ± 0.109	-16.6*	2.085 ± 0.135
Total lipids (mg/g wet wt.)	333.3± 21.23	+49.86*	499.5± 41.10	+0.28 NS	500.9± 40.13

Mean± SD of six individual observations; + and – indicates percent increase and decrease respectively over control; * indicates P<0.001 the level of significance; **indicates P<0.01, NS indicates Non significant changes.

Table 3: Lipid profiles in **uterus** of young, old and old + estradiol treated rats

Parameter	Young (1)	% Change Significance (1&2)	Old (2)	% Change significance (2&3)	Old + Treatment (3)
Total Cholesterol (mg/g wet wt.)	1.84 ±0.09	-28.80*	1.31 ±0.11	+106.8*	2.71±0.13
Phospholipids (mg/g wet wt.)	29.63 ±1.89	+33.61*	39.59 ±2.32	-66.38*	13.34 ±0.89
Free fatty acids (mg/g wet wt.)	29.92 ±1.89	+15.3**	34.5 ±2.46	+16.14**	40.07 ±2.01
Triglycerides (mg/g wet wt.)	3.095 ±0.223	-11.4****	2.75 ±0.234	+22.7**	3.357 ±0.312

Mean± SD of six individual observations; + and – indicates percent increase and decrease respectively over control; * indicates P<0.001 the level of significance; **indicates P<0.01; **** indicates 0.02; NS indicates Non significant changes.

Table 4: Gravimetric analysis in **vagina** of young, old and old + estradiol treated rats

Parameter	Young (1)	% Change Significance (1&2)	Old (2)	% Change significance (2&3)	Old + Treatment (3)
Total organ weight (g)	0.069 ±0.004	+5.79 NS	0.073 ±0.006	-43.83*	0.041 ±0.001
T S I	0.034 ±0.02	-14.70*	0.029 ±0.001	+44.82*	0.016 ±0.001
Dry matter (mg/g wet wt.)	70.3 ±6.2	+1.42 N S	70.9 ±5.7	+14.28*	80.6 ±6.2
Water content (%)	300.9 ±25.6	-0.03 N S	300.6 ±22.7	-33.3*	200.7 ±18.9

Mean± SD of six individual observations; + and – indicates percent increase and decrease respectively over control; * indicates P<0.001 the level of significance; NS Non significant changes.

Table 5: Proximate analysis in **vagina** of young, old and old + estradiol valerate treated rats

Parameter	Young (1)	% Change Significance (1&2)	Old (2)	% Change significance (2&3)	Old + Treatment (3)
Total proteins (mg/g wet wt.)	61.92 ±4.73	+25.17*	46.33 ±3.94	-12.92***	40.34 ±3.26
Total carbohydrates (mg/g wet wt.)	2.293 ±0.201	+3.009 NS	2.362 ±0.136	-14.08*	2.008 ±0.121
Total lipids (mg/g wet wt.)	100.5 ± 6.3	+65.77*	166.6 ±55.7	-9.54 NS	150.7± 42.7

Mean± SD of six individual observations; + and – indicates percent increase and decrease respectively over control; * indicates P<0.001 the level of significance; ***indicates P<0.05; NS indicates Non significant changes.

Table 6: Levels of lipid profiles in vagina of young, old and old + estradiol treated rats

Parameter	Young (1)	% Change Significance (1&2)	Old (2)	% Change significance (2&3)	Old + Treatment (3)
Total Cholesterol (mg/g wet wt.)	1.39±0.11	+10.79 [*]	1.54 ±0.14	+22.07 [*]	1.88±0.16
Phospholipids (mg/g wet wt.)	53.71±4.12	-65.6 [*]	18.45±1.11	+99.2 [*]	36.76±2.12
Free fatty acids (mg/g wet wt.)	32.05 ±2.78	-6.7 ^{***}	29.9±1.87	+10.03 ^{****}	32.9±2.07
Triglycerides (mg/g wet wt.)	2.928 ±0.199	+1.63 NS	2.976 ±0.233	-6.41 NS	2.785 ±0.267

Mean± SD of six individual observations; + and – indicates percent increase and decrease respectively over control; * indicates P<0.001 the level of significance; ***indicates P<0.05; **** indicates 0.02; NS indicates Non significant changes.

There was no significant change in the weight of the vagina by age but with treatment the weight is significantly decreased (-43.83%). TSI were decreased (-14.7%) with increment (+44.82%) by treatment. There is no change in dry matter and water content by age but slight increase (+14.28%) and decreased (-33.3%) with treatment.

The female reproductive system includes the vagina, uterus, ovaries and external genitalia. The ovarian-uterine interrelationship forms an essential prerequisite for normal operation of sexual cycles in mammals. As women age, the ovaries are less able to make estrogen. The ovaries begin to shrink. Fat and other materials start to replace the cells that make hormones.²³ Without enough estrogen, the uterus gets smaller. The muscles of the uterus shrink and are replaced by fat and other materials. The glands in the uterus also get smaller. The uterine lining loses its elasticity and becomes thinner. The uterine tubes get smaller and weaker with age as well. The most bothersome age-related changes usually occur in the vagina. The vagina gets narrower and shorter with age. The walls become thin and less elastic or stretchable. The glands that normally wet the vagina shrink and secrete less lubricant. This makes the vagina become dry. Thus the dry matter in vagina is enhanced by the treatment.²⁴

The Uterus weight was increased by age and decreased by the treatment. The vagina shows there was no significant change in the total organ weight but treatment decreases the organ weight. In uterus & vagina the treatment enhances (two fold in uterus) dry matter and lowered the water content. These observations revealed that there was some influence on the structural composition of the organs by estradiol treatment.

In sex accessory organs like uterus (+15.58%; P< 0.01) and vagina (+25.17%) the protein content enhanced by age and reduced (-14.27%; -12.92%; P < 0.05) by treatment (Table 2 & 5).

Proteins, carbohydrates and lipids are important components of the organism. Each is essential to keep the body functional and active. The metabolic components like total proteins are decreased less significantly in both uterus and vagina by treatment with some significant reduction was noticed in carbohydrates. The lipids were significantly enhanced in ovary and no change in uterus with reduced lipids in vagina.

The decreased uterine & vaginal proteins by treatment confirm the increased protease activity. Hence the protein biosynthetic machinery seems to have been slower in uterus and vagina.²⁵ Thus the decreased proteins by the EV treatment suggest there was no risk of uterine cancer by the treatment.

In uterus and vagina the treatment lowered the carbohydrate (-16.6% & -14.08%) levels. The carbohydrate metabolism in general and glycogen metabolic wing in particular play an important role in reproductive tissue functions²⁶ and the carbohydrate metabolism was shown to be dependent on the levels of gonadotropins and gonadal hormones.¹² The estradiol treatment does not show any significant effect on ovarian carbohydrates. But in uterus and vagina records different trend as it lowered. Hence it can be concluded that the estradiol have direct influence on sex accessories as estradiol-17 β is secreted from uterus. The reduced carbohydrates were also coinciding with the reduced plasma glucose levels as suggested by Crook et al 1991.²⁷ Hence hyperglycemia was not recommended by the estradiol treatment.

In uterus & vagina the lipids were enhanced (+49.8%, +65.77%) with no changes by treatment. The menopause is associated with potentially adverse changes in lipids and lipoproteins, independent of any effects of ageing. These changes may in part explain the increased incidence of coronary heart disease seen in postmenopausal women.²⁸ In the present study lipids were elevated significantly in both uterus and vagina in old aged rats. Forbes 1986²⁹ suggested that estrogens increase lipid mobilization from adipose tissue to offset the negative energy balance by diverting non esterified fatty acids away from the adipose depots to serve as energy precursors of various tissues. So due to the lowered estrogens in old rats the lipids were accumulated and there by enhanced. The treatment does not shows any effect on uterus but reduced vaginal lipids were observed by the treatment.

Cholesterol levels (Table 3 & 6) were increased (+10.79%) in vagina by age and by the treatment (+22.07%). In uterus there was decreased (-28.8%) cholesterol in old rats and four fold enhancement (+106.8%) by treatment.

The reduced uterine cholesterol in old rats suggests the reduced endometrial proliferation. EV treatment enhances the uterine endometrial proliferation, thus

fourfold enhancement of cholesterol was noticed in uterus.³⁰ The vaginal cholesterol was significantly enhanced, which is also enhanced by EV treatment. The degree of increase being higher in the EV treated rats indicates the vaginal proliferation³¹ by treatment. Since cholesterol biosynthesis and its metabolic clearance in vagina are not clearly understood, the increased cholesterol fractions can be attributed to the increased circulating cholesterol levels observed during this period³². The vagina is not a seat of cholesterol synthesis, active uptake of cholesterol from the circulating fluid might be responsible for their accumulation in the tissue.³³

The uterine phospholipids were shows significant increase by age (+33.61%) and significant decrease (-66.38%) with treatment. But in vagina reduction (-65.6%) in old age two fold enhancement (+99.2%) with treatment were noticed.

In uterus the phospholipids were elevated in old rats where it is reduced by the EV treatment. In old age uterine lipid metabolism seems to be oriented towards lipid oxidations resulting in increased phospholipids. This can be correlated to decreased oxidative metabolism owing to low level of estrogen in circulation. The studies on uterine phospholipids like phosphatidyl choline, sphingomyelin, phosphatidyl inositol, phosphatidyl ethanolamine, cardiolipin, and phosphatidic acid inhibited the binding of estradiol and estrogen receptors.³⁴ Hence the decline estrogen levels also responsible for accumulation of phospholipids in old aged rat uterus. However EV treatment reduces the phospholipids in uterus. There are some reports estradiol enhances the serum phospholipids.³⁵ This may be reason for reduced uptake of phospholipids by uterine tissue. Thus phospholipids were averted by the EV treatment in turn reduces uterine prolapsed.

In vagina, opposite trend was observed. The phospholipids were lowered by age and elevated by EV treatment. The hypo estrogenic changes are associated with the vaginal walls become thinner and vaginal dryness in old aged rats.³¹ The decreased phospholipids may be the reason for vaginal dryness. This vaginal dryness may be averted by the EV treatment by increasing the phospholipids, in turn arrest the vaginal prolapsed.

The uterine free fatty acids were increased significantly (+15.3% & +16.14%). In vagina significant (-6.7%) reduction by age and P<0.02 significant increase by treatment was noticed. The free fatty acids in vagina were significantly decreased in old aged female rats. The decreased vaginal FFAs are due to lowered estrogen levels by age^{36,37} suggest their mobilization towards oxidative metabolism and ketogenesis. But in uterus there was significant increase by both age and treatment. The increased uterine FFAs indicate the relation of 17 β -estradiol which is present in uterus at low levels³⁸, thus leads to inhibition of oxidation & ketogenesis.

The uterine triglycerides were reduced in aged rats and enhanced by estradiol treatment. The vaginal triglyceride does not show any significant effect. In uterus the decreased uterine triglycerides suggests its utilization in uterus by estradiol-17 β which is secreted from uterus itself. The treatment increases the same. The vaginal triglyceride does not show any changes both by age and treatment. Thus the estreadiol valerate reduces the uterine and vaginal prolapsed in old aged rats.

Acknowledgements: The authors were grateful to UGC, New Delhi for financial assistance.

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Source of Support: Nil, **Conflict of Interest:** None.

