Effect of coumestrol on the biochemical constituents of ovary and uterus of pups of Female albino rats

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ABSTRACT

In the current research, attempts were undertaken to evaluate the impact of coumestrol on some biochemical constituents of rats of F1 generation when these animals reached the ages of 2, 4, and 6 months. The administration of coumestrol at different doses of 5, 10 and 25 μg/kg during different days of pregnancy (Group-1 on the 10th and 13th day) and (Group-2 on the 15th and 18th day) caused a substantial rise in different parameters like the wet weight of uterus and ovary, protein contents, glycogen contents, the acid phosphatase, and alkaline phosphatase activity even at the age of 6 months.

Introduction

Phytoestrogens are the estrogenic compounds that are present in plants and mimic the estrogenic action similar to that of steroidal origin. Coumestrol is one of the phytoestrogens which is present in a number of plants and mimic the estrogenic effect. Biochemical constituents in the reproductive organs and genital tract of female rats are subjected to fluctuate during various phases of the oestrous cycle. It is well known that when pregnant female consumes such phytoestrogen orally it causes significant changes in their reproductive organs. It is also well established that phytoestrogen produces an inhibitory effect on gonadotropin secretion which may influence biochemical constituents in the ovary and uterus.

Female sex hormones, estrogens, and progesterone of ovarian origin are well known to bring about morphological, histological & biochemical changes in the uterus. Estrogen on one side proliferates the uterine endometrium and on the other side induces oedematous changes. It is also known to increase protein synthesis, the activity of alkaline phosphatase and ATPase, and some carbohydrate-metabolizing enzymes like glucose synthetase. When estrogen, especially the diethylstilbestrol (DES), is given to pregnant rats in different amounts, throughout early pregnancy and later pregnancy, it influences the growth of the fetus in vitro. The effect of estrogen on the gestation period and its effect on neonatal pups delivered from exposed mothers is also established. Phytoestrogens also exert their effect on neonatal pups. Coumestrol is one of the phytoestrogens, which is present in a number of plants, and mimic the estrogenic effects. The Morphological and histological changes seen in the uterus and the ovary are mediated through the biochemical parameters and consequently, in this study attempts have been made in order to evaluate the impact of coumestrol on some biochemical constituents of rats of F1 generation when these animals reached to the age of 2, 4 and 6 months.

Materials and Methods

Male rats with proven fertility and healthy virgin females were kept in the cage for the whole night in a 1:2 ratio. The next day in the morning these were examined for the presence of vaginal plug and spermatozoa. Females who were found positive were selected and the day was marked to be the first day of pregnancy. Animals were splitted into two different groups. The first group functioned as control and received vehicles only and 2nd group was considered experimental and was further divided into two groups. Animals of Group-1 received different doses viz. 5, 10 and 25 μg/kg on the 10th and 13th days of pregnancy and animals of Group-2 received these different doses...
on the 15th and 18th days of pregnancy. After delivery, female pups were observed and were sacrificed at 2, 4, and 6 months. Ovaries and uterus were removed, freed from any adhering tissues, blotted on filter paper and weighed to the nearest of 1 mg on a monopan balance, and processed for the biochemical assessment of the acid and alkaline phosphatase Activity. Protein and Glycogen were examined statistically with a student's t-test.

Preparation of Homogenates: The homogenates were prepared by taking already weighed tissue at the concentration of 50 mg/ml with a chilled hypotonic solution of sodium bicarbonate (0.05%). For this purpose, the tissues were minced and then transferred into a chilled glass homogenizer along with the required quantity of hypotonic solution, with the help of a powerful stirring motor. The pestle of the homogenizer was rotated at 600-1000 rpm. All the subsequent steps in the procedure were carried out from 0°C to 4°C. The prepared homogenate was kept in ice and suitable aliquots were taken for various estimations. For the estimation of glycogen contents, fresh tissue was processed.

In order to estimate Glycogen, the approach was followed with some alterations for glycogen estimation.

Similarly for assessment of the overall protein approach was followed with some alterations. For assessment of the acid and alkaline phosphatase activity, the enzymatic activity of acid and alkaline phosphatase was assessed.

Results
(a) Influence on wet weight-
Table 1 shows the influence of coumestrol on the "wet weight" of the ovary and uterus of rats of F1 generation when given different doses on the 10th and 13th day of gestation (Group-1). It shows that the administration of coumestrol at 5, 10, and 25 μg/kg caused a substantial increase in ovarian and uterine wet weight in 2 months old rats. Similarly, 4 months old rats showed increased wet weight in the ovary and uterus at all three doses. Even at 6 months of age, the animals showed relatively higher wet weight in both the organs when compared to their respective groups (P Vs. respective control < 0.05). When 3 doses were compared, the response was almost similar. When coumestrol was administered on the 15th and 18th days of gestation (Group -2) similar results were obtained. It is important to note that the wet weight of the ovary was markedly improved at 6 months of age due to the presence of a tumour in both ovaries.

Effect on Biochemical constituents
1. Glycogen Contents -
Table 2 revealed the influence of coumestrol on the glycogen contents of the ovary and uterus of 2-, 4- and 6-monthold rats of the F1 generation. When coumestrol was administered at 5, 10, and 25 μg/kg
doses on the 10th and 13th day of gestation. It is seen that the administration of different doses led to a significant increase in ovarian and uterine glycogen contents. Similarly, 4 months old rats indicated a substantial rise in glycogen content in both ovary and uterus at all three doses. At the age of 6 months, rats displayed relatively increased glycogen contents when compared to their respective group (P Vs control < 0.05). When coumestrol was administered on the 15th and 18th (Group-2) day of gestation all 3 doses of 5, 10, and 25 μg/kg showed similar responses as in rats of Group-I.

2. Protein content-

Table - 3 revealed 5, 10, and 25 μg/kg doses of coumestrol on total protein contents of ovary and uterus of 2-, 4-, and 6-monthold rats of F1 generation showed that administration of coumestrol increased the protein contents remarkably, which was maintained even at 4 and 6 months of age. Protein contents of the ovary and uterus were remarkably increased at 2 and 4 months of age but at the age of 6 months, protein contents were increased in the ovary (P Vs control < 0.05). In the animals of Group-2 treatment of coumestrol resulted in a marked rise in protein levels in the ovary and uterus at 2, 4, and 6 months of age. (P Vs control < 0.05).

3. Acid Phosphatase Activity-

Table - 4 showed the influence of coumestrol on the acid phosphatase activity in the ovary and uterus of 2 months old rats of F1 generation when coumestrol was administered at different doses on the 10th and 13th day of gestation (Group-1). It showed that the administration at 5, 10, and 25μg/kg caused a substantial rise in the acid Phosphatase activity of the ovary and uterus. Similarly, 4 months old rats showed raised activity of acid phosphatase in the ovary and uterus at all three doses. Even at 6 months of age animals showed relatively higher acid phosphatase activity in both organs when compared to their respective group (P Vs control < 0.05). When coumestrol was given on the 15th and 18th day of gestation, the response of all three doses was similar to that of animals of Group - 1.

Table - 5 showed the influence of coumestrol on the alkaline phosphatase activity in the ovary and uterus of rats of F1 generation when coumestrol was administered at different doses on the 10th and 13th days of gestation under Group1, it showed a substantial rise in the alkaline phosphatase activity in ovary and uterus of 2 months old rats at all 3 doses. At 4 and 6 months of age, animals showed relatively higher alkaline phosphatase activity in the ovary & uterus when compared to their respective group (P Vs control < 0.05). When coumestrol was given on the 15th and 18th day of gestation at 5,10 and 25 μg/kg doses, responses in alkaline phosphatase activity of ovary and uterus of 2, 4 and 6 months old rats were almost similar to that of rats of Group -1.

Discussion

Biochemical constituents in the reproductive
organs and genital tract of female rats are subjected to fluctuate during various phases of the oestrous cycle. Ovarian and uterine alterations in the morphological features are mediated through biochemical modifications. The ovary of cyclic female rats undergoes changes in biochemical constituents starting from the formation of primordial germ cells to the maturation of follicles and finally at ovulation. It is a known fact that follicular fluids which accumulate in the central cavity of a follicle, contain basic biochemical constituents, which are rich in enzymes. Accumulation of more fluid in the lumen helps in further development proteolytic enzymes are known to exert their effect to induce ovulation. Similarly, uterine biochemical constituents are responsible for the growth of different cells and maintain a proper endometrial echo in rats. These alterations are exclusively hormone dependent. It is known that estrogen and progesterone of ovarian origin bring about changes in biochemical constituents in the uterus.

Phytoestrogens are the estrogenic compounds, which are present in plants and mimic the estrogenic action similar to that of steroidal estrogen. There is a number of phytoestrogens that are known to exert their effect on the ovary and uterus of cyclic rats. A group of isoflavonoides, which belongs to daidzein and genistein, is considered a potent phytoestrogen. Coumestrol is another phytoestrogen, which also behaves like isoflavonoides. These phytoestrogens are consumed through diet and high dietary phytoestrogens are related to bone, mineral density, and other metabolic disorders in women. Similarly, phytoestrogens produce inhibitory effects on gonadotropin secretion, which may influence biochemical constituents in the ovary and uterus.

There are a number of medicinal plants which are known to possess estrogenic activity and are consumed by animals.

Some of the potent medicinal plants are Embelia ribes, Ferula Jaeschkeana, Pueraria tuberosa, Hibiscus rosasinensis, Daucus carota and Moringa oleifera etc. The wet weight of the reproductive organs has been considered one of the important parameters to assess the role of hormones. Investigators observed6 that the wet weight of the uterus and ovary was reduced when the extract of Hibiscus rosasinensis was administered. Alcoholic extract of Dacus carota reduced the wet weight of the uterus and ovary5. A worker has17 reported that extract of Pueraria tuberosa increased the wet weight of the vagina, cervix, and uterus while no noticeable difference was seen in the ovary’s wet weight. Some workers18 noticed that aqueous extract administration of the Moringa oleifera caused a rise in the uterine wet weight of ovariectomized rats. An investigator reported1 that the ethanolic extract administration of Ferula jaeschkeana enhanced the wet weight of the ovary and uterus at single and or multiple doses. Medicinal plant extracts having estrogenic properties are also recognized to increase the wet weight

<table>
<thead>
<tr>
<th>Months</th>
<th>Organ</th>
<th>Control M ± Sc</th>
<th>5 µg/kg</th>
<th>10 µg/kg</th>
<th>25 µg/kg</th>
<th>Control</th>
<th>5 µg/kg</th>
<th>10 µg/kg</th>
<th>25 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Months</td>
<td>Ovary</td>
<td>10.5 ± 0.45</td>
<td>12.5 ± 0.52*</td>
<td>12.9 ± 0.42*</td>
<td>13.0 ± 0.46*</td>
<td>11 ± 0.46</td>
<td>13.5 ± 0.42*</td>
<td>14 ± 0.55*</td>
<td>14.5 ± 0.46*</td>
</tr>
<tr>
<td>2 Months</td>
<td>Uterus</td>
<td>12 ± 0.47</td>
<td>13.8 ± 0.49*</td>
<td>14 ± 0.50*</td>
<td>14.8 ± 0.51*</td>
<td>12.8 ± 0.57</td>
<td>14.8 ± 0.49*</td>
<td>15.5 ± 0.51*</td>
<td>16 ± 0.55*</td>
</tr>
<tr>
<td>4 Months</td>
<td>Ovary</td>
<td>11.3 ± 0.44</td>
<td>13.4 ± 0.46*</td>
<td>13.6 ± 0.48*</td>
<td>13.9 ± 0.49*</td>
<td>12.0 ± 0.48</td>
<td>14.2 ± 0.51*</td>
<td>14.8 ± 0.54*</td>
<td>15.2 ± 0.55*</td>
</tr>
<tr>
<td>4 Months</td>
<td>Uterus</td>
<td>12.5 ± 0.50</td>
<td>14.5 ± 0.52*</td>
<td>14.9 ± 0.51*</td>
<td>15.4 ± 0.54*</td>
<td>13.2 ± 0.52</td>
<td>15.2 ± 0.55*</td>
<td>16.5 ± 0.61*</td>
<td>16.9 ± 0.56*</td>
</tr>
<tr>
<td>6 Months</td>
<td>Ovary</td>
<td>12 ± 0.47</td>
<td>14.9 ± 0.58*</td>
<td>16 ± 0.55*</td>
<td>16.5 ± 0.53*</td>
<td>13 ± 0.51</td>
<td>14.8 ± 0.51*</td>
<td>15.2 ± 0.61*</td>
<td>16.5 ± 0.55*</td>
</tr>
<tr>
<td>6 Months</td>
<td>Uterus</td>
<td>14 ± 0.49</td>
<td>16 ± 0.54*</td>
<td>16.2 ± 0.56*</td>
<td>16.7 ± 0.60*</td>
<td>14.4 ± 0.61</td>
<td>16.6 ± 0.56*</td>
<td>16.5 ± 0.43*</td>
<td>16.5 ± 0.53*</td>
</tr>
</tbody>
</table>

TABLE-3: Effect of Coumestrol in total protein contents of ovary and uterus of 2, 4 & 6 months old rats of F1 generation, Values are Mean ± SE and expressed as mg/100g body weight 5 animals were used in each group.

Statistical Analysis
*P Value V/s respective Control < 0.05.
of reproductive organs. Moreover, the rise in the uterine wet weight is related to the protein synthesis in these organs. Several phytoestrogens and medicinal plant extracts are also recognized to increase the total protein contents in the uterus. An investigator has noted that the administration of ethanolic extracts of *Ferula jaeschkeana* remarkably increased protein synthesis, especially in the uterus due to its estrogenic effects. A worker has reported that administration of hexane extract of *Ferula jaeschkeana* does not indicate any substantial raise in the protein content of the ovary during the first days of treatment, however, a substantial increase was observed on days 4 and 5 post coitum.

In general, estrogens and progesterone of ovarian origin alter the glycogen metabolism in the uterus. It is a well-known fact that the administration of estrogens increases uterine glycogen in adult rats. Many other plants and phytoestrogens are known to increase the glycogen contents in the uterus. An investigator has noted that the administration of *Ferula jaeschkeana* 's ethanolic extract in single or multiple doses enhanced the uterine glycogen contents.

Ovarian hormones estrogens and progesterone regulate the activity of some enzymes like acid phosphatase and alkaline phosphatase within the genital tract. A worker noted that hexane extract administration of *Ferula jaeschkeana* indicated no substantial changes in the acid phosphatase activity of the ovary range from day 1 to 5 post coitum. Extracts or medicinal plants were reported to change the acid & alkaline phosphatase activity in the uterus viz. *Pueraria tuberosa*, *Juniperus communis*, *Terminalia arjuna*, *Ferula jaeschkeana*, and *Nerium odorum*. A worker described that the effect of *Ferula jaeschkeana* ethanolic extract at single or multiple doses is duration dependent. It was noted that single oral administration of extract increased the acid phosphatase activity within the uterus even after 6 days of administration and the effect was not observed in the ovary, however, daily administration of extract maintained the high acid phosphatase activity in the ovary and uterus. Alkaline phosphatase activity is also increased.

**TABLE- 4: Effect of Coumestrol on activity of acid phosphatase of ovary and uterus of 2, 4 & 6 months old rats of F1 generation, Values are Mean ± SE and expressed as mg/100g body weight 5 animals were used in each group.**

<table>
<thead>
<tr>
<th>Months</th>
<th>Organ</th>
<th>Control M ± Sc</th>
<th>5 µg/kg</th>
<th>10 µg/kg</th>
<th>25 µg/kg</th>
<th>Control</th>
<th>5 µg/kg</th>
<th>10µg/kg</th>
<th>25 µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Months</td>
<td>Ovary</td>
<td>200 ± 8.33</td>
<td>283 ± 9.02*</td>
<td>305 ± 8.99*</td>
<td>326 ± 8.9*</td>
<td>2.10 ± 9.48</td>
<td>275 ± 9.13*</td>
<td>296 ± 10.2*</td>
<td>313 ± 10.8*</td>
</tr>
<tr>
<td>2 Months</td>
<td>Uterus</td>
<td>112 ± 4.54</td>
<td>130 ± 4.70*</td>
<td>140 ± 4.86*</td>
<td>167 ± 5.17*</td>
<td>115 ± 4.53</td>
<td>138 ± 4.61*</td>
<td>151 ± 4.94*</td>
<td>186 ± 4.51*</td>
</tr>
<tr>
<td>4 Months</td>
<td>Ovary</td>
<td>232 ± 9.56</td>
<td>295 ± 9.86*</td>
<td>320 ± 10.8*</td>
<td>330 ± 10.8*</td>
<td>245 ± 9.59</td>
<td>301 ± 9.95*</td>
<td>323 ± 10.3*</td>
<td>367 ± 10.4*</td>
</tr>
<tr>
<td>4 Months</td>
<td>Uterus</td>
<td>128 ± 4.86</td>
<td>152 ± 6.51*</td>
<td>158 ± 6.04*</td>
<td>168 ± 6.22*</td>
<td>133 ± 5.22</td>
<td>156 ± 6.25*</td>
<td>165 ± 6.37*</td>
<td>170 ± 7.60*</td>
</tr>
<tr>
<td>6 Months</td>
<td>Ovary</td>
<td>236 ± 9.88</td>
<td>298 ± 10.8*</td>
<td>325 ± 10.2*</td>
<td>346 ± 10.3*</td>
<td>248 ± 10.4</td>
<td>295 ± 10.3*</td>
<td>327 ± 11.1*</td>
<td>352 ± 10.5*</td>
</tr>
<tr>
<td>6 Months</td>
<td>Uterus</td>
<td>139 ± 4.29</td>
<td>156 ± 4.71*</td>
<td>160 ± 6.11*</td>
<td>169 ± 6.12*</td>
<td>126 ± 5.86</td>
<td>159 ± 6.47*</td>
<td>167 ± 6.60*</td>
<td>186 ± 4.51*</td>
</tr>
</tbody>
</table>

Statistical Analysis
*P Value V/s respective Control < 0.05.

The effect of coumestrol on the biomarkers, when administered in utero has been studied. The coumestrol was administered in the diet to pregnant rats during their gestation and the animal was sacrificed. Reproductive organs, liver, and blood were studied for enzymes and plasma hormonal levels. Authors have reported that coumestrol administration significantly affected hepatic glutathione, s-transferase (GST). The level of 17-estradiol in offsprings from coumestrol exposed dams was substantially higher when compared to control. These studies have indicated that the administration of coumestrol in diet during gestation is transported to the foetus through the placenta and thus influences the growth of the foetus, however, authors have not recognized for how long the level of 17-estradiol remains high. Similarly, prenatal exposure to estrogenic compounds is known to alter the expression patterns.
TABLE- 5: Effect of Coumestrol on activity of alkaline phosphatase of ovary and uterus of 2, 4 & 6 months old rats of F₁ generation, Values are Mean ± SE and expressed as mg/100g body weight 5 animals were used in each group.

<table>
<thead>
<tr>
<th>Months</th>
<th>Organ</th>
<th>Control</th>
<th>5 μg/kg</th>
<th>10 μg/kg</th>
<th>25 μg/kg</th>
<th>Control</th>
<th>5 μg/kg</th>
<th>10 μg/kg</th>
<th>25 μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Months</td>
<td>Ovary</td>
<td>210 ± 8.74</td>
<td>298 ± 8.38*</td>
<td>329 ± 9.36*</td>
<td>334 ± 9.37*</td>
<td>215 ± 8.8</td>
<td>308 ± 9.09*</td>
<td>324 ± 8.29*</td>
<td>339 ± 9.86*</td>
</tr>
<tr>
<td>2 Months</td>
<td>Uterus</td>
<td>256 ± 9.56</td>
<td>328 ± 10.4*</td>
<td>355 ± 10.6*</td>
<td>362 ± 10.6*</td>
<td>252 ± 10.1</td>
<td>325 ± 9.75*</td>
<td>355 ± 10.6*</td>
<td>369 ± 10.2*</td>
</tr>
<tr>
<td>4 Months</td>
<td>Ovary</td>
<td>233 ± 9.87</td>
<td>326 ± 10.2*</td>
<td>339 ± 9.4*</td>
<td>343 ± 10.8*</td>
<td>246 ± 9.79</td>
<td>329 ± 9.99*</td>
<td>357 ± 10.4*</td>
<td>359 ± 10.04*</td>
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<tr>
<td>4 Months</td>
<td>Uterus</td>
<td>278 ± 10.9</td>
<td>348 ± 10.9*</td>
<td>393 ± 11.4*</td>
<td>390 ± 10.9*</td>
<td>280 ± 11.5</td>
<td>348 ± 11.08*</td>
<td>395 ± 11.3*</td>
<td>395 ± 12.9*</td>
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<tr>
<td>6 Months</td>
<td>Ovary</td>
<td>235 ± 9.60</td>
<td>339 ± 9.84*</td>
<td>347 ± 10.2*</td>
<td>359 ± 10.4*</td>
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<td>348 ± 10.4*</td>
<td>363 ± 10.9*</td>
<td>398 ± 10.6*</td>
</tr>
<tr>
<td>6 Months</td>
<td>Uterus</td>
<td>280 ± 10.86</td>
<td>351 ± 10.7*</td>
<td>387 ± 11.3*</td>
<td>398 ± 11.4*</td>
<td>282 ± 11.6</td>
<td>357 ± 11.3*</td>
<td>397 ± 12.1*</td>
<td>410 ± 21.1*</td>
</tr>
</tbody>
</table>

Statistical Analysis
*P Value V/s respective Control < 0.05.

and growth factor receptors in the reproductive organs in rats.

Some investigators have reported the influence of coumestrol on ovarian wet weight and insulin receptors in ovarioctomized rats and have compared the response with the known estrogens. Authors have also reported that zearalenone and coumestrol caused a marked rise in uterine wet weight similar to the influence of estrone action. Similarly, there are a number of authors who have reported that phytoestrogens affect the uterine wet weight and also many biochemical constituents in the uterus and the action is mediated through the gonadotropin-releasing hormone of pituitary origin. Some workers have studied estradiol type coumestrol activity in mature and immature ovarioctomized rats on the basis of uterotrophic assays. Authors clearly reported that uterotrophic activity of coumestrol in such animals is not associated with uterine hyperplasia and DNA contents.

Some others have reported potential adverse effects of phytoestrogens. The administration of dietary phytoestrogens is known to affect the estrus cycle in adult female rats. Developmental stages were also examined on neonatal pups which were delivered by mothers fed on coumestrol and were studied after birth at 10, 21, and 132 days of age. Findings showed a broad range of actions. Some other workers have reported that phytoestrogens found in clover, alfalfa, and soyabeans induced reproductive toxicity in several mammalian species through the alteration in the uterine wet weight and biochemical enzymes. Although the effect of phytoestrogen including coumestrol on postnatal pups has been studied by many authors, however, the formation in the development till 6 months is not well known.

In these findings; the authors have observed that administration of coumestrol in diet led to a noticeable alteration in reproductive organs of female rats of the F₁ generation. Oral administration of coumestrol on the 10th and 13th day of gestation (Group -1) at different doses of 5, 10, and 25 μg/kg caused a substantial rise in ovarian and immature ovarioctomized rats on the basis of uterotrophic assays. Authors clearly reported that uterotrophic activity of coumestrol in such animals is not associated with uterine hyperplasia and DNA contents.

When coumestrol was administered on the 15th and 18th days of gestation (Group -2) at different doses (5, 10, and 25 μg/kg) caused increased wet weights of ovary and uterus of 2 and 4 months old, rats of the F₁ generation, at the age of 6 months wet weight was markedly increased due to the tumor formation in ovary. This tumour appeared due to the estrogenic nature of coumestrol. Several phytoestrogens and extracts of medicinal plants have been shown to increase the total protein contents in the uterus. When coumestrol was administered at 5, 10, and 25 μg/kg doses on the 10th and 13th day of gestation protein content was remarkably increased.
Glycogen contents were increased in the ovary and uterus of 2, 4 and 6 months old rats when their mother consumed coumestrol (at 5, 10 and 25 μg/kg doses) on the 10th and 13th day of pregnancy. When pregnant rats consumed coumestrol on the 15th and 18th day of gestation at 5, 10 and 25 μg/kg doses, the acid phosphatase activity of the ovary and uterus of 2 months old rats was significantly increased. Similarly, at the age of 4 months, the ovary and uterus showed increased acid phosphatase activity at all three doses. Even at 6 months of age animals indicated relatively higher activity of acid phosphatase in both organs. When coumestrol was administered on the 15th and 18th day of gestation, the response of all three doses was similar to that of animals of Group -1. When coumestrol was given to pregnant rats on the 10th and 13th day of gestation at different days of gestation at 3 different doses caused a substantial rise in the estrogenic dependent parameters such as ovarian and uterine wet weight, protein, glycogen contents, and activity of acid and alkaline phosphatase. It is interesting to note that all three doses of coumestrol exert the same response. Therefore, even smaller doses of such agents may bring about significant changes in the reproductive organs. Although the exact mechanism of those alterations is not known but looking into the nature of the response of coumestrol it is certain that coumestrol acts in the manner of frank estrogens similar to that of diethylstilbestrol.

References


