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EFFECTS OF *CLEOME GYNANDRA* LINN. LEAF EXTRACT ON OVARIAN FOLLICULOGENESIS OF ALBINO MICE

KEYWORDS: *Cleome gynandra*, folliculogenesis, phytoestrogen, nutraceuticals

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ABSTRACT *It is well known that the plant kingdom contains numerous bioactive substances affecting the regulation of reproduction. Cleome gynandra plant extracts contain phytoestrogenic compounds. These compounds act as agonist or antagonist estrogen receptors, thus affecting the steroid hormones level. In traditional medicine, Cleome gynandra is used by lactating females for enhancement of milk and as a housing drug. The aim of this study was to investigate the effects of methanolic extract of Cleome gynandra leaves on the folliculogenesis of female albino mice. The effect of Cleome gynandra methanolic leaf extracts on folliculogenesis was studied in sixteen (N=16) sexually matured female albino mice with regular oestrus cycle. Mice were randomly divided into four (4) groups of four (n=4) mice per group. The experimental groups were treated as follows: Group I treated with 250mg/kg, Group II with 500mg/kg, Group III with 0.01mg/kg 17β estradiol and Group IV with 1% Tween 80 (control). Follicular growth and changes were studied through standard histological protocols. For each ovary, every 12th and 20th section was examined for counting primordial, primary, secondary, graafian and atretic follicles respectively to obtain an overall view of the follicular populations per ovary. In this experiment, the dose of 500mg/Kg B.W. /day showed a significant ($p<0.05$) decrease in primordial, primary, secondary and Graafian follicle compared to that of normal control mice. Significant increase in number of atretic follicle was recorded in dose of 500mg/Kg B. W. /day compared to normal control mice. The dose of 250 mg/Kg B. W. /day showed a similar decrease in primordial , primary, secondary and Graafian follicle compared to that of control mice ($p<0.05$). 17β Estradiol treated group showed a statistically significant ($p<0.05$) decrease in number of primordial, primary, secondary and Graafian follicle compared to that of normal control mice.*

Introduction:

The relationship between female fertility and ovarian follicle development is well recognized (Takizawa and Mattison, 1983). Studies in mice (Mattison *et al.,* 1983; Takizawa *et al.,* 1984; Mattison *et al,* 1989; Weitzman *et al,* 1992) and rats (Toaff *et al,* 1979; Flaws *et al,* 1994) suggest that differential follicle counts may provide a sensitive means of estimating the extent of ovarian toxicity in females exposed to xenobiotics. As reported in a preliminary study (Heindel *et al,* 1989), a three stage classification system based on follicle diameter and structure (Pedersen and Peters, 1968, as adapted by Mattison and Nightingale, 1982) appears to provide a quantifiable screening procedure for use in subchronic toxicity bioassays.

In 1989, DeFelice hypothesised the occurrence of biological interventions not related to pharmacological methods and wrote about "nutraceutical" products, i.e., "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease" (DeFelice, 1995; Kalra, 2003). The original hypothesis was that these foods can protect human body from adverse events because of the beneficial effects of some phytochemicals. Several studies have reported the validity of this idea in clinical practice (Estruch *et al*., 2013; Massaro *et al*., 2010), (Scicchitano *et al*., 2014).

Certain synthetic or natural compounds present in the environment mimic, enhance or inhibit endogenous hormones. These compounds are called as environmental estrogens (Odum *et al.,* 1998). These chemicals have been a source of concern because of their possible health threats to human being in particular. These are also called as xenoestrogens which cause change in cellular function of animals' body by binding with estrogen receptor sites (Kummerer, 2001; Fent *et al.,* 2006). These chemicals present in the environment interfere in the biosynthetic pathway of the endogenous hormones or modifying hormone metabolism thereby which an animal maintain a normal homeostatic system with which it responds to its surrounding environment (Kummerer, 2001).

Estrogens inhibit mouse oocyte nest breakdown and follicle assembly (Chen *et al.,* 2007). Estrogenic action reduces follicle assembly leading to fewer primary and subsequent developing follicles. Thus, the study of follicular populations provides important information about the function of the ovary, in particular the relationship between folliculogenesis and also environmental factors having estrogenic property that regulate it (Raymond-Whish *et al*., 2007).

Plant kingdom contains numerous bioactive substances affecting the regulation of reproduction in animals and humans. *Cleome gynandra* plant extracts contain numerous bioactive compounds. There are reports that these compounds act as agonist or antagonist of estrogen receptors, thus affecting the steroid hormones level. In traditional medicine, *Cleome gynandra* was used by lactating females for enhancement of milk and as a housing drug. The aim of the present study was to investigate the effects of methanolic extract of *Cleome gynandra* leaves on the ovarian folliculogenesis in albino mice.

Figure 1: *Cleome gynandra* Linn. at natural habitat

Materials and method:

Collection of plant materials:

Fresh leaves of *Cleome gynandra* was collected from different parts of Kamrup district. They in fresh condition were washed under running tap water and then again with distilled water. The plant material was air dried in shade for 5 days and then homogenized to fine powder and stored in airtight bottles with proper labeling.

***Preparation of extract*:**

Powdered plant materials were collected and weighed carefully. 50g of the plant material was weighed and soaked in 300 ml of methanol. The mixture was kept in shaker for 48 hours and filtered. The filtrate was kept in rotary evaporator in low

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temperature under reduced pressure till dryness. Extract thus obtained was examined chemically and screened for phytochemical screening. The extract was kept in refrigerator when not in use.

Treatment procedure and route of administration:
Two doses of 250mg/kg B.W. and 500mg/kg B.W. of plant extracts respectively were used which corresponds to a $1/12^{th}$ and $1/6^{th}$ respectively of the highest tested dose (3000mg/kg B.W.). 1% v/v Tween-80 (P8074, CAS 9005-65-6) which is a polyethylene sorbitol ester was used to prepare the extract suspension of the test plant extract. To prepare the 17β-Estradiol stock solution, the same was dissolved in Ethanol (analytical grade) and it was diluted with normal saline to prepare the desired dose of working solution.

Animals were exposed to the test compounds through standard gastric gavages feeding syringe (Feedy-I, FG-05). The doses were administered at 24 hours of interval for a period of 21 days.

Morphological classification of follicles:
Ovaries of five animals of each group were taken for the follicular studies. Ovaries were selected based on the stage and comparability of the weight with respective control ovaries. The ovaries were fixed in Bouin's fluid, embedded in paraffin and sectioned at 6 µm thickness. The sections were separated for every 10th section and stained with hematoxylin and eosin. Sections of the ovary were examined under a light microscope and the general histologic appearance of the ovary was assessed. All serial sections of the ovary were counted for various stages of development of follicles as described by Moawad *et al.* (1965) and Bolon *et al.* (1997). Follicles and atretic follicles were classified according to the method described by Swartz and Mall (1989) and Bucci *et al.* (1997).

Quantification of follicles:
To determine the total population of different types of follicles per ovary the method used by Pedersen and Peters (1968) and Butcher and Kirkpatric-Keller (1984) were followed. At an average, about 200 serial sections were obtained and for each ovary, every 12^{th} and 20^{th} section was examined for counting smaller (primordial, primary and secondary) and larger (graafian and atretic) follicles respectively to obtain an overall view of the follicular populations per ovary (Myers *et al.*, 2004).

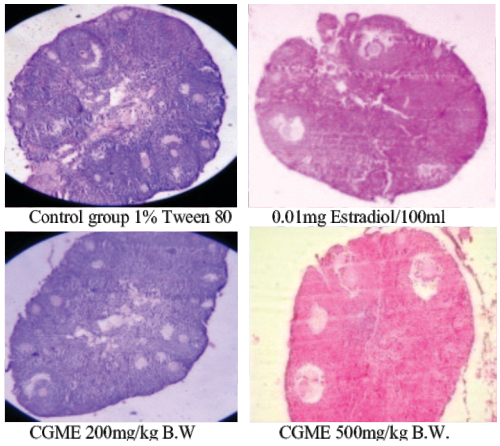
Statistics:
Statistical analyses for all the data of animal experimentations were performed using MS Office Excel 2007. The results were expressed as mean ± standard error (SE) of mean. The means in both negative as well as positive control versus treated animals were analyzed for significant by Student's independent t- test distribution. A value of $p<0.05$ was considered statistically significant for all the tests.

Result:
The results of the ovarian follicular counting on treatment with CGME were showed in the table. In this experiment, the dose of 500mg/Kg B.W. /day showed a significant ($p<0.0001$) decrease in primordial follicle (892.45 ± 5.97), primary follicle (252.34 ± 4.67), secondary follicle (152 ± 9.78) and Graffian follicle (11.89 ± 2.55) compared to that of normal control mice. Significant increase in number of atretic follicle (48.12± 4.67) was also recorded in dose of 500mg/Kg B. W. /day compared to normal control mice (18.06±3.27). The dose of 250 mg/Kg body weight/day showed a similar decrease in primordial follicle (928.43±4.11), primary follicle (268.51±5.39), secondary follicle (159.72±2.17) and Graffian follicle (16.44± 5.13) compared to that of olive oil control mice ($p<0.0001$). 17β Estradiol treated group showed a statistically significant ($p<0.0001$) decrease in number of primordial follicle (816.75 ± 44.47), primary follicle (179.42 ±11.56), secondary follicle (142.51 ±6.33) and Graffian follicle (12.34 ±7.21) compared to that of normal control mice.

Table: Effect of CGME on the ovarian follicular population after 21 days of treatment.

Treatment group	primordial follicle	primary follicle	Secondary follicle	Graffian follicle	Atretic follicle
Control, 1% Tween 80	983.62± 10.52	286.37± 6.48	169.26± 8.32	19.43.±4.86	18.06±3.27
Estradiol treated, 0.01mg/100ml	816.75± 44.47***	179.42± 11.56***	142.51± 6.33**	12.34±7.21**	52.81±1.57***
Low dose, 250mg/kg BW	928.43± 4.11	268.51± 5.39	159.72± 2.17	16.44±5.13	39.68±2.41***
High dose, 500mg/kg BW	892.45± 5.97 ***	252.34± 4.67**	152.53± 9.78	13.89±2.55**	48.12±4.6***

Significance was estimated by student's t-test and compared with the untreated control group (*$p<0.01$, **$p<0.001$, ***$p<0.0001$). Values are shown as mean ± SEM, (n=5).



PF=Primary follicle, SF=Secondary follicle, TF=tertiary follicle, AF=Atretic follicle

Figure 2: Photomicrographs of hematoxylin and eosin stained representative section of the ovarian tissue illustrating the interstitial cell masses, follicular remnants and oocyte.

Discusion:
In this experiment, the dose of 500mg/Kg B.W. /day showed a significant ($p<0.05$) decrease in primordial follicle (892.45 ± 5.97), primary follicle (252.34 ± 4.67), secondary follicle (152 ± 9.78) and Graafian follicle (11.89 ± 2.55) compared to that of normal control mice. Significant increase in number of atretic follicle (48.12± 4.67) was also recorded in dose of 500mg/Kg B. W. /day compared to normal control mice (18.06±3.27). The dose of 250 mg/Kg body weight/day showed a similar decrease in primordial follicle (928.43±4.11), primary follicle (268.51±5.39), secondary follicle (159.72±2.17) and Graafian follicle (16.44±5.13) compared to that of tween 80 control mice ($p<0.05$). 17 Estradiol treated group showed a statistically significant ($p<0.05$) decrease in number of primordial follicle (816.75 ± 44.4), primary follicle (179.42 ±11.56), secondary follicle (142.51 ±6.33) and Graafian follicle (12.34 ±7.21) compared to that of normal control mice. The results showed a clear vision of the effect of CGME on the ovrian folliculogenesis of the albino mice. It is evident that the higher dose of CGME has much reductive activity on it than those of lower dose. The increasing number of atretic follicles due to the higher dose reveals that there are certain phyto-compounds in CGME which affect normal folliculogenesis. These compounds must have definite estrogenic property which affects the steroid hormone level.

The experimental results of the present investigation showed a reducing effect upon the follicular development in all the

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experimental groups of female mice with respect to control upon treated with *Cleome gynandra* leaf extract (methanolic) for a period of consecutive 21 days. The number of primordial, primary, secondary and graafian follicles decreased significantly in both the CGME treated groups along with the Estradiol treated group, whereas degenerative nature was seen clearly resulting in the increase in the number of atretic follicles. Another study reported that methanolic extract of *Rumex steudelii* has potential to disrupt ovarian folliculogenesis when administered orally for 30 consecutive days by inhibiting further development of the recruited ovarian follicles (Solomon, 2010). Many other investigations reported the disrupting effect of various plant extracts on ovarian folliculogenesis. The number of primordial follicle reduced in the ovaries of gerbils when treated with Cannabis extract at 2.5mg/day for 60 days (Dixit, 1976). There was a total loss of primordial follicles in the ovaries of rats treated with an aqueous extract of dried seed powder of *Sapindus trifoliatus* at dose level of 50, 100 and 150mg/100kg B.W. for consecutive 30 days (Singh and Singh, 1994) resembling the effect of hexane extract of *Ferula jaeschkeana* in guinea pigs (Pathak *et al*., 1995). Another study revealed the reducing effect of nicotine on the number of graafian follicles at a dose level of 0.3mg/kg for 15 days (Patil *et al*., 1998). A study by Roop *et al*., 2005, reported the significant reduction at 6mg polar fraction of Azadirachta extract treatment. This significant reduction in the number of healthy follicles in all the experimental groups leads to the assumption of disruption of the process of follicle selection due to atresia (Guraya, 1997). These changes in the oocyte growth and maturation have been influenced by gonadotrophins and steroids along with maturation promoting and growth factors (Guraya, 2000; Driancourt and Thuel, 1998) suggesting the possible reason of the inhibitory effect of these plant extracts on the folliculogenesis in female.

Conclusion:

Lots of researches are going on the phytochemicals of *Cleome gynandra* L. and its application in different aspects of human welfare. My aim was to investigate the estrogenic property of *Cleome gynandra* L. on ovarian folliculogenesis of the albino mice.

References:

1. Anderson LD and Hirshfield AN. An overview of follicular development in the ovary: from embryo to the fertilized ovum in vitro. Md. Med. J. 1992; 41: 614-620.
2. Bolon B, Bucci IJ, Warbritlon AR, Chen JJ, Mattison DR and Heindel JJ. Differential follicle counts as a screen for chemically-induced ovarian toxicity in mice: results from continuous breeding bioassays. Fundam. Appl. Toxicol. 1997; 39: 1-10.
3. Bucci TJ, Bolon B, Warbritton AR, Chen JJ and Heindel JJ. Influence of sampling on the reproducibility of ovarian follicle counts in mouce toxicity studies. Reprod. Toxicol. 1997; 11: 689-696.
4. Butcher RL and Kirkpatric-Keller D. Pattern of follicular growth during the four-day oestrus cycle in the rat. Biol Reprod. 1984; 31: 280-286.
5. Butcher RL and Kirkpatrick-Keller D. Patterns of follicular growth during the four-day oestrus cycle of the rat. Biology of Reproduction 1984; 31: 280-286
6. Chen Y, Jefferson WN, Newbold RR, Padilla-Banks E and Pepling ME. Estradiol, progesterone,and genistein inhibit oocyte nest breakdown and primordial follicle assembly in the neonatal mouse ovary in vitro and in vivo. Endocrinol. 2007; 148: 3580-3590.
7. Chen YT, Mattison DR, Feigenbaum L, Fukui H and Schulman JD. Reduction in oocyte number following prenatal exposure to a diet high in galactose. Science. 1981; 214: 1145-1147.
8. DeFelice, S. L. (1995). The nutraceutical revolution: Its impact on food industry R&D. Trends in Food Science and Technology, 6, 59–61.
9. Dixit VP, Arya M and Lohiya NK. Mechanism of action of chronically administered Cannabis extract on the female genital tract of gerbils Meriones hurrianae. Indian Journal of Physiology and Pharmacology 1976; 20: 38-41.
10. Estruch, R., Ros, E., Salas-Salvado, J. (2013). Primary prevention of cardiovascular disease with a Mediterranean diet. New England Journal of Medicine, 368, 1279–1290.
11. Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 2006; 76: 122-159.
12. Flaws JA, Doerr JK, Sipes IG. and Hoyer PB. Destruction of preantral follicles in adult rats by 4-vinyl-cyclohexene diepoxide. Reprod Toxicol. 1994; 8: 509-514.
13. Guraya SS. Comparative Cellular and Molecular Biology of Ovary in Mammals. Fundamental and Applied Aspects. Oxford & IBH Publishing Co. Pvt. Ltd., 2000. New Delhi, and Calcutta, India.
14. Guraya SS. Ovarian Biology in Buffaloes and Cattle. Directorate of Information and Publications of Agriculture, Indian Council of Agricultural Research, 1997. New Delhi, India. 332
15. Halpin DM, Jones A, Fink G and Charlton HM. Postnatal ovarian follicle development in hypogonadal (hpg) and normal mice and associated changes in the hypothalamic-pituitary-ovarian axis. J. Reprod Fertility. 1986; 77: 287-296.
16. Haque A, Kalita JC, Deka DD and Baruah BK. Effect of effluent water downstream to the Nagaon Paper Mill, Assam on ovarian follicular population of immature female C3h mice. The Bioscan. 2010; 02: 529-535.
17. Heindel JJ, Thomford PJ and Mattison DR. Histological assessment of ovarian follicle number in mice as a screen for ovarian toxicity. In Growth Factors and the Ovary (A. N. Hirshfield, Ed.), 1989; 421-426. Plenum, New York.
18. Kalra, E. K. (2003). Nutraceutical–Definition and introduction. AAPS PharmSciTech, 5, E25.
19. Kummerer K. Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. 3rd ed. Berlin: Springer publisher 2001.
20. Maheshwar A and Bhattacharya S. Ovarian ageing and fertility-review. Current Women's Health Reviews. 2007; 3: 63-67.
21. Massaro, M., Scoditti, E., Carluccio, M. A. (2010). Nutraceuticals and prevention of atherosclerosis: Focus on omega-3 polyunsaturated fatty acids and Mediterranean diet polyphenols. Cardiovascular Therapeutics, 28, e13–e19.
22. Mattison DR and Nightingale MS. Oocyte destruction by polycyclic aromatic hydrocarbons is not linked to the inducibility of ovarian aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity in (DBA/2N X by guest on August 8, 2016 http://toxsci.oxfordjournals.org/ Downloaded from 10 BOLON ET AL. C57BL/6N)F1 X DBA/2N backcross mice. Pediatr. Pharmacol. 1982; 2: 11 - 21.
23. Mattison DR and Thomford PJ. The mechanisms of action of reproductive toxicants. Toxicol. Pathol. 1989; 17: 364-376.
24. Mattison DR, Plowchalk DR, Meadows MJ, Miller MM, Malek A and London SN. The effect of smoking on oogenesis, fertilization and implantation. Semin. Reprod. Endocrinol. 1989; 7: 291-304.
25. Mattison DR, Shiromizu K and Nightingale MS. Oocyte destruction by polycyclic aromatic hydrocarbons. Am. J. Ind. Med 1983; 4: 191 - 202.
26. Moawad AH, Rakoff AE and Kramer SA. histologic study of the effects of lowdosage irradiation of rabbit ovaries. Fertil. Steril. 1965; 16: 370-381.
27. Myers M, Britt KL, Wreford NGM, Ebling FJP and Kerr JB. Methods for quantifying follicular numbers within the mouse ovary. Reprod. 2004; 127: 569-580.
28. Odum J, Ashby J, Sumpter JP. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicology and Applied Pharmacology.1998; 153: 12–19.
29. Osman P. Rate and course of atresia during follicular development in the adult cyclic rat. J. Reprod. Fertil. 1985; 73: 261-270.
30. Pathak S, Jonathan S and Prakash AO. Timely administration of extract of Ferula jaeschkeana causes luteolysis in the ovary of cyclic guinea pig. Indian Journal of Physiology and Pharmacology, 1995; 39: 395-399.
31. Patil SR, Ravindra Patil SR, Londonkar R and Patil SB. Nicotine induced ovarian and uterine changes in albino mice. Indian Journal of Physiology and Pharmacology, 1998; 42: 503-508.
32. Pedersen RA and Peters H. Proposal for a classification of oocytes and follicles in the mouse ovary. J.Reprod Fertil. 1968; 83: 555-557.
33. Raymond-Whish S, Loretta PM, O'Neal T, Martinez A, Sellers MA, Christian PJ, Marion SL, Begay C, Propper CR, Hoyer PB and Dyer CA. Drinking water with uranium below the U.S. EPA Water Standard causes estrogen receptor dependent responses in female mice. Env. Health Perspect. 2007; 115: 1711-1716.
34. Roop JK, Dhaliwal PK and Guraya SS. Extracts of Azadirachta indica and Melia azedarach seeds inhibit folliculogenesis in albino rats. Brazilian Journal of Medical and Biological Research, 2005; 38: 943-947.
35. Scicchitanoa, P., Cameli, M., Maiello, M., Modesti, P. A., Muiesan, M. L., Novo, S., Palmiero, P., Saba, P. S., Pedrinelli, R. and Ciccone, M. M. (2014). Nutraceuticals and dyslipidaemia: Beyond the common therapeutics. Journal of functional foods, 6: 11–32.
36. Singh SP and Singh K. Effect of Sapindus trifoliatus seed on the fertility of female albino rats. Cell Signalling and Ova Implantation (Abstracts). The International Symposium on Cell Signalling and Ova-Implantation. All India Institute of Medical Science, New Delhi, India, 1994; 21-23.
37. Solomon T, Largesse Z, Mekbeb A, Eyasu M and Asfaw D. Effect of Rumex steudelii methanolic root extract on ovarian folliculogenesis and uterine histology in female albino rats. African Health Sciences, 2010; 10(4): 353 – 361.
38. Swartz WJ and Mall GM. Chloredecone induced follicular toxicity in mouse ovaries. Reprod. Toxicol. 1989; 3: 203-206.
39. Takizawa K and Mattison DR. Female reproduction. Am. J. Ind. Med. 1983; 4: 17-30.
40. Tilly JL. Ovarian follicle counts – not as simple as 1, 2, 3. Reprod Biol. Endocrinol. 2003; 1: 11.
41. Toaff ME, Abramovici A, Sporn J and Liban E. Selective oocyte degeneration and impaired fertility in rats treated with the aliphatic monoterpene, citral. J. Reprod. Fertil. 1979; 55: 347-352.
42. U. S. Environmental Protection Agency. Health Effects Test Guidelines, OPPTS 870.3800, Reproduction and Fertility Effects. EPA 712-C-98-239. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, DC. 1998.
43. U. S. Food and Drug Administration. Redbook 2000. Toxicological Principles for the Safety Assessment of Food Ingredients. IV.C.9.a. Guidelines for Reproductive Studies. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC. 2000.
44. Weitzman GA, Miller MM, London SN and Mattison DR. Morphometric assessment of the murine ovarian toxicity of 7,12-dimethylbenz(a)anthracene. Reprod Toxicol. 1992; 6: 137-141.