ORIGINAL ARTICLE

PREVENTIVE ROLE OF OCIMUM BASILICUM AGAINST CYCLOPHOSPHAMIDE INDUCED PRIMORDIAL FOLLICULAR ATROPHY IN ADULT FEMALE ALBINO RATS

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ABSTRACT

Background: Cyclophosphamide, the alkylating chemotherapeutic drug is commonly used in treatment against wide variety of carcinomas. This drug has known antifertility effects on gonads of young female patients. A natural herb known as *Ocimum Basilicum* has been found rich in polyphenols and is known to significantly improve fertility because its strong antioxidant potential.

Objectives: To assess the preventive ability of the natural herb, *Ocimum basilicum* extract against atrophic changes induced by cyclophosphamide in primordial follicles.

Methods: Total number of female albino rats used was 45. All were evenly distributed into three groups: Group A was control, group B and group C were experimental. There were 15 rats present in each one of these groups. On the 8th day of the experiment, rats in group A were administered a single intraperitoneal injection of normal saline (150 mg/kg). Group B rats were administered, on day 8 of experiment, a single intraperitoneal injection of cyclophosphamide (150 mg/kg). Rats in group C were fed with methanolic extract of basil seeds for 7 days. After that, rats were injected with a single intraperitoneal dose (150 mg/kg) of cyclophosphamide on day 8. The rats were euthanized and dissected after 48 hours of administration of cyclophosphamide.

Results: Results showed the significant improvement in state of primordial follicles between groups B and C (p-value 0.003), in terms of number of rats showing atrophic primordial follicles i.e., 11 rats (80%) in experimental group B and only 4 (26.7%) in experimental group C.

Key Words: Cyclophosphamide, Anti-cancerous alkaloid, oxidative stress, Antioxidant, Primordial follicular atrophy, *Ocimum Basilicum*

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INTRODUCTION

A widely used and effective anticancer alkaloid, Cyclophosphamide, is a potent drug known for its immunosuppressive activity. When tested as treatment agent in clinical cases of 33 different type of tumors, it demonstrated the best results compared to 1,000 other agents.[1] This alkylating agent, belonging to the oxazophosphorine group, was introduced long ago by Bourseaux, Arnold, and Brock back in 1958.[2] Anticarcinogenic cyclophosphamide exerts its cytotoxic

effects by transferring its alkyl group to different components of carcinogenic cells, particularly DNA, leading to cell death. A common result of this alkylation is the modification of guanine, causing abnormal pairing with thymine, which ultimately leads to the formation of defective DNA strands.[3]

Case studies make it obvious that females treated with adjuvant chemotherapy are on risk of developing temporary or permanent amenorrhea depending upon age and combination of drugs used.[4] Early menopause and ovarian failure are associated with early osteoporosis in women.[5] A study on male mice showed that chemotherapeutic agents caused DNA damage of germ cells resulting in removal of these cells by apoptosis causing decreased number of mature spermatozoa.[6]

Various studies on Ocimum Basilicum have proven its strong antioxidant potential. Aqueous extract of basil is seen protecting bones in experimental group when given along with glucocorticoid which induces osteoporosis by increasing serum parathyroid hormone, alkaline phosphatase, acid phosphatase and significantly decreasing antioxidant enzymes. In this study one group of rats which received dexamethasone along with 400 mg/kg basil extract, on comparison with the group receiving dexamethasone alone, showed significant improvement in levels of antioxidant enzymes.[7]

Methanolic extract of Basil at dose of 0.5gm/kg also acted as antioxidant agent against oxidative damage produced by electromagnetic field on brain tissue. It significantly raised the levels of antioxidant enzymes i.e., superoxide dismutase, glutathione, catalase, and peroxidase.[8]

Ocimum Basilicum causes reduction in induced oxidative damage in gonads by increasing antioxidant enzymes. This ability can improve fertility because free radicles causing oxidative gonadal tissue damage constitute a prominent factor in secondary infertility.[9] These medicinal herbs can also increase sperm count, forward movement, and normal sperm morphology to increase pregnancy outcome.[10]

Cyclophosphamide has been increasingly used in recent years for the treatment of various malignant conditions. A significant adverse effect of cyclophosphamide noted in young females undergoing breast cancer treatment is development of secondary infertility, which occurs due to its direct toxic effects on primordial and growing ovarian follicles, leading to oxidative damage.[9] Former studies have demonstrated that natural antioxidants exert a protective effect when administered alongside cyclophosphamide.[11] Therefore, the present study was designed to evaluate the antioxidant potential of basil seed extract in protecting against cyclophosphamide-induced ovarian toxicity.

METHODS

The present study was experimental and was conducted in the Anatomy Department of Shaikh Zayed Postgraduate Medical Institute. Calculation of sample size was done by using a software (Power and Precision 3.0), based on 0.48 effect and the error standard deviation was 2.28. Total female rats included in study were 45 (age: 3–4 months, weight: 190–240 grams). The animals were contained inside cages in the animal facility of the Anatomy department, Punjab Postgraduate Medical Institute, Lahore, with easy access to food and clean water. They were fed a standard commercial chick feed No. 1, supplemented with wheat flour, molasses, and fish meal. Environmental conditions included maintaining a light/dark cycle of 12-hour and optimal temperature (22–25 °C).

Extract was prepared by using basil seeds and methanol by using solvent extraction method. Weighted seed samples were placed in flasks filled with solvent and agitated continuously for 48 hours with regular 3-hour intervals. The mixture was then filtered through filter paper. Whole solvent was removed first by rotary evaporation and then by air drying. The final extract was stored in a freezer to preserve its antioxidant properties.[12]The solution was prepared for oral dose by dissolving the extract of basil seeds in normal saline. The drug used in experiment was purchased from a local pharmacy, and an appropriate dose of 150 mg/kg of the body weight of rats was calculated.[13]

The rats were randomly divided into three groups (A, B, and C) with 15 rats each. They were assigned numbers from 1 till 15 in each group using a lottery method. In each group rats ware marked with a permanent marker and housed accordingly. Their weights were carefully recorded using a weighing machine and noted in a proforma. Following treatment protocol was decided:

Group A (Control): All rats received no basil seed extract or cyclophosphamide, only their regular diet. On day 8, they were given a single injection of normal saline through intraperitoneal route (150 mg/kg body weight).

Group B (Experimental): Rats received the regular diet for 7 days. They were injected with a single intraperitoneal dose of cyclophosphamide (150 mg/kg) on experiment day 8.

Group C (Experimental): Rats were fed with basil seed extract orally via gastric intubation at a dose of 1.5 g/kg/day for 7 consecutive days. Then they were injected with a single dose of cyclophosphamide through intraperitoneal route (150 mg/kg) on day 8. The extract was administered daily at a fixed time.

48 hours after cyclophosphamide administration in experimental groups, all animals received analgesia consisting of ketamine and xylazine intraperitoneally. Following anesthesia, dissection of rats was done and

both ovaries were removed and preserved. Microscopic slides were prepared by using Hematoxylin and eosin from serial sections (five per slide) of each ovary. Primordial follicles were observed for signs of atresia, which was characterized by pyknotic nuclei in follicular cells, irregular cell orientation around the oocyte, and displacement of follicular cells from the basement membrane.

Statistical Analysis: Qualitative parameters of primordial follicles were presented in the form of frequencies and percentages for each group. Comparison among groups was recorded using the Chi-square test. The p-value of less than 0.05 was taken statistically significant.

RESULTS

Primordial follicles in all rats of group A were seen as primary oocyte surrounded by a well aligned single layer of flattened cells. Atrophy was observed in 12 (80%) rats in group B. There were only 4 (26.7%) rats in group C that showed atrophic changes in primordial follicles. The difference recorded among the three groups came out highly significant (p-value <0.001). (Fig. 1, 2, 3)

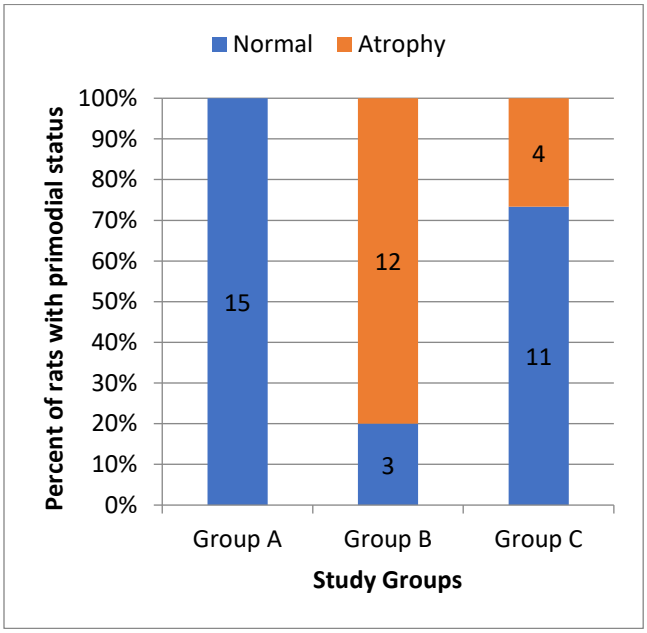

Figure.1 Percentage component bar diagram showing appearance of primordial follicles in groups (A, B, and C)

Highly significant difference was observed when groups A and B were compared for histological changes in primordial follicles (p-value <0.001). The results showed significance when group A was compared with group C (p value 0.013). P-value was 0.003, when the results of groups B and C were analyzed and comparison was made. It revealed that difference was significant between two groups showing marked improvement (Table. 1)

Table: 1 roup wise comparison between groups A, B, and C

(I) Group	(J) Group	Chi-square	p-value
A	B	20.0	< 0.001**
	C	6.16	0.013*
B	C	8.57	0.003*

KEY: A Control. B Experimental. C Experimental
** Highly significant statistical difference (p-value <0.001.
* Significant difference (p-value >0.05)

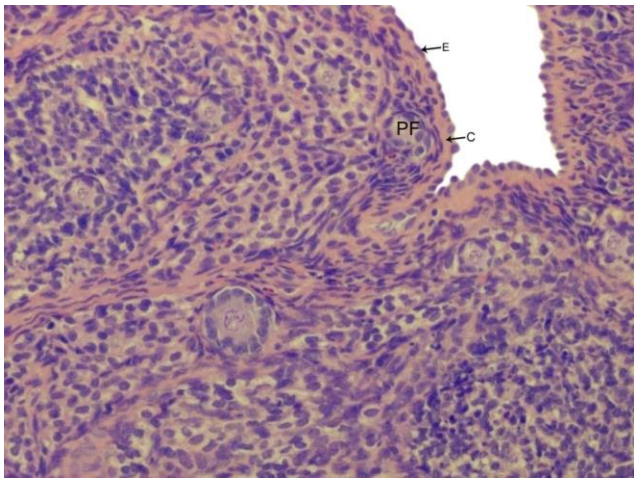

Figure 2: Photomicrograph of rat ovary of experimental group B showing appearance of normal simple cuboidal epithelium (E), normal ovarian capsule (C) and multiple atretic primordial follicles (White arrow) and few normal primordial follicles (black arrow) in the ovarian cortex, (H & E, 40x).

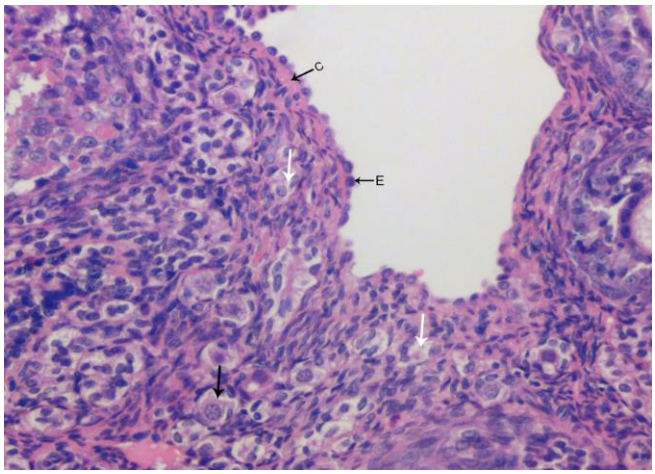

Figure.3 Photomicrograph (20x) showing appearance of normal ovarian germinal epithelium (E), normal ovarian capsule (C) and multiple normal primordial follicles (black arrow) and few atrophic primordial follicles (white arrow) in the ovarian cortex of group C

DISCUSSION

Detailed histological structure of ovaries of all rats of group A showed different stages of follicular maturation including primordial follicles, secondary follicles, and

mature Graafian follicles. The term primordial follicle was used to describe the oocyte lined by a single layer of flattened follicular cells.[14] Granulosa cells were recorded atretic when seen with pyknotic nuclei, irregular arrangement around central oocyte, and detachment from their basement membrane under light microscope.[15]

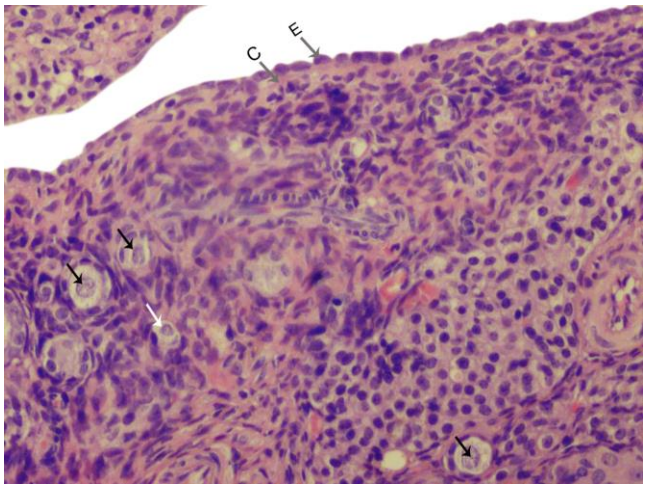

Figure 4: Photomicrograph showing appearance of normal ovarian germinal epithelium (E), normal ovarian capsule (C) and multiple normal primordial follicles (black arrow) and few atrophic primordial follicles (white arrow) in the ovarian cortex of group C, (H & E, 20x).

All rats of group A had normal primordial follicles with well-arranged flattened follicular cells as a single layer around the oocyte.(Figure 1) In 12 rats of group B, follicles showed atrophic follicular cells.(Figure 1) When observed in group C, only 4 rats had atrophic primordial follicles with the similar changes as observed in group B, while the 8 rats had normal shaped primordial follicular cells with normal arrangement around oocyte showing significant improvement when compared with group B. (Table 1, Figure 3,4) Atrophy of primordial follicles in group B, treated with cyclophosphamide, supports the study performed by Jalalie L and Spears N to observe the histological effect of cyclophosphamide on ovarian follicles. They collected data from rodents and found that primordial follicles are highly sensitive to cyclophosphamide induced toxicity.[16, 17] This can be a result of overproduction of free radicles along with diminished levels of antioxidant enzymes, possibly caused by oxidative effects of cyclophosphamide resulting in beginning of cell damage followed by planned apoptosis of injured cells.[18]

The significant histological improvement in appearance of primordial follicles in preventive group can be best clarified by previously depicted antioxidant and free radical hunting potential of extract from all parts of natural herb (*Ocimum Basilicum*), that can be responsible for prevention of oxidative destruction in gonads. [19-24]

CONCLUSION

The results of the present study evidenced that methanolic extract of basil seeds can defend ovarian tissue when given as a preventive treatment in combination with cyclophosphamide treatment through improvement in the histological appearance of primordial follicles. The results also recommend the possible clinical use of *Ocimum Basilicum* seeds extract in future to prevent the secondary infertility in young female cancer patients undergoing treatment with various cyclophosphamide regimens

LIMITATIONS OF STUDY

Limitation of time was a big factor for brief study duration. The resources and finances were also limited labelling the present research project a preliminary study. This study can open the ways to more detailed research projects in future using other biochemical and histological parameters. Further research is suggested to be carried out with simultaneous assessment of serum gonadotrophins levels.

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ETHICAL APPROVAL

Ethical approval of article was granted by the Institutional Ethical Review Board of Federal Postgraduate Medical Institute/Sheikh Zayed Hospital National Health Research Complex, vide reference No F-39/NHRC/Admin/IRB/187 dated 09 August, 2016.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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AUTHOR'S CONTRIBUTIONS

SS: Manuscript writing, data collection
HZ: Data collection, proof reading
AN: Manuscript writing, statistical analysis
MS: Research supervisor, critical review
NI: Manuscript writing, data analysis
KS: Data analysis, Critical review
All Authors: Approval of the final version of the manuscript to be published

REFERENCES

1. Emadi A, Jones R.J., Brodsky RA. Cyclophosphamide and cancer: golden anniversary. Nature reviews Clinical oncology. 2009; 6 (11):638-647.

2. Chighizola C, Ong VH, Denton CP. Cyclophosphamide as disease-modifying therapy for scleroderma: pros and cons. International Journal of Clinical Rheumatology. 2011; 6(2):219-227.
3. Edward C. Alan C. Sartorelli. Cancer chemotherapy, In: Bertram G. Katzung, Susan B. Basic and Clinical Pharmacology 12th edition. New York, USA: Mc Graw Hill Companies; 2012.p. 953.
4. Rugo HS, Rosen MP. Reducing the long-term effects of chemotherapy in young women with early-stage breast cancer. JAMA. 2011; 306(3):312-4. 83
5. Kalder M, Hadji P. Breast cancer and osteoporosis-management of cancer treatment-induced bone loss in postmenopausal women with breast cancer. Breast Care. 2014; 9(5):312-7.
6. Kanno TY, Sensiate LA, Paula NA, Salles MJ. Toxic effects of different doses of cyclophosphamide on the reproductive parameters of male mice. Brazilian Journal of Pharmaceutical Sciences. 2009; 45(2):313-319.
7. Hozayen WG, El-Desouky MA, Soliman HA, Ahmed RR, Khaliefa AK. Antiosteoporotic effect of Petroselinum crispum, Ocimum basilicum and Cichorium intybus L. in glucocorticoid-induced osteoporosis in rats. BMC complementary and alternative medicine. 2016; 16(1):165.
8. Khaki A. Protective Effect of Ocimum basilicum on brain cells exposed to oxidative damage by electromagnetic field in rat: Ultrastructural study by transmission electron microscopy. Crescent Journal of Medical and Biological Sciences. 2016; 3(1):1-7.
9. Alizadeh H, Khaki A, Farzadi L, Nouri M, Ahmadi-Asrbadr Y, Seyed-Ghiasi G et al. The therapeutic effects of a medicinal plant mixture in capsule form on catalase levels in the semen of men with oligospermia. Crescent Journal of Medical and Biological Sciences. 2015; 2(1):1-4.
10. Ouladsahebmadarek E, Giasi GS, Khaki A, Ahmadi Y, Farzadi L, Ghasemzadeh A et al. The effect of compound herbal remedy used in male infertility on spermatogenesis and pregnancy rate. International Journal of Womens Health and Reproduction Sciences. 2016; 4(4):185-8
11. Khaki A, Khaki AA, Ezzatzadeh A, Hamidreza A. Effect of Ocimum basilicum on ovary tissue histopathology after exposure to electromagnetic fields (EMF) in rats. African Journal of Pharmacy and Pharmacology. 2013; 7(25): 1703-1706.
12. AYDEMIR T, Becerik S. Phenolic content and antioxidant activity of different extracts from Ocimum basilicum, Apium graveolens and Lepidium sativum seeds. Journal of food biochemistry. 2011; 35(1):62-79.
13. Rasekh HR, Hosseinzadeh L, Mehri S, Kamli-Nejad M, Aslani M, Tanbakoosazan F. Safety assessment of ocimum basilicum hydroalcoholic extract in wistar rats: acute and subchronic toxicity studies. Iranian journal of basic medical sciences. 2012; 15(1):645.
14. Pedersen T, Peters H. Proposal for a classification of oocytes and follicles in the mouse ovary. Journal of reproduction and fertility. 1968; 17(3):555-557.
15. Yener NA, Sinanoglu O, Ilter E, Celik A, Sezgin G, Midi A et al. Effects of spirulina on cyclophosphamide-induced ovarian toxicity in rats: biochemical and histomorphometric evaluation of the ovary. Biochemistry research international. 2013; 764262.
16. Plowchalk DR, Mattison DR. Ovarian morphometric changes following cyclophosphamide treatment.Growth factors and the ovary. New York, 1989:427-431.
17. Meirow D, Lewis H, Nugent D, Epstein M. Subclinical depletion of primordial follicular reserve in mice treated with cyclophosphamide: clinical importance and proposed accurate investigative tool. Human Reproduction. 1999; 14(7):1903-7.
18. Devine PJ, Perreault SD, Luderer U. Roles of reactive oxygen species and antioxidants in ovarian toxicity. Biology of reproduction. 2012; 86(2):27,1-10.
19. Kaurinovic B, Popovic M, Vlaisavljevic S, Trivic S. Antioxidant capacity of Ocimum basilicum L. and Origanum vulgare L. extracts. Molecules. 2011; 16(9):7401-14.
20. Gajendiran A, Thangaraman V, Thangamani S, Ravi D, Abraham J. Antimicrobial, antioxidant and anticancer screening of Ocimum basilicum seeds. Bulletin of Pharmaceutical Research. 2016; 6(3):114-9.
21. Fekri N, Khayami M, Heidari R, Jamee R. Chemical analysis of flaxseed, sweet basil, dragon head and quince seed mucilages. Research Journal of Biological Sciences. 2008; 3(2):166-70.
22. Stevanović ZD, Radanović D. Proceedings of the Seventh Conference on Medicinal and Aromatic Plants of Southeast European Countries, (Proceedings of the 7th CMAPSEEC). Institute for Medicinal Plant Research and Association for Medicinal and Aromatic Plants of Southeast European Countries (AMAPSEEC). Subotica, Serbia, 2012: 267-273.
23. Hussain AI, Anwar F, Sherazi ST, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food chemistry. 2008; 108(3):986-95.
24. Shirazi MT, Gholami H, Kavoosi G, Rowshan V, Tafsiry A. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of T agetesminuta and Ocimum basilicum essential oils. Food science & nutrition. 2014; 2(2):146-55.