ORIGINAL ARTICLE

EFFECT OF OCIMUM BASILICUM IN PREVENTION OF MURINE CYCLOPHOSPHAMIDE INDUCED VASCULAR CONGESTION OF OVARIAN MEDULLA

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ABSTRACT

Background: Cyclophosphamide is one of the alkylating chemotherapeutic drugs used in cancer patients that exhibits antifertility effects on female gonads. *Ocimum Basilicum* is a natural herb rich in polyphenols and is known to improve fertility.

Objective: To determine the role of a natural herb, *Ocimum basilicum* extract, as a protective agent against cyclophosphamide induced congestion in ovarian medulla.

Methods: This experimental study was done in Sheikh Zayed Postgraduate Medical Institute, Department of Anatomy. Forty-Five adult female albino rats were divided in control group A, experimental groups B and C each having 15 rats. Group A rats received single dose of 150 mg/kg normal saline intraperitoneally on 8th day of experiment, while group B was given single intraperitoneal dose of 150 mg/kg cyclophosphamide at day 8 of experiment. Group C rats were pretreated with methanolic basil (*Ocimum basilicum*) seeds extract for 7 days followed by single intraperitoneal dose of 150 mg/kg cyclophosphamide at day 8 of experiment. All the rats were dissected 48 hours after the last dose.

Results: Cyclophosphamide caused congestion of blood vessels in ovarian medulla in group B and C. Congestion of ovarian medulla in group B when compared with control group A was highly significant with p-value <0.001. When observed in experimental group C, there was slight improvement in congestion of blood vessels. When compared with the group B, the difference was not significant having p-value 0.229.

Conclusion: The results of the present study showed that basil seeds showed slight improvement in vascular congestion in ovarian medulla, when used with Cyclophosphamide as a preventive agent.

Keywords: Cyclophosphamide, oxidative stress, ovarian medulla, congestion, basil.

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INTRODUCTION

Cyclophosphamide is a widely used and effective anticancerous alkaloid and an effective immune-suppressive agent first synthesized 60 years ago. Cyclophosphamide has showed the best desired result when used against 33 common tumours in comparison with multiple other drugs[1]. It is known as a useful alkylating agent that belongs to oxazophosphorine group. It was first introduced by Arnold, Bourseaux, and Brock in 1958[2]. Cyclophosphamide has a nitrogen mustard group that is attached to oxazophosphorine ring. It is water soluble and can also be dissolved in ethanole and saline[3].

It is a chemotherapeutic agent that acts as a cytotoxic drug mainly through a mechanism involving formation of intrastrand and interstrand abnormal DNA cross links and thus inhibiting DNA replication of tumour cells and limiting their growth and division[4]. This drug also induces cytotoxicity through shifting its alkyl group to the tumour cells constituents especially their DNA resulting in cell death. Alkylation of guanine results in abnormal pairing of guanine and thymine resulting in formation of abnormal DNA[5]. Absorption of cyclophosphamide occurs both from parenteral route and gastrointestinal tract[6]. Cyclophosphamide is non cytotoxic in its original form until it is converted into its active metabolites. Peak plasma level of these alkylating metabolites reaches 2-3 hours after administration of drug[7].

Cyclophosphamide is a commonly used chemotherapeutic agent that is widely used in patients with malignant breast carcinoma, scleroderma, Hodgkin's lymphoma, glomerulonephritis, systemic lupus erythematosus and autoimmune cytopenias[8,9,10,11,12]. Cyclophosphamide, like other chemotherapeutic drugs, has dose dependant adverse effects including immunosuppression, nausea, hair loss, vomiting, haemorrhagic cystitis, myelosuppression, weight loss, loss of appetite, and permanent infertility[13, 14] .

It caused hemorrhagic cardiomyopathy in patients at dose of 50mg/kg body weight when given for consecutive 4 days. Possible mechanism was the toxic effects of drug metabolites on myocardium and endothelial cells of its capillaries causing extravasation of vascular fluid and blood cells in interstitium.[15] Cyclophosphamide treatment also results in haemorrhagic bladder toxicity because of acrolein.[16] Increasing use of chemotherapeutic drugs for treatment of lupus nephritis and breast carcinoma in young females is responsible for premature menopause and secondary infertility due to post treatment ovarian failure[17,18]. Cyclophosphamide causes significant oxidative damage especially in male and female gonads due to overproduction of reactive oxygen species resulting in apoptosis of ovarian follicular cells and vascular endothelium[19].

Basil is a plant that belongs to the species *Ocimum basilicum* and Genus Ocimum and comes under Lamiacaea family[20]. Seeds of this plant are traditionally being used as anti-inflammatory, to increase circulation of blood and to enhance immunity. These beneficial effects are linked to its active components flavonoids and phenols[21]. High content of phenolics in extract of various parts of this basil give it significant antioxidant potential in various in-vitro studies[22]. Maximum antioxidant activity was observed with methanolic extract of basil seeds in comparison with other extracts[23]. This was due to higher phenolic content of methanolic extract of basil seeds and phenolic concentration is directly proportional to the free radical scavenging activity of basil seeds[24]. Rameshrad M et al. conducted an animal study after inducing paw inflammation in rats with carrageenan followed by different doses of *Ocimum basilicum* extract. Results depicted that ethanolic extract of basil is very effective anti-inflammatory[25]. The underlying anti-inflammatory mechanism involved the blockage of lipoxygenase and cyclooxygenase pathways that are responsible for metabolism of arachnoid acid[26].

Methanolic extract of Basil acts as antioxidant when tested against oxidative stress induced in brain tissue by significantly increasing levels of antioxidant enzymes, superoxide dismutase, glutathione peroxidase and catalase[27]. Aqueous extract of different parts of Basil also proved its antioxidant potential in deltamethrin induced nephrotoxicity by decreasing markers of lipid peroxidation and increasing superoxide dismutase and catalase levels and also significantly improving renal tubular epithelial degeneration, glomerular atrophy and vascular congestion[28]. A study on female rats also showed the antioxidant potential of its methanolic extract against toxicity induced by the electromagnetic field and results showed significant improvement in various parameters of ovaries including granulosa cell apoptosis, fibrosis and vascular congestion[29].

Cyclophosphamide commonly used in young females having carcinoma breast is linked to major adverse effects including ovarian toxicity and secondary infertility due to oxidative damage of growing follicles and ovarian blood vessels. It is obvious from past studies that natural antioxidants have protective effect when used in combination with cyclophosphamide. [30] Present study is therefore, designed to see the antioxidant potential of Basil seeds extract against cyclophosphamide induced toxicity of blood vessels in ovarian medulla.

METHODS

This experimental study was done in Sheikh Zayed Postgraduate Medical Institute, Department of Anatomy. The sample size was calculated by using power and precision 3.0 software with 0.48 effect using and 2.28 as error SD [30]. Based on these a total 45 adult healthy female albino rats with average weight 190-240 grams were used in this study. All animals

were kept in separate labeled cages in the animal house, Punjab PGMI, Lahore. The animals were allowed free access to food and water. A commercial brand of chick feed no. 1 was provided to rates. In every 5 kg of feed, wheat flour 2.5 kg, molasses 1 kg, fish meal 100 grams and water were added. A 12:12 light: dark cycle was maintained. Temperature was maintained between 22-25 ℃.

Basil seeds extract was prepared with methanol. Nonvolatile compounds were extracted through solvent extraction method. Weighted samples were taken in a flask and it was filled with the solvent. The whole sample was shaken for 48 hours with interval of 3 hours. The sample was then filtered with the help of filter paper and subjected to rotatory evaporation to remove solvent followed by air evaporation. Dried sample was stored in freezer to avoid loss of antioxidant compounds[31]. Dosage solution was prepared for oral administration by dissolving extract in normal saline[32]. Cyclophosphamide was purchased from Pharmacy and dose of drug was calculated as 150mg/kg body weight for each rat.

The rats were divided in 3 groups A, B and C. Each group contained 15 rats that were further named as A1-A15, B1-B15 and C1-C15 through lottery method. Each rat was assigned its number and marked with permanent marker and placed in the specific group cage. The weight of each rat was carefully recorded, with the help of weighing scale, in a proforma.

The dose was given according to following schedule:

Group A (Control)

The rats of this group were not given any extract or toxic drug. These rats were provided with routine oral food daily and only given the single dose of normal saline 150 mg/kg body weight intraperitoneally on day 8 of experiment[32].

Group B (Experimental)

The rats of this group received routine oral food with other rats for 7 days followed by only a single dose of 150 mg/kg cyclophosphamide intraperitoneally at day 8 of experiment[30].

Group C (Experimental)

This experimental group received basil seed extract as dose of 1.5 g/kg/day through gastric intubation for 7 days followed by single 150 mg/kg intraperitoneal dose of cyclophosphamide at day 8 of experiment. The extract was given on the same fixed time daily.

The animals of each of group A, B and C were given proper analgesia at the end of experiment with 50mg/kg ketamine followed by 50mg/kg xylazine through intraperitoneal route. After dissection, ovaries were removed from rats and hematoxylin and eosin slides were prepared for both right and left ovary of each rat.

All slides were observed for congestion in ovarian medulla. Medulla was labeled congested when vessels were dilated showing extravasated blood outside the blood vessels.

The data for qualitative variable, congestion of blood vessels, was reported by using frequency and percentage for each group. Comparison was made among the groups by using Chi-square test. P value <0.05 was considered significant.

RESULTS

Ovarian medulla of 14 rats in group A was normal and no congestion was seen. In experimental group B, 12 rats were having congestion of blood vessels in ovarian medulla and 3 rats were having normal ovaries. In group C, 9 rats had congested ovaries showing dilated blood vessels, (fig. 1, 2, 3). The difference among these three groups was highly significant statistically with p value <0.001, (table. 1).

Table 1: Congestion of blood vessels in control group A, experimental group B given cyclophosphamide alone and group C given both basil extract and cyclophosphamide.

Group	Congestion in Blood Vessels					
	Present		Absent		Total	
	N	%	N	%	N	%
Group A	1	6.7	14	93.3	15	100.0
Group B	12	80.0	3	20.0	15	100.0
Group C	9	60.0	6	40.0	15	100.0

P-value < **0.001**[**]

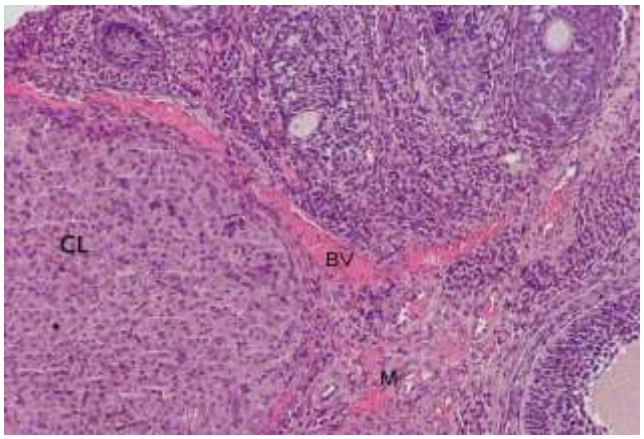

Figure 1: Photomicrograph of ovarian medulla (M) in rats of control group A showing normal blood vessels (BV) and corpus luteum (CL), (H & E, 10x).

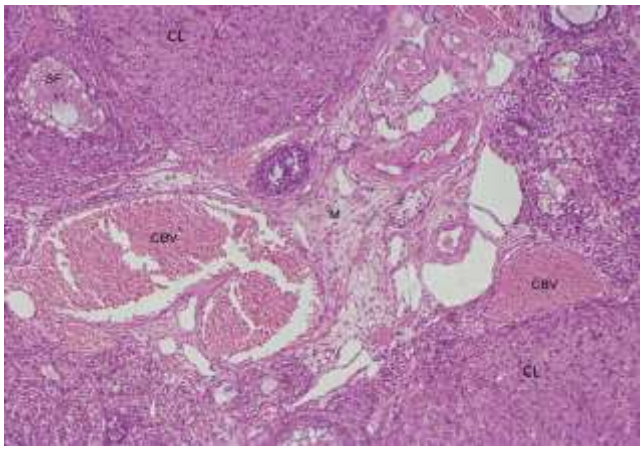

Figure 2: Photomicrograph of ovarian medulla (M) in rats of group B showing congested blood vessels (CBV) and normal corpus luteum (CL), (H & E, 10x).

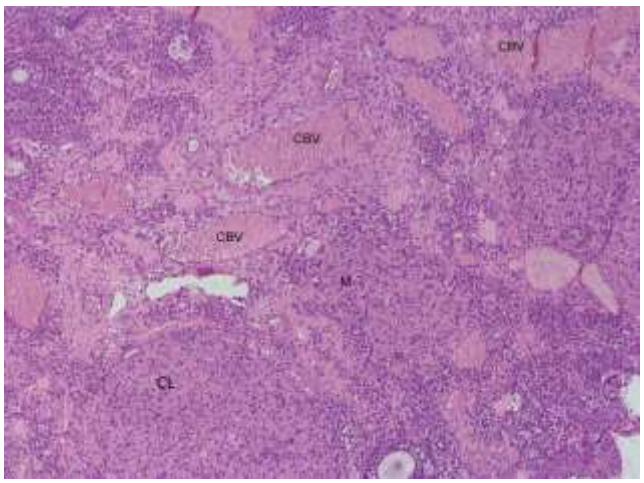

Figure 31: Photomicrograph of ovarian medulla (M) in rats of group C showing congested blood vessels (CBV) and normal corpus luteum (CL), (H & E, 10x).

When group wise comparison was made between control and experimental groups, results showed that there is highly significant number of rats with ovarian congestion in group B when compared to group A with p-value of <0.001. Comparison between control group A and experimental group C was also significant with P-value 0.002 showing some improvement in group C. The difference between both experimental groups B and C was insignificant with p-value 0.229, (table. 2).

Table 2: Group wise comparison for congestion in ovarian blood vessels between control group A, experimental group B given cyclophosphamide alone and group C given both basil extract and cyclophosphamide.

(I) Group	(J) Group	P-value
Group A	Group B	< 0.001**
	Group C	0.002*
Group B	Group C	0.229+

DISCUSSION

In the present study cyclophosphamide caused congestion of blood vessels in ovarian medulla in group B and C, (fig. 2, 3). Congestion of ovarian medulla in group B when compared with control group A was highly significant with p-value <0.001, (table 1). A similar study performed by Meirow et al. also showed that cyclophosphamide causes fibrosis of ovarian cortex and damage to proliferating blood vessels. According to this study, it causes hyalinization and thickening of vascular walls resulting in luminal narrowing as well as dilatation of other mature blood vessels causing congestion.[33] The results of present study coincide with another study performed in 2016 showing the damaging effects of chemotherapy on ovaries in rats resulting in dilatation and congestion of blood vessels[34]. Another study explains the role of cyclophosphamide in damaging blood vessels through apoptosis of vascular endothelial cells causing extravasation of blood[35]. This may be due to its ability to form abnormal DNA cross linkages in living cells resulting in cell death and apoptosis[4,5]. When observed in preventive group C, there was slight improvement in congestion of blood vessels (fig. 3). When compared with the group B, the difference was not significant (table 2). The results of present study suggest that basil seed extract administration has no role in prevention of cyclophosphamide induced congestion of ovarian blood vessels. These results did not coincide with the study performed by Arash Khaki et al. in 2013, which states that *Ocimum basilicum* extract improves electromagnetic field induced vascular congestion in ovaries[29]. The difference in results is possibly due to use of electromagnetic field to

induce toxicity in study done by Arash Khaki while cyclophosphamide in the present study.

CONCLUSION

The results of the present study showed that basil seeds showed slight improvement in vascular congestion in ovarian medulla, when used with Cyclophosphamide as a preventive agent.

ETHICAL APPROVAL

The study was approved by the Institutional Review Board of Shaikh Zayed Postgraduate Medical Institute, SKZ Medical and Dental College, Lahore, vide Reference No. F-39/NHRC/Admin/IRB/228 Dated 06.11.2014

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AUTHOR'S CONTRIBUTIONS

SA: Manuscript writing
SS: Statistical analysis, Proof Reading
KS, MM, MS: Proof Reading
ZF: Research Compilation