Effect of Melphalan on Ovarian cell and its hormone on female albino rat

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Abstract

It is more difficult to determine how chemotherapy affects female reproductive function as there is no direct way of monitoring toxic effect on the ovaries. Gonadal damage is often manifest by amenorrhea, low estrogen levels, and increased concentrations of FSH and LH, which resemble the hormonal changes seen at menopause. As in men, alkylating agents appear to be the most toxic. Primary ovarian failure has been reported with both melphalan. Present study designed to understand, effects of melphalan (Alkeran) on ovary and their correlations with hormones and related physiological and reproductive functions. It is more difficult to determine how chemotherapy affects female reproductive function as there is no direct way of monitoring toxic effect on the ovaries.

KEYWORDS: Ovaries, alkylating agent, menopause, melphalan.

IntroductionMelphalan (Alkeran) , L-Phenylalanine mustard is a alkylating agent used in the treatment of ovarian carcinoma (Dominique et.al. 1992). Studies on the biological alterations of the result suggest that non nitrogen mustard is the active alkylating agent.it is well absorbed orally and have effect on cell cycle phase non-specific. These drugs remain active in blood for approximately 6- hours in human blood. Early toxicological study indicate that it has a effect on gastrointestinal tract with anorexia, nausea and vomiting in the patient (Spitzer et.al., 1986). It has been generally used for the treatment in ovarian carcinoma, multiple myeloma, breast carcinoma, testicular seminoma and in the malignant melanoma (Sutton, 1994).

It is more difficult to determine how chemotherapy affects female reproductive function as there is no direct way of monitoring toxic effect on the ovaries. Gonadal damage is often manifest by amenorrhea, low estrogen levels, and increased concentrations of FSH and LH, which resemble the hormonal changes seen at menopause. As in men, alkylating agents appear to be the most toxic. Primary ovarian failure has been reported with both melphalan . Present study designed to understand, effects of melphalan (Alkeran) on histology of ovary and their correlations with hormones and related physiological and reproductive functions.

Materials and methods In the present study sexually matured healthy albino virgin female rats of 180 + 05 of body weight were used for present experiments. All animals acclimatized in the laboratory for 10 days prior to the experiment. Animal maintenance and experimental procedure strictly followed by “Principles of laboratory animal care (NIH)’’ and also as per the local “Ethical regulations’’.Melphalan (Alkeran) of 100% purity, chemist purchased from local chemist as which is marketed by Wellcome pharmaceutical S A, from Glaxo group. Substrate and enzyme kits were obtained from commercial manufactures. Most of the fine chemicals used in the present study were obtained from M/S Sigma chemicals, U

S A.; M/S British drug house, England; M/S Merck's Transasia company, Accurex Ltd.¸ as analytical reagents.

Experimental protocol Animals divided into two groups. Group I – Control and Group II –Treated. Animals orally fed with 0.25 mg/animal/day for four (4) and eight (8) weeks of Melphalan to treated groups of 14 animals. The control groups of 14 animals were also fed orally the same volume of vehicle (distilled water). After completion of treatment period animals sacrificed at an interval of four (4) and eight (8) weeks period.

Animals given light ether anesthesia and blood were drawn carefully from the blood vessel of eye using retro-orbital technique. Blood collected in clean dry test tubes for separation of serum and plasma were collected, labelled and processed for further investigations. Animals required for autopsy were sacrificed in CO_2 chamber as per the guideline strictly followed by ethical regulation. The required organs were quickly excised, cleared off the adhering fat, blotted and weighed and inserted in appropriate fixative for histological studies. In hormonal study serum FSH, LH, Prolactin, assayed by antibody coated test tube assay kits, which were obtained from commercial manufacturers. Hormones then measured by RIA technique and results were compared favorably with, ovary and accessory reproductive structure.

Light microscopy After autopsy, reproductive organs were fixed in aqueous bovin's fixative for twenty-four hours. Tissues were washed in running tap water and then dehydrated through ascending grades of alcohol then were kept in xylene for clearing then transferred to paraffin wax (m.p.58 -60^0) for impregnation and infiltration. The tissues then embedded in paraffin wax and blocks were prepared. Sections taken on rotary microtome at 5-7 micron thickness. The sections were arranged on glass slides, then dipped in xylene for deparaffinisation, passed through descending grades of alcohol and water for hydration, stained with Haematoxylin, counter stained with eosin then passing through ascending grades of alcohol cleared in xylene and mounted in DPX. The stained slides then photographed. Prints of 35 mm negative been made to obtain six times of the original microscope magnification for further study.

Results and Discussion Melphalan is an alkylating agent of the bis-chloroethylamine type that exerts a cytotoxic effect through the formation of interstrand or intrastrand DNA cross-links or DNA protein cross- links via its two chloroethyl groups (Smith et al. 1989; Rauschecker et al. 1991; Samuels and Bitran 1995; Pinguet et al. 2000; Malahyde information system 2005).Serum follicle stimulating hormone (FSH), Luteinizing hormone (LH) and Prolactin (PRL) were studied to know the increase or decrease in their levels of these hormones in treated animals. The results are compared with the control hormones level. Hormone as serum FSH register significant decreased in their levels. Serum luteinizing hormone levels after the treatment of melphalan register markedly decreased in four and eight weeks of treated animals. Serum prolactin levels registered significant increased in both the groups of four and eight weeks treated animals. In women, LH stimulates estrogen and progesterone product from the ovary. Surge of LH in the mean menstrual cycle is responsible for ovulation and continue LH secretion subsequently stimulate the corpus luteum to produce progesterone by enhancing the conversion of cholesterol to pregnenolone. In women, LH stimulates estrogen and progesterone product from the ovary. Present observations are in good agreement with the earlier observations made by Muller and Stahel (1993); Glacer, (1994); Sutchffe, (1979); Johnson et al. (1985)

and Shenns, (1993). In the present study serum PRL level in female registered increase significantly in both the groups of four and eight weeks treated indicate hyperprolactinemia due to the treatment of melphalan drug effect which may cause additive effect of this compound.

The present results support with the earlier observations made by Larsen et al. (2003) who able to show the treatment of chemotherapy from such alkylating agent can cause reduction in ovarian function with further ovarian failure, had follicle depleted (Wallace et al. 1989, Byrne et al. 1992; Thibaud 1998 and Chiarellii et al. 1999). It also supports with the earlier observations made by Oropesa et al. (2014) that melphalan has a strong reproductive effect in humans (amenorrhea in women and azoospermia in men).

Present study indicates significant increase in prolactin level in both the groups of four and eight weeks treated animals, significant increase in prolactin level in the present study indicate hyperprolactinema. Alkeran can cause suppression of ovarian function in pre-menopausal women resulting in hyperprolactinema and it may cause the permanent sterility (Kauppila et al. 1988; Meirow and Nugent 2001). Hyperprolactinema disturbs the development and function of ovarian follicles leading to different menstrual disorders and fertility (Archer, 1980; Pepperel 1981; Kauppila 1982) and increased in serum prolactin concentration can interferes with ovarian function (Kauppila 1984) and cause infertility as reported by Ben-David et al (1983) and Schettini, (1983). Present observation are in good agreement with earlier observation made by Archer (1980); Kauppila et al. (1982), (1988), Kauppila (1984) and Ben-David et al. (1983) who are able to show development and function of ovarian follicles, menstrual cycle and related disorders and fertility (Kreuser and Hetzel 1990).

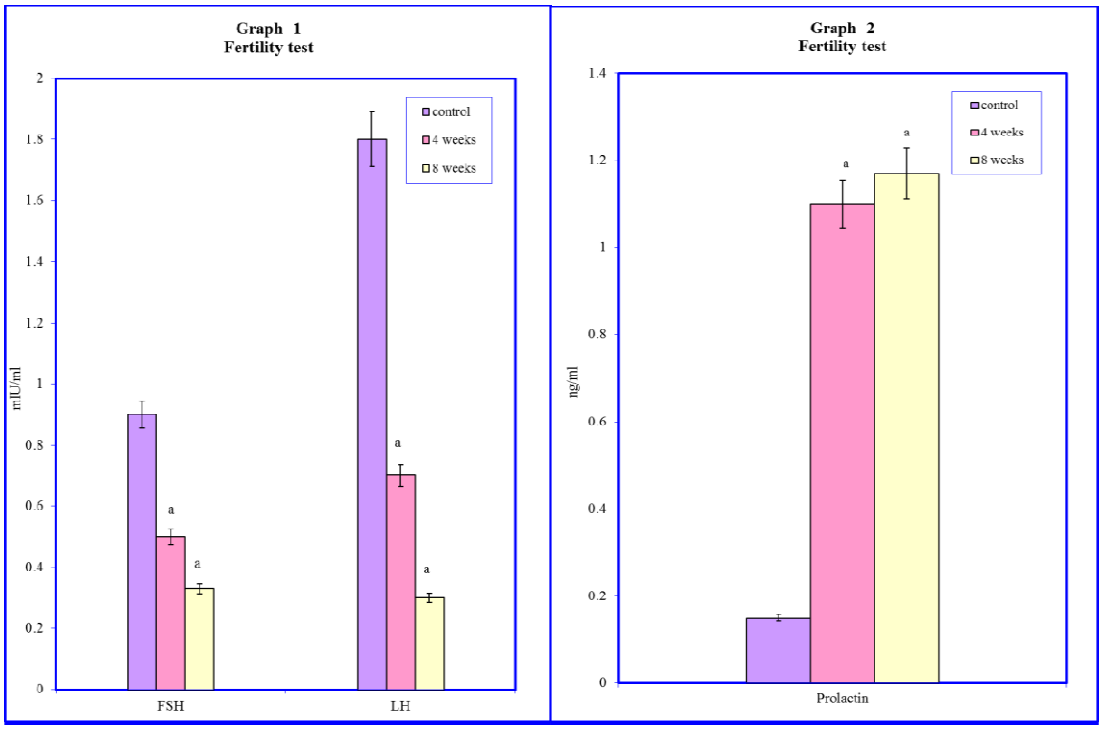
Present study registered decrease in estrogen level indicate failure of ovarian functions. The present observation are in good agreement with the earlier observation made by Kauppila (1988) who able to show hyperprolactinemia disturbs development and function of ovarian follicle leading to different disorders and infertility. Present observations are also in good agreement with observation made by Kauppila (1988); Archer (1980); and Pepperell (1981). Ovary is normally the major source of estrogen although the conversion of androgen precursors in other tissue is clinically important after the menopause and in some with disorders with ovarian function. Ovary also produces and secrete large amount of progesterone during the luteal phase of the cycle. It is also a source of small amount of testosterone and other androgens that sources not only as precursors to estrogen synthesis but also are release into the circulation to act on peripheral tissue (Ross 1985; Greenspan 1995; Goldfien and Monroe 1991).The only known bio-effects of LH and FSH are in the gonads. LH and FSH stimulate cell growth and maintenance in both the ovaries and testis. As classically defined LH stimulates steroidogenesis in both sexes particularly from thecal cells in the female. FSH stimulates follicular development and estrogen secretion. LH also induces ovulation from the mature follicle in the females; LH and FSH were name for their initially described roles in female. Both gonadotrophins act through classic protein hormone receptor mechanism (Catt et al. 1980). They bind to the cell membrane receptor and exert cellular effect of gonadotropin.

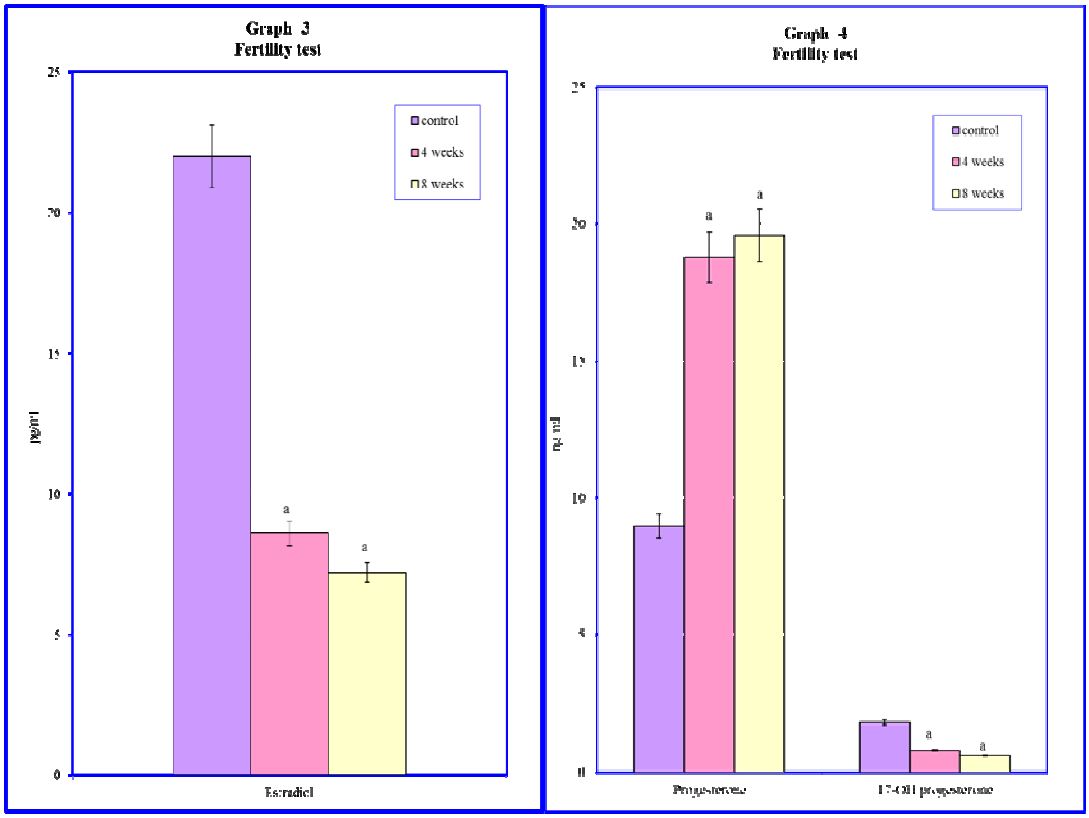
In women, LH stimulates estrogen and progesterone product from the ovary. Surge of LH in the mean menstrual cycle is responsible for ovulation and continue LH

secretion subsequently stimulate the corpus luteum to produce progesterone by enhancing the conversion of cholesterol to pregnenolone. The development of ovarian follicle is largely under the FSH control, secretion of estrogen from these follicles depend on both FSH and LH (Greenspan, 1995; Findling and Tyrrell, 1991).Following the treatment for four weeks the portion of ovary contains only luteinizing follicles and atriatic follicles with corpus lutei, atriatic follicle seen with different stages of atresia. Following treatment of eight weeks ovary showed late primary follicles and atriatic follicles with appearance of corpus luteum. The atriatic follicle seen in ovary with corpus luteum and degenerating cell mass with increased in interspaces. The medullary region showed appearance of prominent cyst. Cortex region with stroma showed markedly cellular hypertrophy.

Further it showed only late primary follicles and atriatic follicles with appearance of corpus luteum but the development of Graafian follicles was totally not seen. Present observations indicate effect of melphalan on ovarian development and function as a suppressive effect, of chemotherapy which reduces ovarian function (Larsen et al. 2003). Ovarian failure had follicle depleted and non-detectable ovaries, elevated FSH and LH and immeasurable inhibin B (Thibaud 1988; Wallace et al. 1989; Byrne et al. 1992; Chirarelli 1999; Larsen et al. 2003).Ovarian follicle depletion and ovarian failure in relation to treatment modalities (Levy and Stillman 1991; Levitt and Jenny, 1998; Howell and Shalet 1998; Bathe et al. 2002).Treatment of melphalan for eight weeks affects an ovarian follicle with slow down of development indicates additive effect. The ovarian follicles showed affected mitochondria and ruptured nuclear membrane with effect on zonapellucida and hypertrophy of follicular cells. Present study indicates treatment of melphalan have additive effects on follicles to slow down development and some follicles with hypertrophied nucleus. At some places nuclear membrane seen ruptured. Present study suggests treatment of melphalan may cause the effect on development of ovarian follicle with affected nucleus with hypertrophied and ruptured nuclear membrane indicates effect of this compound is also on to arrest the growth and development of follicles, indicating defect in functional unit of the ovary with atresia or degeneration.

Observations





Changes in the hormonal levels of control and treated animals.Levels are expressed in mIU/ml; mean + SE of six animals in each group.Figures in parenthesis denotes number of estimations.

Parameter	Control(6)	4weeks treated(6)	8weeks treated (6)
Follicle stimulating hormone(FSH)	0.9 ± 0.17079	0.5 [a] ± 0.0816	0.33 [a] ± 0.0115
Luteinizing hormone (LH)	0.02 ± 0.0042	0.7 [a] ± 0.1390	0.3 [a] ± 0.0465
Prolactin (PRL)	0.15 ± 0.0128	1.1 [a] ± 0.1390	1.17 [a] ± 0.0085
Estradiol	22 ± 1.8073	8.6 [a] ± 0.2394	7.2 [a] ± 0.1132
Progesterone	9 ± 0.9661	16.8 [a] ± 0.1291	18.8 [a] ± 0.1154
17-OH progesterone	1.8 ± 0.1712	0.8 [a] ± 0.1707	0.59 [a] ± 0.0121

Data analysed by Data Annova analysis test.
P > 0.05 i.e. P value is statistically Significant.a= significant

I indicates ± SE of 6 animals.

Fig. 1

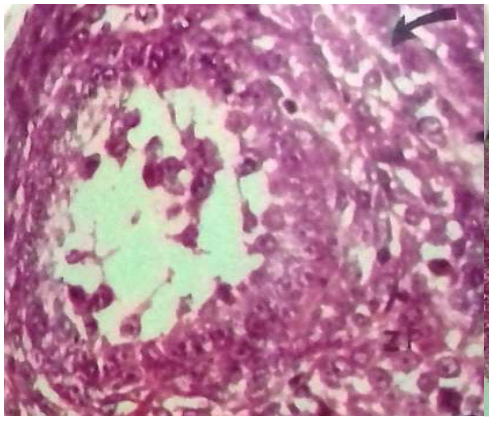


Fig.2

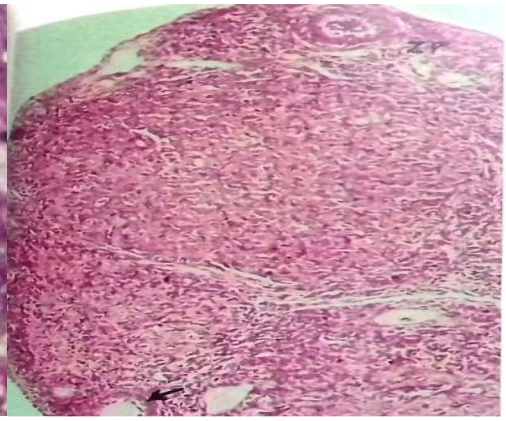


Fig.3

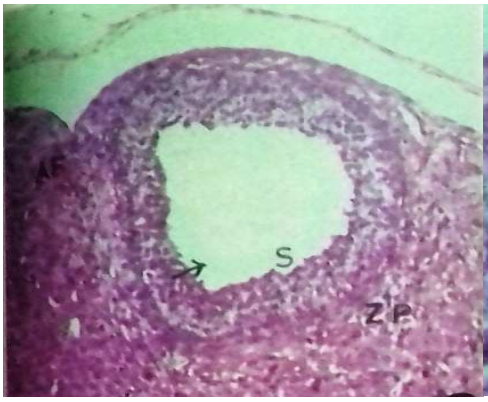


Fig.4

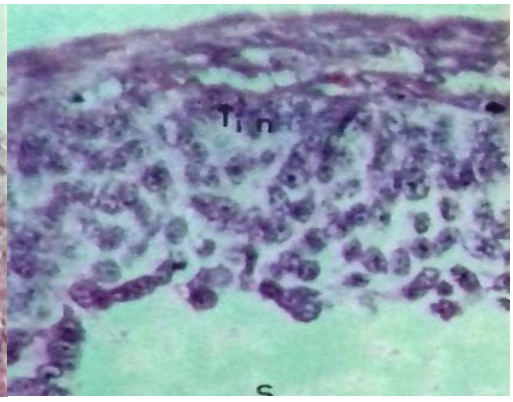


Fig.5 **Fig.6**

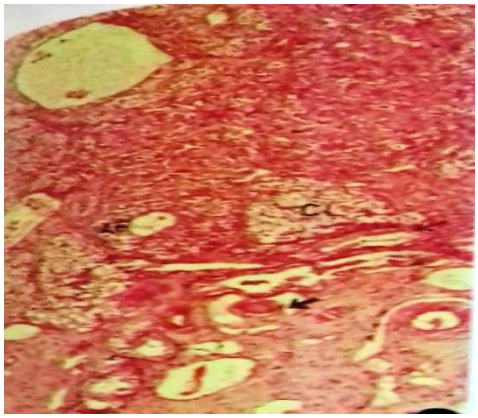
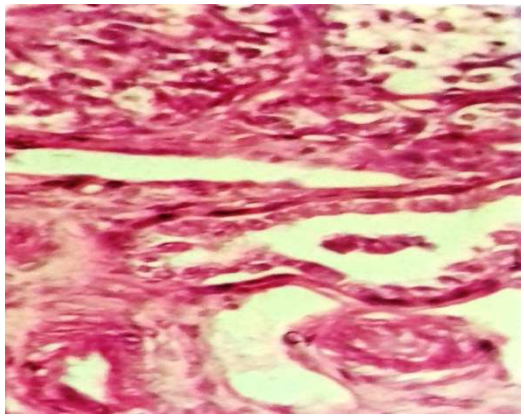



Fig. 1 &2 A low power light micrograph of control rat ovary showing various stages of follicle such as primary, late primary follicle, secondary follicle with zonapellucida (ZP), multiple layers of follicle cells (thick arrow) also atriatic follicles(AF) and thick thecal layer (curve arrow). X320 & X80

Fig. 3& 4 Light micrograph of rat ovary treated with melphalan for four weeks showing atriatic follicles (AF) without membranagranulosa layer.Theca interna (Tin) with empty cytoplasm with darkly stained nuclei., endoplasmic stroma (S) i.e., empty cytoplasm withdarkly stained nuclei. X80 &X320.

Fig.5& 6 Light micrograph of rat ovary treated with Melphalan for eight weeks.low power micrograph showing atriaticfollicles(AF),corpus lutium showing degenerative cell mass with increase interspaces(Arrows),also shows the prominent cyst on the medullary region of the ovary. X80 & X320.

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