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PROLIFERATIVE EFFECT OF VINCRISTINE SULFATE AND ESTRADIOL ON FEMALE MICE

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ABSTRACT
Many chemical agents like vincristine sulfate and estrogen have an important role in veterinary and humane application to produce successful treatment of malignant and non-malignant disease. This study was conducted to determine the pathological effect of estrogen and vincristine sulfate on female genital organs (uterus, ovary, and mammary glands). A total of 100 females mouse are taken at age of 21day and divided into two groups, each groups contain (40) mouse and two groups of control (10 mouse in each group).The first group of experimental animals was treated with estrogen hormone diluted by olive oil as a vehicle with subcutaneous injection for (12weekes) at a daily manner. While the second group of experimental animals is treated with vincristine sulfate diluted with distal water as a vehicles with intraperitonial injection for (12 weeks), twice weekly. The control group of each one of them is injected with the vehicles of it in the same manner of injection and the period. The result of the first group which is treated with estrogen showed a metaplasia of the uterine epithelia and hyperplasia of endometrial glands under effect of estrogen hormone, endometritis appear prominent. While the ovarian tissue of this group showed different stage of ovarian follicles without maturation. The mammary gland which is subjected to the same treatment produces prominent hyperplasia of ductal epithelia. The results of second group treated with vincristine sulfate in the same period showed a metaplasia and hyperplasia of epithelial tissues with extensive hemorrhage, while the endometrial gland showed different lesion from the first group. The endometrial gland appeared atrophied. Lutiniztion of the ovarian tissue and atratic follicles is clear. On other hand, hyperplasia of the epithelial duct and acini of mammary tissues appeared clearly.

KEYWORDS: vincristine sulfate, estrogen, ovarian follicles, mouse.

INTRODUCTION

The ability of the cell to maintain genomic integrity is vital for cell survival and proliferation. The lack of fidelity in DNA replication and maintains can results in cell division (Rodney *et al*., 2016). Environmental factors such as exposure to chemicals, dietary habits, and lifestyle have direct effect on the genomic mammalian cell .Chemical compound such as vincristine sulfate and Estrogen hormone which is widely distributed in veterinary medicine and human application to improve successful treatment of cancer and other disease which have direct effect on cell proliferation. One of these chemical compound which is classified as chemotherapy is vincristine sulfate return to the oldest group of these compound, Which is called vinca alkaloid obtained from the Madagascar periwinkle plant called (*Cantharanthus roseus*) (Sahelian *et al*., 2011; Brogan, 2010).The medical application of this compound lead to monitoring of them to treat hypoglycemic activity, diabetes, high blood pressure and these drug have been used as disinfectant, but the main use of vincristine sulfate as anticancer (Bennouna *et al*., 2008). In veterinary application, vincristine used as chemotherapeutic agent to treat various neoplastic disorder such as lymphoma, leukemia and sarcomas (Dobsan *et al*., 2008). Vincristine sulfate exert cytotoxic activity by disrupting cellular microtubules formation including the replication of cancer cell (Coppoc, 2009). In human vincristine sulfate has been approved to treat acute leukemia, rhabdomysarcoma, neroblastoma, willm's tumor, Hodgkin's disease and other lymphoma. Another characteristic of vincristine sulfate has been reported to treat several non- malignant hematologic disorders such as refractory autoimmune thrombocytopenia, hemolytic uremic syndromes and thrombotic thrombo cytopenia purpura (Rath, and Kozielski, 2012: Kufe *et al*., 2003).The main mechanism of vincristine sulfate is to interaction with function, particularly of microtubules. The mitotic spindle apparatus, directly which causes metaphase arrest (Jacsone *et al*., 2007).On the other hand chemical compound such as estrogen hormone which is act most importantly on the reproductive organs and on other organ system such as cardiovascular, skeletal, immune, gastrointestinal and neural sites (Doroo and Korach, 2006; Nelson *et al*., 2013). Estrogen is a well-known female steroid hormone synthesized in the ovary that controls the estrous or menstrual cycle in females, therefore estrogen is imperative for female reproduction, (Chaturved *et al*., 2008). Estrogen receptors play a crucial role in reproduction and normal physiology. The two sub-types of ER (ERα and β) are expressed in various levels in different tissues and selective cell types. Estrogen is not only important in female reproduction but also in male reproduction and in numerous other systems including the neuroendocrine, skeletal and immune systems in males and females. Along with the influence of estrogen on many physiological processes, it is also implicated in many different diseases including obesity, metabolic disorder, cancer, osteoporosis, endometriosis and fibroids The predominant mechanism of estrogen action is through nuclear estrogen receptor (ER) expression in estrogen

target organs The biological and genomic actions of Estrogen is mediated through two distinct ER proteins, ERα and ERβ (ERs) (Burns and Korach, 2012) . The Estrogen is mediated via estrogen receptors which are protein that bind estrogen with high affinity specify. These receptors are a member of nuclear receptor (Hauny and Rastingyad, 2010). Estrogen used in human combined with progesterone as hormonal replacement therapy which is given either in cyclical or combined manner other application used in the contraceptive.

MATERIALS & METHODS

Experimental animals: (Mice) One hundred female with an average weight (15gm), were obtained from the Animal house colony of the (National Center for Drug Control and Research). They were housed in plastic cages 50×30×10 cm and placed in the room for 10 days for adaptation. The room temperature was maintained at 21 ± 3˚C; the air of the room was changed continuously by using ventilated vacuum. The litter of the cages was changed every day. The animals were housed in the animal house of the College of Veterinary Medicine, Baghdad University, Department of pathology and were fed on pellet *ad libitum*. The current study was end in the three months.

Experimental design: One hundred female mouse, in aged 3 weeks were divided into 3 main groups that treated as following;

The 1St group (n=40) mouse:-This group of animals received estrogen treatment diluted in olive oil at a dose of 0.1 ml. The rout of administration is subcutaneous (S/C), daily along the period of experiment (12weeks).

The 2nd group (n=40) mouse:-This group of animals received vincristine sulfate treatment diluted in distal water injected at a dose 0.1ml.The rout of administration is intraperitonial (IP),twice weekly along the period of experiment(12weeks).

Control group

The 1St group (n=10) mouse:- This group of animals received olive oil at a dose of 0.1 ml. The rout of administration is subcutaneous (S/C). This group injected with olive oil daily along the period of experiment (12 weeks).

The 2ndgroup (n=10) mouse:- This group of animals received distal water injected at a dose 0.1ml.The rout of administration is intraperitonial (IP). This group injected with distal water twice weekly along the period of experiment (12 weeks).

Postmortem Examination

Macroscopical examination: Postmortem examination was done after killing all the mice and at the end of the experiment. The macroscopic appearance was recorded to detect any abnormal gross changes in the organs.

Histopathological examination: at the end of the experiments (12weeks), all animals were sacrificed gross examination was done to detect the pathological lesions. Specimens was taken from the target organs including the ovary, uterus, and mammary gland of the mice. The tissue section was fixed in 10% buffer formaldehyde for 72 hrs. The specimens were washed with tap water and then processing was done routinely by using the Histokinette, with an increasing alcoholic concentration from 70%, 80%, 90% to absolute 100% for 2 hrs. In each concentration to remove water from the tissues' xylen, then the specimens were infiltrated with semi-liquid paraffin wax at 58 Ċ on two stages, the blocks of specimens were made with paraffin wax and sectioned by rotary microtome at 5µm thickness for all tissues. All tissues slides were deparaffinized in xylen and rehydrated in ethanol (Luna, 1968).Then the slides was stained with Heamatoxyllin and Eosin (H & E) stains and the histopathological changes were observed under a light microscope and examined in Pathology Department, College of veterinary medicine, Bagdad University.

RESULTS

Macroscopical Examination: The gross lesions of each group are examined by naked eye after sacrificing all the animals to determine the gross lesion of the target organs of all groups (uterus, ovaries and mammary glands).The first group which is subjected to Estrogen hormone, for 12 weeks showed abnormal features. The uterus of this group showed enlarged, congested and filled with pus (pyometra) (Fig.-1), the uterus of control group appear normal in size and architectures (Fig.-2).The ovaries of the same group appears edematous, enlarge and congested (Fig3). Mammary glands on the other hand appear enlarged and congested (Fig.-4). Second group which is injected with vincristine sulfate (VCR) for 12weeks showed gross lesions appear to be differently from the first group. The uterus which is the most affective organ in this group appear to be atrophied and small in size (Fig.- 5), in the same feature ovaries and uterus appear atrophy (Fig.- 6), mammary glands of this group appear atrophied and congested (Fig.- 7).

Histopathological Examination: The results of Histopathological examination demonstrate many changes which is variant according to the compound used in this study. Histopathological examination has been done by light Microscope with Haematoxyllin and Eosin stain. The target organs include (uterus, ovaries and mammary glands) of the first group which is injected with Estrogen hormone at a daily manner for 12 weeks demonstrate multiple changes associated with Estrogen treatment. The uterus of this group showed morphogenetic alteration that include changes in the morphology of epithelial cells of endometrium, which produce hyperplasia with hyper chromatic nuclei (Fig.- 8). Our observative study recorded that under effect of Estrogen the number of endometrial glands increased in numbers with different shapes (Fig.- 9) and squamous metaplasia of endometrial glands (Fig.-10). Other glands showed cystic dilatation filled with secretion and inflammatory reaction within stroma (Fig.-11). Cervix appear to be more affected with Estrogen which showed cervicitis and infiltration of inflammatory exudate within endometrial glands (Fig.-12). Metritis characterized by infiltration of inflammatory cell within stroma and fibrosis (Fig.-13). Ovaries of this group showed increase number of follicles with different development stages (Fig. 14), on the other hand ovaries of this group showed cystic follicles and hyperplasia of granulosa cell (Fig.-15), and the same organs showed increased number of cystic follicles (Fig. 16). Mammary glands showed ductal hyperplasia with papillary projections in the lumen of duct (Fig.-17), the same lesion showed hyperplasia with hyper chromatic

nuclei (Fig. 18). The second group which is injected with vincristine sulfate twice weekly for 12 weeks showed different Histopathological changes. The uterus appears to be the Maine organ which is affected by vincristine sulfates the epithelia of uterus showed hyperplasia with papillary projection inside the lumen (Fig.-19). Squamous metaplasia of the endometrial glands is the most important changes under effect of vincristine sulfate with infiltration of inflammatory cell in all stroma of uterus (Fig.-20) .The most prominent lesion observed in endometrial glands which appear atrophied, and extensive hemorrhage in the stroma (Fig 21). Ovaries of this group showed atratic follicles and extensive hemorrhage of ovarian tissues (Fig.-22) and leutinization (Fig. -23) extensive hemorrhage of the ovarian tissues (Fig.- 24) ductal hyperplasia appear in all sections of mammary tissues (Fig. 25).

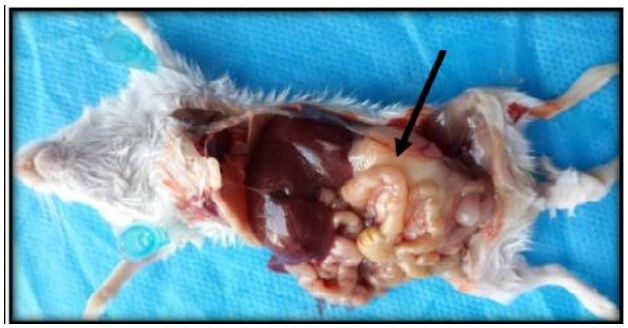

FIGURE 1: Gross section of mouse uterus treated with Estrogen for 12weeks showed enlarged, congested and filled with pus (pyometra).

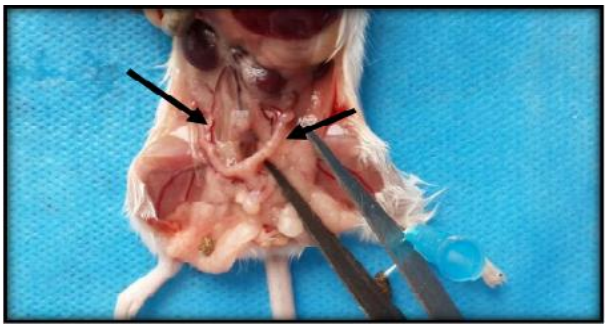

FIGURE 2: Gross section of normal uterus and ovary in mouse of control group which appear normal in size and artituchures.

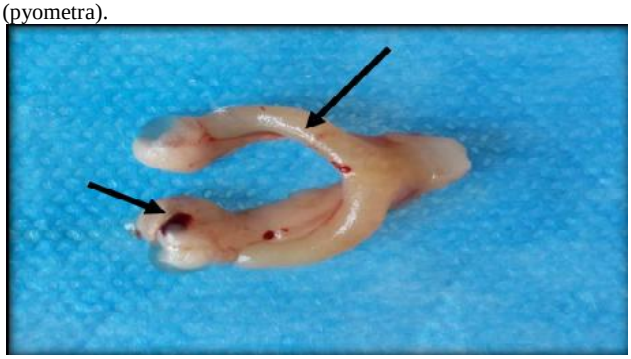

FIGURE 3: Gross section of ovary and uterus of mouse treated with Estrogen for 12 weeks which appear edematous, enlarged, and congested.

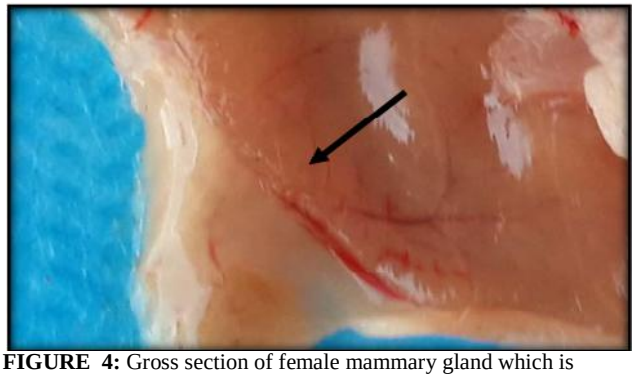

FIGURE 4: Gross section of female mammary gland which is treated with Estrogen for 12 weeks appear enlarged and congested

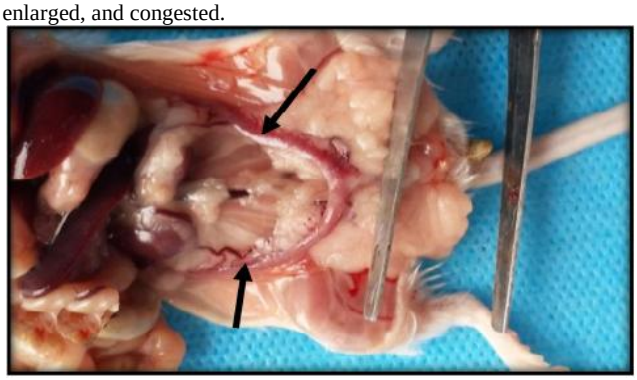

FIGURE 5: Gross section of female genital organs of mouse treated with vincristine sulfate for 12 weeks, show atrophied uterus and ovary and small in size.

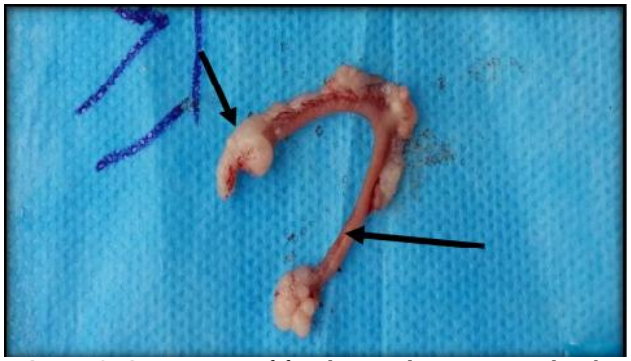

FIGURE 6: Gross section of female genital organs, treated with vincristine sulfate for 12 weeks show atrophied ovary and uterus.

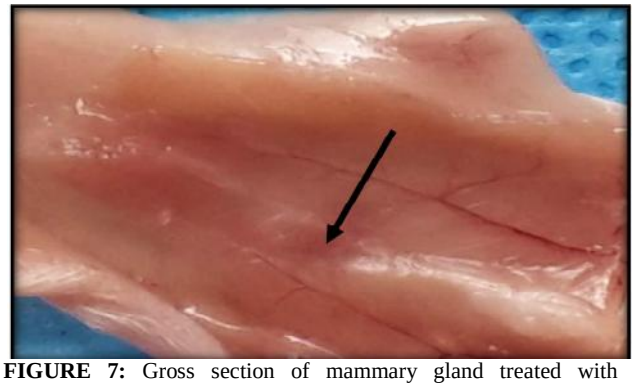

FIGURE 7: Gross section of mammary gland treated with vincristine sulfate for 12 weeks appear slightly atrophied and congested.

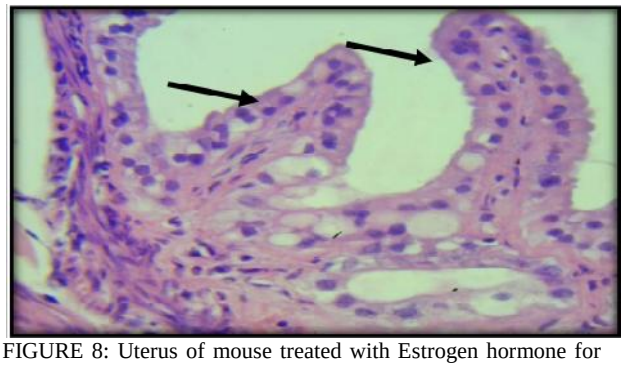

FIGURE 8: Uterus of mouse treated with Estrogen hormone for 12 weeks showed hyperplasia of epithelial lining with hyper chromatic nuclei (H&E) X400.

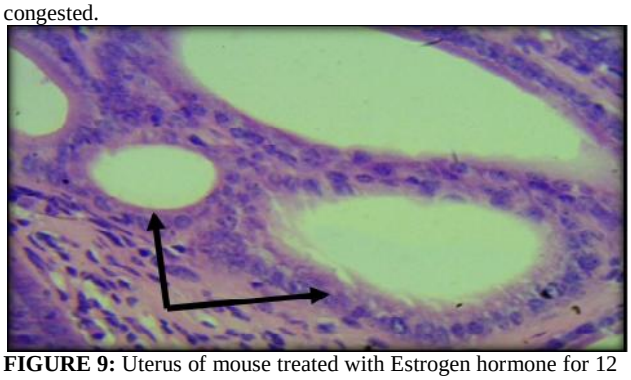

FIGURE 9: Uterus of mouse treated with Estrogen hormone for 12 weeks showed increased number of endometrial glands with different shape (H&E) X100.

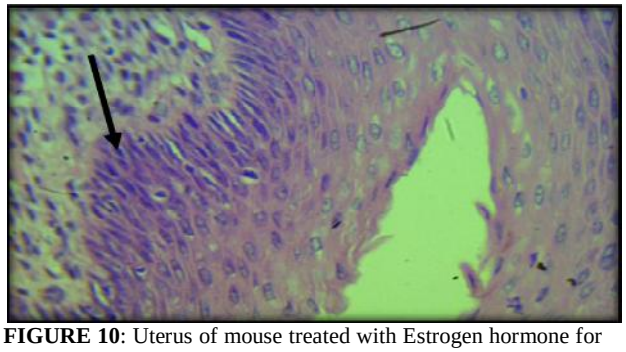

FIGURE 10: Uterus of mouse treated with Estrogen hormone for 12 weeks showed squamous metaplasia of endometrial glands (H&E) X400

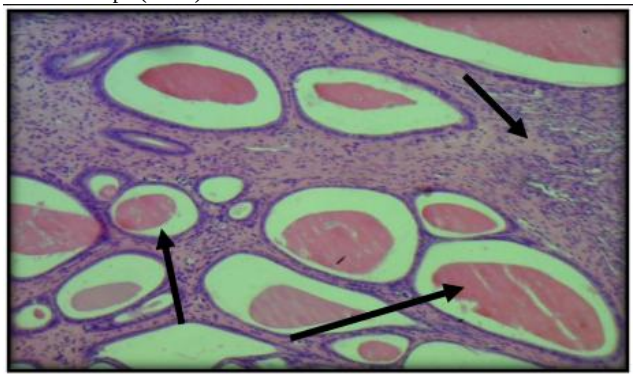

FIGURE 11: Uterus of mouse treated with Estrogen hormone for 12 weeks showed cystic dilatation of endometrial glands glands filled with secretion and inflammatory reaction within stroma (H&E) X100.

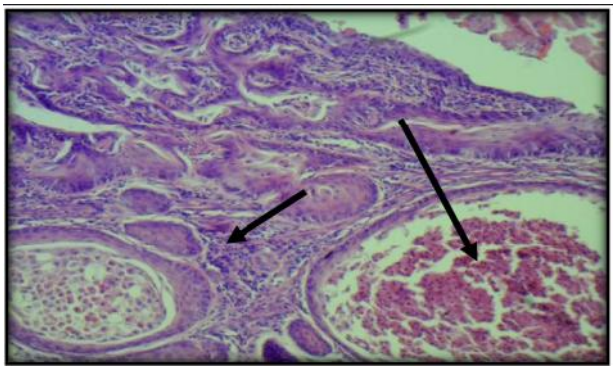

FIGURE 12: Uterus of mouse treated with Estrogen hormone for 12 weeks showed cervicitis with infiltration of inflammatory cell within stroma and inflammatory exudates within endometrial glands (H&E) X100

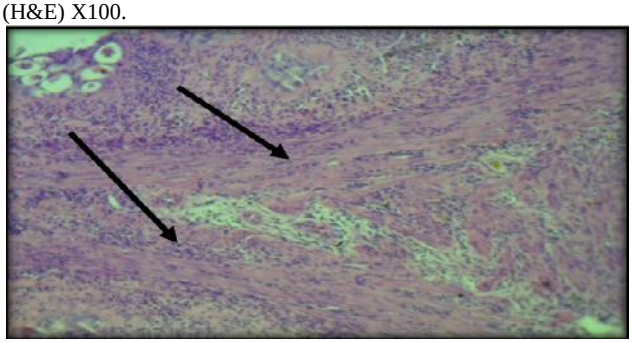

FIGURE 13: Uterus of mouse treated with Estrogen hormone showed metritis and fibrosis with infiltration of inflammatory cell within stroma (H&E) X100

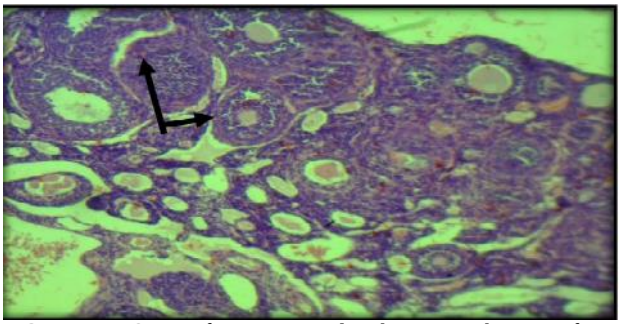

FIGURE 14: Ovary of mouse treated with Estrogen hormone for 12 weeks showed increased number of follicles with different development stages (H&E) X100.

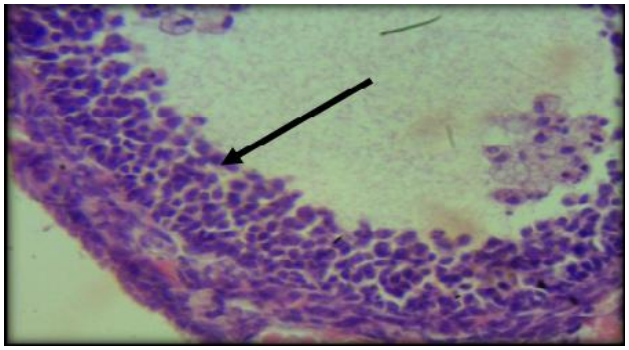

FIGURE 15: Ovary of mouse treated with Estrogen showed cystic follicles' with hyperplasia of granulosa cell and hyperchromatic nuclei (H&E)X400.

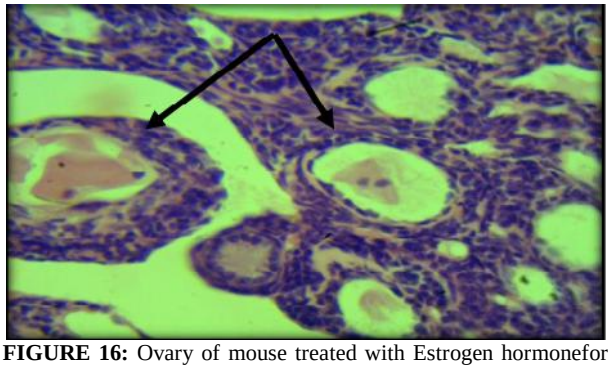

FIGURE 16: Ovary of mouse treated with Estrogen hormonefor 12 weeks showed cystic follicles with hyper chromatic nuclei of granulose cells (H&E) X100

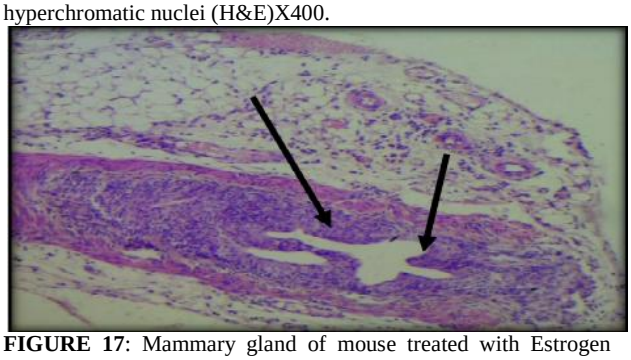

FIGURE 17: Mammary gland of mouse treated with Estrogen hormone for 12 weeks showed hyperplasia and papillary projection of epithelial duct with mitotic figure (H&E) X100.

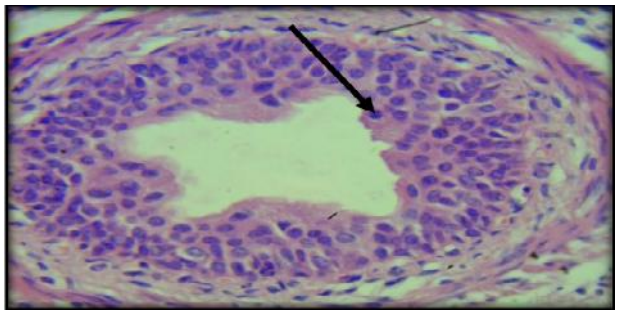

FIGURE 18: Mammary gland of mouse treated with Estroge hormone for 12 weeks showed hyperplasia of epithelia duct with hyper chromatic nuclei (H&E) X400.

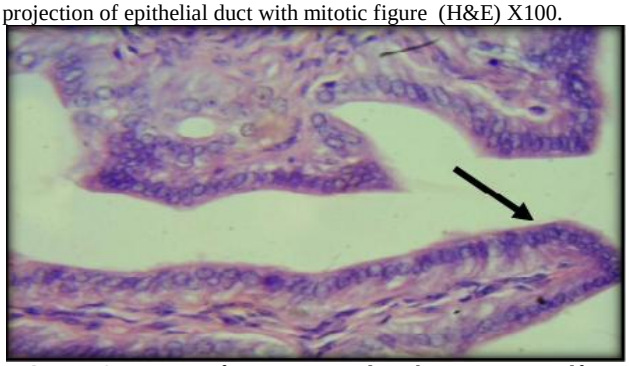

FIGURE19: Uterus of mouse treated with vincristine sulfate (VCR) for 12 weeks showed hyperplasia of epithelial lining Cells with papillary projection (H&E) X100

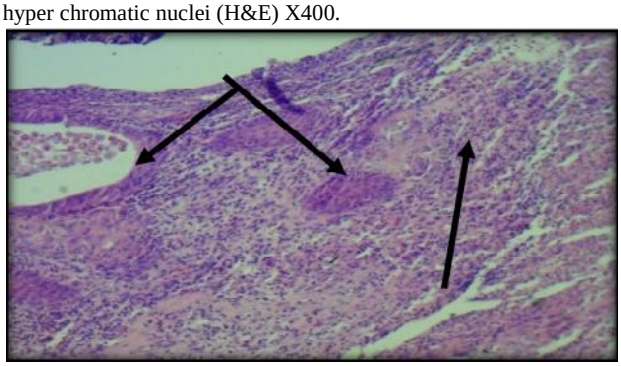

FIGURE 20: Uterus of mouse treated with vincristine sulfate (VCR) for 12 weeks showed squamous metaplasia of endometrial glands with inflammatory reaction within. stroma (H&E) X100

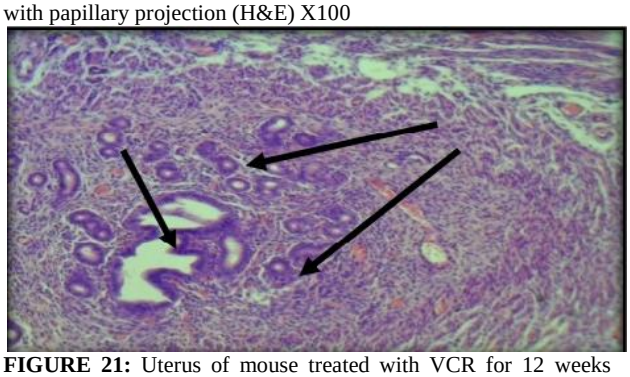

FIGURE 21: Uterus of mouse treated with VCR for 12 weeks Showed atrophy of endometrial gland and hyperplasia of epithelial lining cells with congestion, hemorrhage.

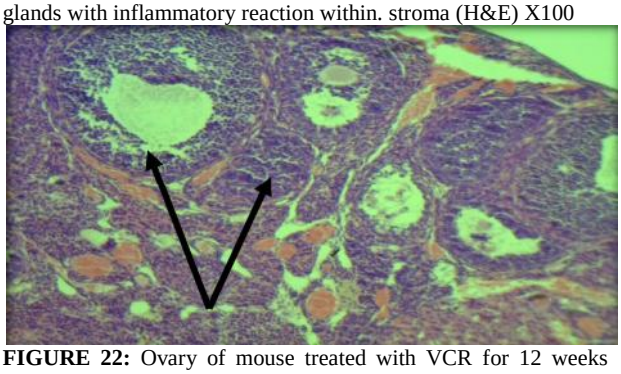

FIGURE 22: Ovary of mouse treated with VCR for 12 weeks showed atratic follicles and hemorrhage of ovarian tissues (H&E) X100

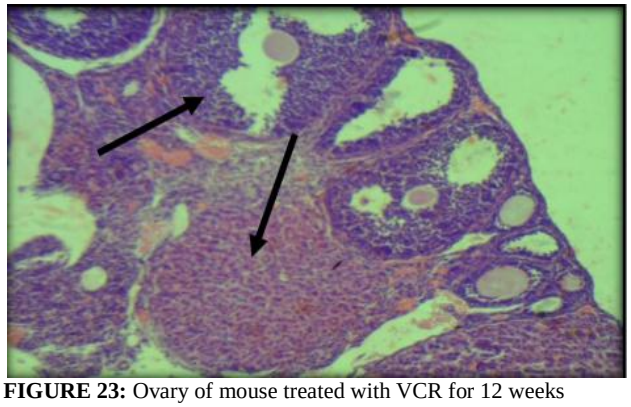

FIGURE 23: Ovary of mouse treated with VCR for 12 weeks Showed leutinization and atratic follicles (H&E) X100

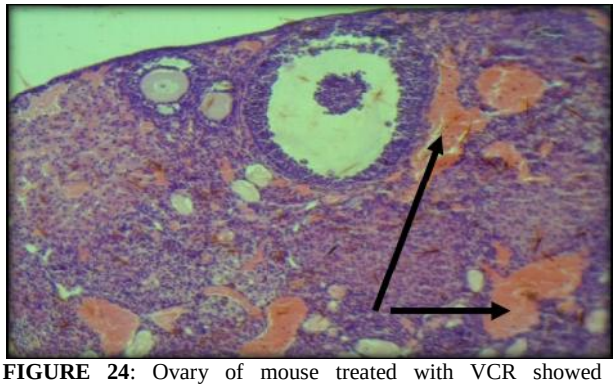

FIGURE 24: Ovary of mouse treated with VCR showed diminished ovarian follicles, with extensive hemorrhage of ovarian tissues (H&E) X100.

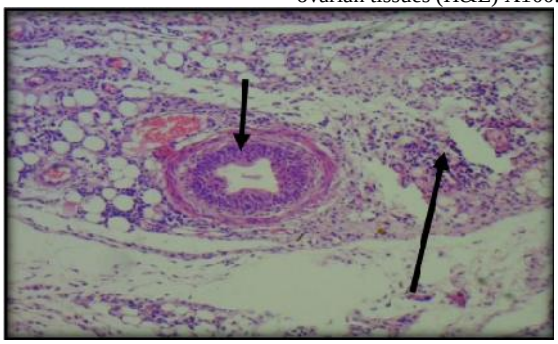

FIGURE 25: Mammary gland of mouse treated with VCR for 12 weeks showed hyperplasia of epithelial duct with increased mitotic figure and inflammatory reaction and hemorrhage (H&E) X400

DISCUSSION

Macroscopical Examination

The result of macroscopicall examination which has been done by naked eyes showed different lesion from each other's. The female genital organs in each group which include (uterus, ovary and mammary glands). The uterus of the first group which is subjected to Estrogen treatment showed the mainly lesion which is characterized by enlargement, congested and filled with pus (pyometra) compared with control group which appeared normal in size and architecture. This feature returned to the immune suppressive effect of Estrogen which has important role in accumulation of pus and fluid in the lumen so the uterus appear hypertrophic and congested as a result of inflammatory reaction. This observation supported by the flowing Histopathological examination which produce endometrial hyperplasia and accumulated inflammatory cell that lead to cause thickness of endometrial wall. Ovaries of this group appeared enlarge and edematous under effect of Estrogen associated with follicle development and abnormal number of follicles and the congestion which appear in the surface of the ovary associated with estrogen which increase the blood supply to the ovarian tissue .Estrogen has the same effect on the mammary glands which is the gross lesion showed enlargement and edematous duo to the hyperplastic change of the mammary tissues. Second group which is treated with vincristine sulfate showed different lesion from the first group .So the main organ which is affected by this compound is the uterus which appeared atrophied and small in size .In some area showed extensive hemorrhage. This is returned to the effect of vincristine sulfate which cause atrophy of endometrial glands and destruction of blood vessels lead to the appearances of hemorrhage in the surface .Ovaries for the same reasons demonstrated shrinkage and atrophy duo to destruction of ovarian tissue and depletion of follicles. Mammary tissues appear enlarged duo to proliferation of ductal epithelia of mammary gland tissues

Histopathological Examination: 1st group which is injected with Estrogen at a daily manner and therapeutic dose showed different histopathological changes of the target organs (uterus, ovary and mammary gland). The uterus is one of the major target organs for steroid hormones action. The uterus consist of multiple cell types including tow layer of smooth muscle tissue, myometrium, that surrounding the glandular endometrium the endometrium can be further divided into the stromal layer, the luminal epithelium, and the glandular epithelia .The uterus of mouse contain both Estrogen receptor ERα and ERβ (Gurber *et al*., 2002) Under effect of Estrogen, endometrium appear to be more sensitive to Estrogen hormone (Gnanagurudasan *et al*., 2017). In the present study the intensity of uterine lesion varied and showed different morphogenetic alteration that include changes in the morphology of epithelium cells of the endometrium which showed hyperplasia with cryptologic atypia in which the epithelial cells appear hyper chromatic with prominent nuclei and increase nuclear to cytoplasmic ratio. This result agree with (Volumer, 2003) who reported that Estrogen is a carcinogenic as well as triggering neoplastic changes in the endometrium these observative is reported by (Montogomory *et al*., 2004).

In human prevalence that persistence hyper estrogenic state in obese women will cause proliferative changes in the endometrium. If the estrogenic effect is prolonged for

a long period this may be lead to type 1 Estrogen dependent adenocarcinoma of the endometrium (Montogomory *et al*., 2004). Our observative study refer that there is an increase in the number of endometrial gland with abnormal shape and abnormal type of epithelium with incidence of endometrial hyperplasia and metaplasia. This is return to the effect of Estrogen action at molecular level and increase the transcription and growth factor signaling pathway and cause malignant changes (Schiff *et al*., 2004) this result also agree with (Takashi *et al*., 2002) who consider this changes as apre-cancerous. Cystic gland which prominent in this group and appear filled with secretion duo to mitosis orientation which is induce by Estrogen, so the mitosis orientation effect the shape of glands which is appear as a simple or cystic gland .It formed from normal simple tubular glands following Estrogen induced alteration morphogenesis. If epithelial cells of glades divide parallel to both the basement membrane and the long axis of gland this should lead to lengthening of glands. If the cells divided parallel to the basement membrane and perpendicular to the long axis of along glands this cause an increase in diameter of glands (dilatation) and formation of cystic glandes. If the cell division is perpendicular to the basement membrane it will lead to the formation of stratified epithelium and/or branches papillae. There is strongly suggestion that the orientation of mitosis perpendicular to the basement membrane which is responsible for the formation of the pre-cancerous changes which is more important changes in proliferation (Gunin, 2001). Epithelial cells were at one time considered to act as physiological barrier in the female reproductive tract to separate the host from potentially harmful bacteria and viral pathogen, but under the effect of Estrogen .This epithelial cells carry out a number of essential function as part of the innate and adaptive immune system in mouse (Wira *et al*., 2004). Our study recorded many changes in these epithelial tissues, one of the prominent lesion appear in the cervix that mean (cervicitis). Cervicitis is the most prominent, because cervix is a part of the female reproductive target tissues that is highly responsive to Estrogen (Dero, 2006). Cervix are necessary for this dynamic changes in cervical epithelium, so infection are more easily to overcome all the uterus section and the supportive exudates filling the lumen of the uterus with infiltration of inflammatory cells within the wall of the uterus and exudates specially neutrophil and macrophage are appear in section under effect of Estrogen. This observation agree with (Wantabe *et al*., 2004) who refer to the effect of Estrogen on the recruitment of different immune cells. Other investigation indicated by (Chieh *et al*., 2015) improve that Estrogen is known to be involve in the production of pro inflammatory component which might participate in the neoplastic transformation and carcinogenesis of the endometrium. Further studies reported that CD45 cells could be involve in the proliferative action induced by Estrogen CD45 cells were homogenously scattered through the endometrial stroma in normal non proliferative endometrium. In aggregation around the glandular epithelium CD45 cells increase in non-atypical hyperplasia and there are marked increased in these cells surrounding the glands and infiltration to the glandular epithelium. Other authors have another opinion about this inflammatory reaction who pointed that under effect of Estrogen cause inhibition of TNF-alpha release from uterine epithelia. Mouse endometrium contains both TNF –alpha and TGF-beta mRNA have been showed to be under estrogen control so epithelial responsive to Estrogen is complicated by the communication between epithelia of uterus and stroma (Pierro *et al*., 2001), which play an important role in the immune protection In the uterus ,cervix against potential pathogen(Crane *et al*., 2005).

Several morphological alterations were observed in the ovaries of animals treated with Estrogen. As compared to control groups, the ovaries of Estrogen treated group showed several changes in the morphology of anatral and preovulatory follicles, hyperplastic corpora lutea and cystic follicles have also been observed in the ovaries which contained follicles with large cavities, and cystic appearance This observation is consistent with that reported previously by (Manni *et al*., 2005) the same result recorded by (Singh, 2005). leutinization of cells in the anatrum and hyperplastic theca layer which is agree with (Beloosesky *et al*., 2004).our observative showed increase number of ovarian follicles.

These changes returned to the effect of Estrogen treatment which regulate expression of angiogenetic factor such as vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthesis (NOS) in the ovary (Choong *et al*., 2010). So the angiogenesis plays critical roles in folliculogenesis such as the growth of follicles and the selection of the dominant follicles (Geva *et al*., 2000). It's believed that sufficient blood supply via active angiogenesis is necessary for the induction of oocyte production. Vascular endothelial growth factor (VEGF) is critical regulator of angiogenesis. It is expressed in the follicles and in ovarian cells (Fraser *et al*., 2005). Enhancing VEGF expression during the follicular phase could be useful in increasing the number of predominant follicles destined for ovulation and angiogenesis (Lijima *et al*., 2005), on the other hand nitric oxide (NO) has been known to be another potent vasodilator and angiogenetic factor and its play an important role in ovarian angiogenesis during folliculogenesis and ovulation (Sengoku *et al*., 2001) its mediate the vascular permeability of VEGF which stimulate the production of NO in endothelial cells (Weiner *et al*.,1994) these results similar to (Kolibianaski *et al*., 2005) who is improve that Estrogen enhance follicular growth and development. Further study has been done on mice by (Fraser *et al*., 2005. Estrogen receptor (ER) is transcription factor that regulate the genetic program of cell progression and growth of mammary tissues and have the ability to promote tumorgenesis directly, through stimulation of the Estrogen receptor (Mavaddat *et al*., 2012). So mammary gland which contains two forms of receptors (ESα and ESβ). ES is the main critical transcriptional factor responsible for development and function (yuxin *et al*., 2007).

Mammary gland on the other hand of Estrogen treated group show hyperplasia of the epithelial duct with hyper chromatic nuclei. This result return to the effect of Estrogen metabolites including catechol Estrogen and reactive semiquine and Quinone which may act as apre-carcinogenes. They might induce direct and indirect free radicals mediated DNA damage, genetic instability and mutation in cell (Jefocoat *et al*., 2000).

This study is supported by (Hayes, 1996; Liehr, 2000) who hypothesized that Estrogen metabolism through the catechol pathway. Phase I in, mice, hamsters, and rats involves several cytochrome P-450 enzymes that catalyze the oxidative metabolism of Estrogen to 2-hydroxycatechol Estrogen (cytochrome P-450 1A1, 1A2, and 3A) or to 4-hydroxycatechol Estrogen (cytochrome P-450 1B1). cytochrome P-450 1B1 is constitutively expressed in the breasts, ovaries, adrenal glands, and uterus, as well as in several other tissues. In human Estrogen associated with incidence of breast cancer in which it is hydroxylated to form the catechol Estrogens 2-hydroxyestradiol (2-OHE1(E2)) and 4-hydroxyestradiol4-OHE1(E2)), a process that is catalyzed by a number of cytochrome (CYP) P450 enzymes, including CYP1A1, CYP1A2,CYP1B1, and CYP3A4. The catechol Estrogens are further oxidized (by the same enzymes) into semiquinone and quinone forms, the latter of which can react with DNA to form adducts. This idea reported by (Huang *et al*., 2012). In vivo studies have demonstrated that exogenous 2-OHE2 and 4-OHE2 can induce uterine and kidney cancers The Estrogen 3,4-quinone can form unstable adducts with adenine and guanine in DNA, leading to depurination and mutation in vitro and in vivo (Gaikwa *et al*., 2009).From another view reduction of Estrogen quinones back to hydroquinones and catechols provides an opportunity for redox cycling to produce reactive oxygen species and probably accounts for the oxidative damage to lipids(Gaikwa *et al*., 2009).

The second group which is inject with vincristine sulfate twice weekly showed many histological changes many of these changes appear in the endometrial epithelia demonstrated hyper chromatic nuclei of the epithelia with simple hyperplasia this opinion have agreement with (Tsai *et al*., 2012) who refer that vincristine sulfate cause oxidative DNA damage by induce reactive oxygen and nitrogen species .this study show that vincristine sulfate cause injury to the epithelial cell. Our study refer that there is hyperplasia and squamous metaplasia in the epithelium of uterus this result is supported by (Jiang *et al*., 2008; Tasi *et al*., 2012) who showed that vincristine sulfate induce DNA misrepair, telomere end fusion, nuclear buds and increase frequency of gene mutation they pointed to the possibility of genotoxicity by vincristine sulfate .On other hand our result showed that the epithelial cells appear hyper chromatic with increased of mitotic figure indicating an antimitotic activity of these compound.

This is consistent with previous results showing that VCR increased apoptotic cell numbers and ratios and decreased the nuclear division showing the cytotoxicity of VCR (Jiang *et al*., 2008: Le Fevre *et al*., 2007). In fact, VCR, is known to exert their cytoxicity via arresting mitosis and going into interphase (Novichkova *et al*., 2003), these pathological changes in epithelia of endometrium associated with VCR treatment support the hypothesis that aneuploidy is one of the possible mechanism for induction of the early steps of neoplastic transformation (Takeki *et al*., 2016). On the other ways VCR are able to induce certain types of DNA alteration such as oxidative and micronuclei, this could be duo to their mechanism of action that is associated with activation of apoptosis through interference with spindle fiber formation and mitochondrial function(Jiang *et al*., 2008). So the damage of DNA speculate that regenerating cell may go through phase genotypic and phenotypic atypia in their way (Brien *et al*., 2007) As a result of these changes hyperplasia and metaplasia consider per neoplastic changes (Takashi, 2002). Metritis and the inflammatory reaction that overcome all the stroma of uterus hat is characterized by infiltration of inflammatory cell returned to the effect of VCR which cause immunosuppressive effect and marked release of CD4 & CD8 resulting decrease immunity and increase susptibility to infection (Eloisic *et al*., 2008). Others showed that VCR cause inhibition of release some type of interleukins in mice and rat (Norikaazu *et al*., 2008). Ovaries of this group showed different changes most of these changes appear in the follicles and granullosa cells; this group showed decreased number of primordial follicles, presence of atratic follicles and severs destruction of ovarian tissue with extensive hemorrhage. These result returned to the effect of VCR on members of caspase gene family in mice which have a role in oocyte destruction so the pathway of potassium influx during ovarian tissue death appears early in oocyte and granulosa cells this may be lead to regulate a number of apoptotic events including caspase activity and inter nucleosomal DNA cleavage (Perez *et al*., 2000). Other authors like (Meirowe *et al*., 2010) consider these pathological changes result in local ischemia, thereby affecting the growth and survival of primordial follicles. So the depletion of primordial follicles, indicating that blood vessel damage results in primordial follicle injury. This may also impair the processes of new vessel formation which are necessary for normal follicles development. This observation explains the hemorrhage and fibrosis of ovarian tissue which is more prominent in this group under effect of VCR. The same result recorded in human by (Brusamolino *et al*., 2000: Soleimani *et al*., 2011) who pointed that VCR associated with high rate of ovarian damage other authors have another opinion about ovarian damage by activation of the PI3K/ Phosphatase and tensin homolog/protein kinase B (PTEN/ AKT) pathway, leading to primordial follicle activation and follicular burnout (kalich & Philosoph, 2013: change, 2015) Under effect of VCR mammary gland also affected which produce hyperplasia of the epithelial duct with hyperchromatic nuclei this alteration of epithelial duct associated with genotoxice effect of VCR which is significantly increased 8-hydroxy-2-deoxy guanosine (8-OHdG) and sister chromatid exchanges (SCEs) this is may be lead to induce DNA damage (Mhaidat *et al*., 2016). The VCR has been shown to increase the frequency of micronuclei in experimental animals and in cultured human lymphocytes this result recorded by (Jiang *et al.*, 2008)In addition, they have also been shown to cause chromosomal mutations in vivo and in cultured cancer cells (Alsatari *et al*., 2012). Beside all the causative agent mention above other opinion refer to the maine effect of VCR to induce reactive oxygen and nitrogen species which have the ability to cause oxidative injury to the epithelia of mammary duct this is pointed by (Tsai *et al*., 2012; Zhao *et al* ., 2014).

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