Comparative pathological and cytogenetical study of ethanolic extract of *Vinca rosea* L. and Vinblastine in treating mammary gland adenocarcinoma implanted mice

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Abstract:

Pathological, cytogenetical comparative study was the main objectives of this project between the influence of ethanolic extract of *Vinca rosea* and Vinblastine (vinca alkaloid) as chemotherapy. Fourty female adult Swiss albino balb/C mice of approximately body weight (20-26g) and age (8-10) weeks were used in whole experiments. In cytogenetic study two parameters mitotic index (MI) and blast index (BI) were used. The results showed significant increase ($p<0.05$) in mitotic index (MI) and blast index (BI) values in groups treated with ethanolic extract of *Vinca rosea*. Also the results showed significant decrease in these values in groups treated with Vinblastine. Histopathological sections in control group showed the presence of tumor growth characterized by formation of acinar like structures, with pleomorphic tumor cells and hyperchromatic nuclei with a tendency to form giant cells and high numbers of mitotic figures with extensive areas of necrosis. The tumor showed metastasis in liver and Lung, with aggregation of tumor associated macrophages in liver. Histopathological sections in groups of tumor-bearing female mice, treated with ethanolic extract, were showed large areas of necrosis, mononuclear cell infiltration and encapsulate with fibrous connective tissue capsule with mononuclear cell infiltrations in lung and formation of early granuloma in liver. In chemotherapy, tissue sections showed vacuolation and necrosis of tumor cells which encapsulated with fibrous connective tissue. The pathological changes characterized by alopecia, and large pneumonic areas with extensive coagulative necrosis and apoptosis in liver with hyperplastic nodules formation. Bone section showed marked depletion of hemopoietic tissue of bone marrow and increased in the number of megakaryocytes, with signs of osteoporosis. We concluded that both ethanolic extract of *Vinca rosea* and Vinblastine have cytotoxic effect on transplanted mammary tumor cells in mice, with no side effect in case of *Vinca rosea* in comparison with vinblastine which cause severe pathological changes in internal target organs with signs of osteoporosis and precancerous lesions, with significant changes in the blood picture and in cytogenetic parameters.

Key words: *Vinca rosea* ؛ Alkaloids ؛ Herbal therapy

دراسة مرضية وخلوية وراثية مقارنة حول تأثير الخلاصة الكحولية لنبات عين البزون والفنبلاستين في معالجة السرطانه الغديه اللبنيه المغروسه في الفئران

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الخلاصة

هدفت الدراسة إلى مقارنة تأثير كل من الخلاصة الكحولية لنبات عين البزون*Vinca rosea* والعلاج الكيميائي الفنبلاستين(احد القلويدات المشتقة من النبات) وذلك من خلال دراسة التغيرات المرضية والتغيرات الحاصلة في معامل الانقسام الخيطي ومعامل التحول الارومي في نخاع عظم الفئران.تم استخدام 40 من إناث الفئران (بوزن= 20-26غم) وبعمر (8- 10) أسابيع حيث تناولت الدراسة مؤشرين من مؤشرات الوراثة الخلوية هما الانقسام الخلوي Mitotic index ومعامل التحول الارومي Blast index . فقد تم دراسة تأثير الخلاصة الايثانولية لنبات عين البزون *Vinca rosea* والفنبلاستين على الخلايا اللمفاوية لنخاع عظم الفئران حيث بينت النتائج ارتفاع قيمة الانقسام الخلوي MI ومعامل التحول الارومي BI للمجاميع المعاملة بالخلاصة الايثانولية لنبات عين البزون *Vinca rosea* تحت مستوى المعنوية ($P \leq 0.05$) . وانخفاضا في قيمة الانقسام الخلوي MI ومعامل التحول الارومي BI للمجاميع المعاملة بالفنبلاستين تحت مستوى المعنوية ($P \leq 0.05$) عند المقارنة مع مجموعة السيطرة.بعد أجراء الصفة التشريحية اظهر الفحص النسجي المرضي لمجموعة السيطرة وجود كتلة ورمية تميزت بالتكاثر العشوائي للخلايا الورمية المتعددة الأشكال وذات النوى الغامقة مع تكوين الخلايا العملاقة وزيادة في عدد الأشكال الانشطارية مع تكوين تراكيب سنخية ووجود مناطق واسعة من النخر وحصول النقائل في الكبد والرئة مع حصول النخر والموت المبرمج للخلايا وارتشاح البلاعم الكبيرة المصاحبة للورم من الكبد. إما المقاطع المرضية النسجية المعاملة بالخلاصة الكحولية لنبات عين البزون*Vinca rosea* فقد أظهرت وجود اوروام حبيبية في الكبد مع ارتشاح خلايا وحيدة النواة في ،الرئة ،الكبد.إما أهم التغيرات المرضية للمجاميع المعاملة بالفنبلاستين فقد تميزت الرئة بوجود مساحات واسعة من ذات الرئة، ووجود النخر التجلطي في الكبد مع زيادة في الموت المبرمج للخلايا الكبدية.لقد أظهرت مقاطع العظام حصول نفاد للنسيج مكون الدم مع وجود علامات لحصول تخلخل العظام، مع ارتشاح أعداد كبيرة من خلايا العدلات في نخاع العظم. نستنتج مما سبق إن الخلاصة الكحولية لنبات عين البزون*Vinca rosea* والفنبلاستين لها تأثيرات سمية خلوية على سرطانة الغدة اللبنية في الفئران وبدون أعراض جانبية في حالة الخلاصة الكحولية للنبات *Vinca rosea* على عكس من المعالجة الكيميائية فقد أدى الفنبلاستين إلى إحداث تغيرات مرضية شديدة في الأعضاء الداخلية المستهدفة من الجسم مع وجود علامات تدل على تخلخل العظام وآفات محتملة التسرطن.

مفاتيح الكلمات :نبات عين البزون ؛ القلويدات ؛ العلاج بالأعشاب.

Introduction:

Cancer is the second leading cause of death worldwide. It affects people at all ages with the risk for most types increasing with ages (1). Breast cancer is the most common form of cancer in women. Globally, it accounts for 22% of all new cancer diagnosis in women, and approximately 10% of all cases when men and women are combined (2). Because

of high death rate associated with cancer and because of the serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and /or complementary methods of treatment.Chemotherapy being a major treatment modality used for the control of the advanced stages of malignancies and as a prophylactic against possible metastasis, exhibits severe toxicity on normal tissues(3). *Catharanthus roseus* is known as the common or Madagascar periwinkle is a perennial, evergreen herb in the dogbane family *Apocynaceae* that was originally native to the island of Madagascar. Due to the pharmaceutical import-ance and the low content in the plant of vinblastine and related alkaloids vincristine, *Catharanthus roseus* become one of the best-studied medicinal plants. Consequently it developed as a model system for biotechnological studies on plant secondary metabolism (4).Vinca alkaloids are anti-mitotic and anti-microtubule agents working by preventing mitosis in metaphase

bind to tubulin, thus preventing the cell from making the spindles it needs to be able to divide (5). It comprises a group of about 130 terpenoid indole alkaloids. Vinblas-tine and vincristine introduced a new era of the use of plant material as anticancer agents. They were the first -agents to advance into clinical use in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancer including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers and Kaposi's sarcoma(6).

The present study was designed to investigate the followings:

1. Comparative pathological study between the Vinblastine and crude ethanolic extract of *Catharanthus roseus*.
2. Cytogenetical study between the Vinblastine and crude ethanolic extract of *Catharanthus roseus*.

Materials and Methods:

Collection and extraction of plant

Vinca rosea plant was obtained locally from Baghdad gardens. Shed and dried at room temperature. A voucher specimen of the plant was deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directo-rate in Abu-Ghraib (Certificate number (2967) in (23/11/2009).plant was (Vinca *rosea L.)*, English name was (Periwinkle) and local name was (Ain albazoon) a member of the family (*Apocyanaceae)* according to certificate.

The dried plant was separated into: aerial parts, the aerial (leaves) parts were grind into powder by coffee electrical grinder (mesh no.50), and the powdered parts were submitted to primary analysis diagnosis for its component (7*)*.

Preparation of ethanolic extracts of *Vinca rosea*

Ethanolic extracts of *Vinca rosea* was prepared according to (8).

Determination of LD_{50}:

Determination of LD_{50} of *V.rosea* ethanolic extract and vinblastine: ten adult mice were used . The procedure employed according to (9). By returned to result of LD50 of ethanolic extract in addition to some references (10) and LD50 for Vinblastin (11). The doses which gave the LD50 and these which gave highly severe pathological changes were lift.

Experimental design: In order to study the pathological and cytogenetics effect, (30) adult Swiss albino BALB/C mice were used at (8-10) weeks of age and (20-26) g of weight and in average of five mice for each group.All mice received treatment for 10 weeks. The group (1) treated with P.B.S. I/P considered as control (-). The group (2) involve tumour bearing mice treated with P.B.S I/P considered as control (+).The third group treated with 1g/kg I/P of ethanolic extract for *V.rosea* while the fourth group contain tumor bearing mice treated with 1g/kg I/P of ethanolic extract for *V.rosea.* The fifth group treated with 0.1mg/kg I/P of vinblastine .The sixth group tumor bearing mice treated with 0.1mg/kg I/P of vinblastine.

Cytogenetic toxicity of *Vinca rosea* extract and vinblastine in bone marrow:

Direct method (MI, BI): The protocol of (12) was done to study the direct method (MI, BI).

Histopathology: At the end of the experiment, the animals were sacrificed. Specimens were taken from liver, lung and bone.. Specimens of bones were taken (humorous) and kept in a neutral buffer of 2% formalin then washed with tap water. After washing bones were put in 10% of formic acid for 2-3 days (for the decalcification process) then treated in the same way as for soft tissues (13).

Statistical analysis:

Data were analyzed by using Complete Randomized Design (C.R.D.) in factorial experiment. Data were subjected to Anova test by using the General linear models (GLM) procedure of SPSS (2006, version 16). Significant values were separated by using the multiple range test of (14).

Results and discussion:

Extraction

In this present study, crude extract of *V.rosea* was used because this is merely a preliminary study to assess the possible cytotoxic effect of *V.rosea* on cancer cell. In addition, biological activity, if proven to exist, might be listed during the process of purification of crude extract (7). The extraction of dried leaves of *Vinca rosea* yielded dark brown pasty product with a yield of 20%. The relative proportion between the amount of plant used for extraction and crude product is variable depending on several factors, such as the method of extraction and solvent used in extraction process as the type of test plant and others (15).(16) was found in phytochemical analysis of

V. rosea extract by 70% ethanolic solution the presence of reaction to alkaloids, flavenoids, glycosides, terpens and tannins. Fruits and vegetables generally possess phytochemicals which are responsible for antioxidant and anticancer activities and the benefit of a diet rich with fruit and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (17).

Determination of LD_{50}

Determination of LD_{50} of *V.rosea* ethanolic extract

Determination of the median lethal dose of a test substance is considered one of the very important steps to be done in experimental animals before any other experimental tests. This is to aid choosing the appropriate dose that can be employed in an experiment (18). Determination of the median lethal dose (LD_{50}) of ethanolic extract of *V.rosea*, through I/P administration in Swiss albino Balb/c mice showed the value 10g/kg BW I/P. The acute clinical signs showed rapid shallow respiration which became more deep and wheezy with general lassitude, losses of appetite, staggered gates, tremors and soft yellow stool .The value of LD_{50} was in agreement with that found by (19) who suggested that LD_{50} for ethanolic extract of *V. rosea* 10000 mg/ kg B.W I/P. (20) mentioned that *V.rosea* ethanolic extract has little toxicity recorded others suggested that it may cause kidney or nerve problems (21). Some of these reports may only be extrapolating from the toxicity of isolated alkaloids which are given in low quantities of any single alkaloid in the plant. The differences in the values of LD_{50} could be attributed to the variation in collection time of the plant, parts of the plants used for extraction, method of extraction as well as the animal species and route of administration used in the research experiments (22).

Determination of LD_{50} of vinblastine

Determination of the median lethal dose (LD_{50}) of vinblastine, after I/P administration in albino/c mice showed the value was 2.7mg/kg B.W I/P. The animals showed the same clinical signs observed after treatment with *V. rosea* ethanolic extract.This experiment was done in order to compare the toxicity effect of vinblastine with ethanolic extract of *V.rosea* which considered is the biological source of this drug on cancer affected and normal animals. The result revealded higher toxicity of vinblastine compared with ethanolic extract of *V.rosea*. Many references considered vinblastine as a toxic drug and this toxicity may be due to neurotoxic substrate of p-glycoprotein (23). The LD_{50} values found in the present study for vinblastine agreed with that obtained by (10) who recorded the LD_{50} in mice equal to 2.7mg/kg B.W I/P.

Cytogenetic Study:

Mitotic and blast Index:

Table (1) showed the effect of ethanolic extract of *Vinca rosea* and Vinblastine on mitotic index and blast index of bone marrow cells. Ethanolic extract of *V. rosea* at dose (1g/kg B.W) gave significant increase ($p<0.05$) in MI reached (1.8±0.1) whereas the control (treated with PBS only) gave (1.5±0.1) and that equal to results of groups (tumor bearing female mice treated with *V. rosea* at dose (1g/kg B.W). The significant ($p<0.05$) decrease in MI value was noticed in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg B.W) gave (0.37±0.1) and in group treated with Vinblastine only at same dose which gave (0.97±0.1). The results showed significant ($p<0.05$) difference in BI value. In group treated with ethanolic extract of *V. rosea* only at dose (0.1g/kg B.W) have significant increase in BI value reached (52.7±0.3) when compared with control group (47.57±0.1). In addition the group of tumor bearing female mice treated with Vinblastine at dose (0.1mg/ kg B.W) gave significant ($p<0.05$) decrease in BI value reached (34.83±0.6) whereas the control tumor gave (43.97±0.6). The most sensitive tests for the effect of potentially mutagenic and carcinogenic agents are the quantifying of cytogenetic parameters including mitotic index (MI %) and blast index (BI %) (24).Results of the present study showed that the ethanolic extract of *Vinca rosea* increased (BI) and (MI) values and that agreed with (16), she recorded increase in the BI and MI value. Antioxidant provide protection to living organism from damage caused by uncontrolled production of free radicals, reactive oxygen species (ROS) and concomitant lipid peroxidation, protein denaturation and DNA- strand breaking (25). A major advantage of antioxidants is that they are generally effective against a wide range of mutagens, both exogenous and endogenous (26). Tannic acid reduced mutagen – induced chromoso-mal aberration in mammalian cell (27). Flavonoids are probably the best known of these substances due to their properties (28). Flavonoids have antioxidant activity (29). It has been recognized that alkaloids and flavonoids showed antioxidant activity and their effects on human nutrition and health care are considerable mechanisms of action of alkaloids are through inhibition of peroxidation (30). Oxindole is one of alkaloids which cause immune stimulation so that mean increase in MI and BI (31). The reason of no changes in MI and BI values with significant difference ($P \leq 0.05$) between treatment groups of ethanolic extract of *V.rosea* and Vinblastine , compared with positive control group, might be attributed to several reasons, ethanolic extract of *V. rosea* have different compounds alkaloids, flavonoids, glycosides and tannins. There compound may cause increase MI in concentration depended manner (32).Vinblastine the pure chemotherapeutic agents derived from vinca alkaloids (33). Vinblastine cause inhibition of DNA synthesis (34). Vinblastine blocks mitosis at the

metaphase/anaphase transition, leading to apoptosis (35). That suppression of microtubule dynamics during mitosis is responsible for the ability of *Vinca* alkaloids to inhibit mitotic progression and cell proliferation. Microtubules are intrinsically dynamic polymers, undergoing two kinds of dynamic behaviors, called dynamic "instability" and "treadmilling." Dynamic instability is the stochastic switching of microtubule ends between episodes of prolonged growing and rapid shortening (36). Treadmilling is net growing at microtubule plus ends and net shortening at minus ends (37). Both extensive dynamic instability and treadmilling (or flux) occurs in mitotic spindles. The rapid dynamics of spindle microtubules play a critical role in the intricate movements of the chromosomes (38) and may play a crucial role in passage through the metaphase/anaphase checkpoint. Vinblastine suppresses both microtubule treadmilling and dynamic instability. In living cells, low concentrations of VBL suppress the growing and shortening dynamics of microtubules during interphase, at the same drug concentrations that block mitosis and inhibit cell proliferation (39). The spindle abnormalities induced by the *Vinca* alkaloids also suggest that the drug may act to alter microtubule dynamics during mitosis (40). Whereas the *Vinca* alkaloids act specifically during mitosis, it has not been possible to visualize the dynamics of individual microtubules in mitotic cells. Suppression of the stretching and relaxation movements of the centromeres correlates with mitotic block in a drug concentration-dependent manner, suggesting that suppression of centromere dynamic movement may lead directly to invoking the spindle checkpoint (41).

Table (1) The result of mitotic and blast index

Test	MI	BI
Control	1.5±0.1 B	47.57±0.1 C
Control-Tumor	1.2±0.1 C	43.97±0.6 F
V.rosea -Tumor 1g/kg B.W I/P	1.5±0.1 B	48.83±0.03 B
V.rosea only 1g/kg B.W I/P	1.8±0.1 A	46.5±0.2 D
VBL- Tumor 0.1mg/kgB.WI/P	0.37±0.1 D	34.83± 0.6 H
VBL only 0.1mg/kg B.WI/P	0.97±0.1 D	42.57±0.7 F

***Different capital letters means significant (p<0.05) results between groups.**

*** I/P: intraperitoneal injection, B.W: body weight, VLB: vinblastine.**

Pathological Study:

Pathology of mammary adenocarcinoma tumor.

Gross lesion of mammary adenocarcinoma tumor in non treated group (control): The gross lesion revealed the presence of large irregular, tumor mass (Fig1).

Gross lesion of mammary adenocarcinoma in treated group with crude extract of *V. rosea*: The gross lesion revealed that the tumor mass become smaller in size, with scantly blood supply. The tumor mass disappeared completely at the end of the experiment (Fig2).

Gross lesion of mammary adenocarcinoma in groups treated with chemotherapy (Vinblastine):

The tumor mass showed similar pathological changes as in groups treated with crude extract of *V. rosea* only with many alopecia (Fig3).

Microscopic lesion of mammary adenocarcinoma tumor in non treated group (control):

Histopathological findings showed that the tumor masses involved the whole mammary lobules. It consisted of acinar like structures, trabeculae and islands of tumor cells which are pleomorphic with large hyperchromatic nuclei and a tendency to form giant cells with presence of large number of mitotic figures (Fig4). The tumor centers showed extensive areas of necrosis with pyknosis, karyorrhexis and karyolysis of nuclei . In other sections there is a wide areas of hemorrhage and inflammatory cells infiltrations mainly macrophages and Neutrophils.

Microscopic lesion of mammary adenocarcinoma in treated group with crude extract of *V. rosea*:

The tumor showed the presence of remnant tumor cells which undergo vacuolation and necrosis surrounded by fibrous connective tissue infiltrated with mononuclear cells (Fig5).Furthermore the mononuclear cells infiltrate the tumor necrotic centers .

Microscopic lesion of mammary adenocarcinoma in groups treated with chemotherapy (Vinblastine):

The therapeutic dose (0.1mg/kg B.W)showed complete disappearance of tumor masses. Lymphocytic infiltration was observed around the tumor cells of treated group with plant extract and this may be attributed to that mice acquired an immunological memory for tumor cells and induction of tumor cells undergo extensive necrosis and the area was surrounded by a thick band of fibrous tissue which was infiltrated by mononuclear cells. It is well known that the main immune cells active in the granulation tissue are macrophages and neutrophils, although other leukocytes are also present. Their works are to protect the healing tissue from pathogenic insult. This is necessary for both to aid the healing process and to protect against invading cancer act as a first line of defense.

Pathology of organs of non-treated (control) group

Lung: Presence of multiple variable sizes of tumor masses within the lung parenchyma showing the same

microscopical picture of mammary adenocarcinoma with a tendency of invasion the adjacent tissues (Fig6) and metastasis to the pulmonary arteries. In other tissue sections there are solid masses of tumor with a tendency to giant cells formation .

Liver: The main microscopical features were the presence of tumor masses within the parenchyma consisting of pleomorphic cells containing large hyperchromatic nuclei (Fig7). Large aggregations of tumor associated macrophages (TAM) around central veins and blood vessels and within sinusoid which undergo severe dilation (Fig 8) . The presence of extensive TAM infiltration was shown to correlate with cancer metastasis and poor prognosis in a variety of human carcinomas. TAMs promote cancer metastasis through several mechanisms including tumor angiogenesis, tumor growth, and tumor cell migration and invasion (42).

Bone marrow: No pathological changes.

Pathology of organs treated with ethanolic extract of V. rosea

Pathology of organs of tumor bearing female mice treated with ethanolic extract of V. rosea.

Lung: Thickening of interalveolar septa due to infiltration of mononuclear cells, with perivascular lymphocytic cuffing (Fig9). Proliferation of pneumocytes type II, with presence of alveolar macrophages.

Liver: Tissue sections showed the formation of early-granuloma within the parenchyma consisting of mononuclear cells aggregation (Fig10). Furthermore there is perivascular lymphocytic cuffing with proliferation of kupffer cells.Organs treated with ethanolic extract groups showed focal infiltrations of mononuclear cells especially in lung and formation of early granuloma in liver; this may be attributed to the active compound like antioxidant alkaloids and flavonoids which may act as immune stimulant our results agreed with , (43). (44) mentioned that the patient was given a better chance at survival if the cancer tissue showed lymphocytic infiltration.

Bone marrow: Moderate hyperplasia of hemopoietic tissue with increase in numbers of megakaryocytes (Fig11). This occurred due to plant's active compound and that agreed with, (45) who reported that triterpenoid stimulate proliferation of hemopoietic tissue and possess immunostimulating activity.

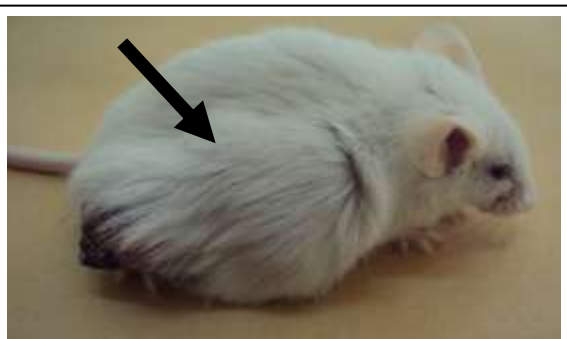
Pathology of organs treated with Vinblastine.

Pathology of organs of tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w):

Lung:Subpleural hemorrhage, congestion of alveolar blood capillaries and pulmonary blood vessels, edema with large pneumonic areas (Fig12). (46) stated that vinblastine may cause active lung disease. Pulmonary edema, dilated vessels with scattered macrophages have foamy cytoplasm.

Liver: Extensive necrosis especially in mid and peripheral zones with increase in apoptosis (Fig13). Other sections showed formation of hyperplastic nodules, in which hepatocytes undergo fatty degeneration. These nodules causing pressure atrophy to adjacent liver parenchyma (Fig14). In addition mononuclear cells infiltration in portal areas with peribiliary fibrosis (Fig15).Coagulative necrosis may be due to increase in hepatic oxygen demand without an appropriate increase in hepatic blood flow. Apoptosis is connected to slight alterations within the plasma membrane causing the dying cells to be attractive to phygocytic cells (47). Apoptosis is an active and highly regulated form of cell death responsible for the cellular default demise of the hepatocytes which occur due to the toxic effect of Vinblastine that which agreed with *(*48) who referred that Vinblastine block mitosis at the metaphase/anaphase transition, leading to apoptosis. Other important change occurred due to Vinblastine treatment were the formation of nodular hyperplasia. The lesion found because of the ability of the liver to replace lost cells through liver regeneration this agreed with previous studies that noticed in all hepatocytes have the potential to re-enter the cell cycle (49).

Bone: Tissue section showed the presence of thin trabculae of calcified cartilage covered by a thin layer of bone. The bars of mineralized cartilage which result from impaired resorption of osteoclasts are wide and project further into the metaphyseal marrow cavity than normal (Fig16). In addition bone marrow showed marked depletion of hemopoietic tissue and increased in the no. of megakaryocytes (Fig17). Other sections showed infiltration of large number of neutrophils (Fig 18). (50) they suggested that the multiple modes of action of chemotherapy drugs suggest a complex and diverse influence on chondrocytes, extracellular matrix and bone cells. Normal bone remodeling involves a delicate balance between bone formation, mediated by osteoblasts, and bone resorption by osteoclasts. Antineoplastic therapy may upset this balance in a variety of ways. In addition bone marrow showed marked depletion of hemopoietic tissue and increase in the number of megakaryocytes. That agreed with (51) vinblastine may cause bone marrow suppression. The infiltrations of neutrophils were related to the immune suppression caused by chemotherapy (52).



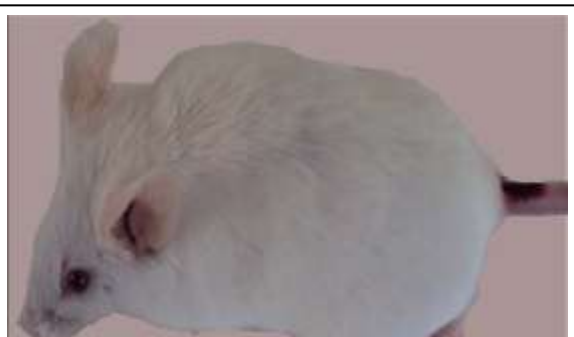
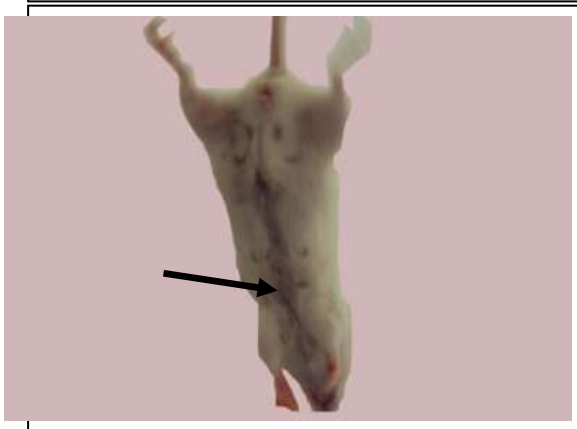


Fig (1) Gross appearance of tumor in female mouse (control group) showing very large irregular highly vascuolarized (➡) tumor mass.



Fig(2): Gross appearance of tumor in female mouse treated with ethanolic extract of *Vinca rosea* showing tumor mass disappeared completely at the end of the experiment.

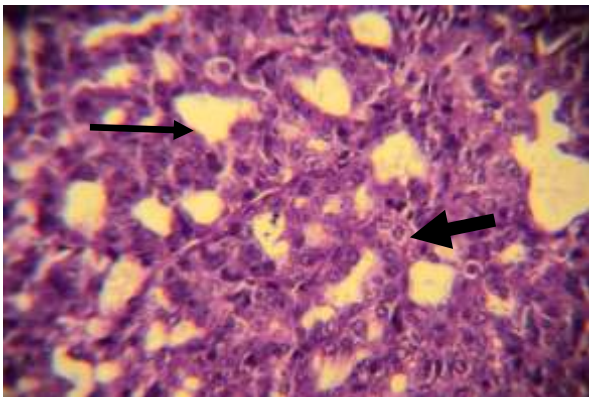
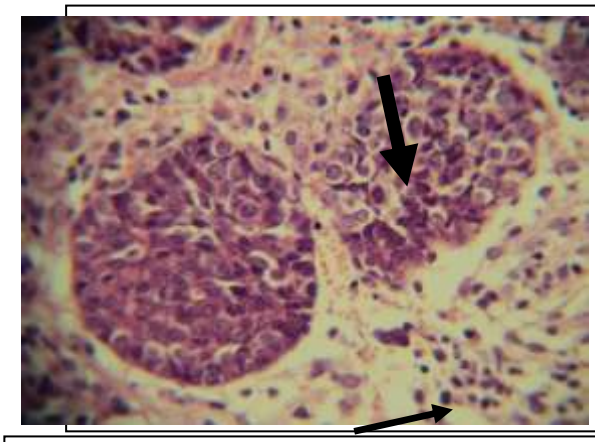


Fig (3): Gross appearance of tumor in female mouse treated with Vinblastine at dose (0.1mg/kg B.W) showing alopecia (➡).

Fig (4): Histopathological section of tumor mass in tumor bearing female mice (control group) showing acinar like structures (➡) with proliferation of pleomorphic cells with hyperchromatic nuclei and high numbers of mitotic figures (➡) (H and E x 400).



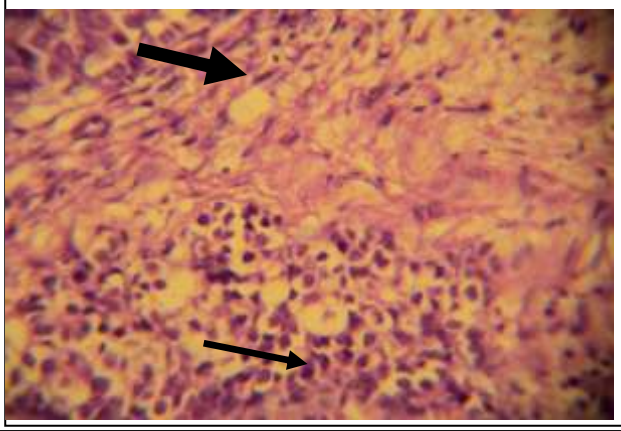
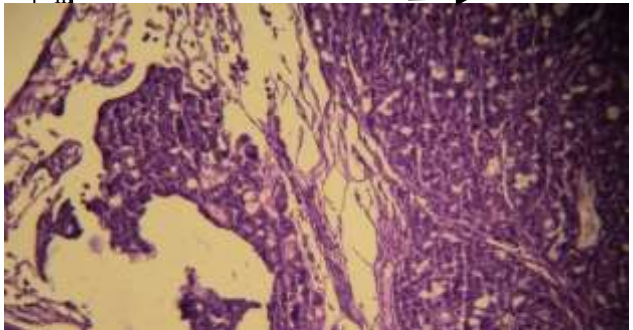


Fig (5): Histopathological section of tumor mass in tumor bearing female mice treated with (1g/kg b.w I/p) of ethanolic extract of *V. rosea* showing the presence of remnant tumor cells which undergo vacuolation and necrosis (➡) surrounded by a thick band of connective tissue which

Fig(6): Histopathological section of tumor mass in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w I/p) Showing vacuolation and necrosis of tumor cells (➡) which encapsulated with fibrous



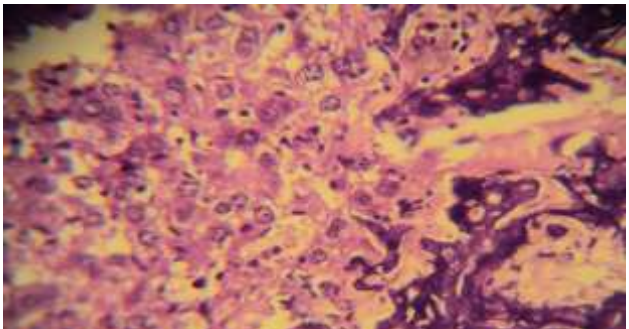
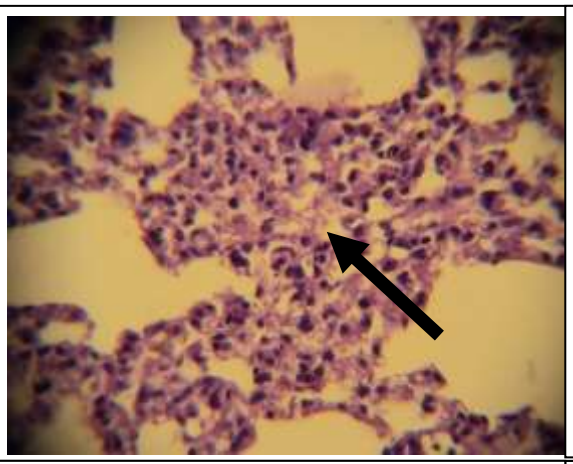


Fig (7): Histopathological section of Lung in tumor bearing female mice showing presence of large tumorous mass causing invasion of adjacent parenchyma ( ) (H & E x400).

Fig (8): Histopathological section of Liver in tumor bearing female mice (control group) showing presence of tumor mass consisting of pleomorphic cells with large hyperchromatic nuclei ( ) (H&E x400).



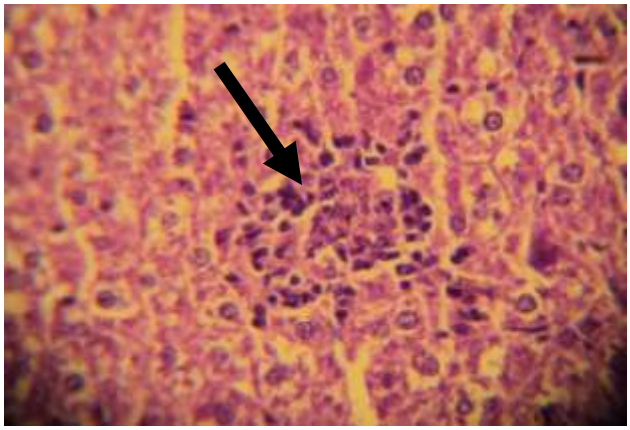
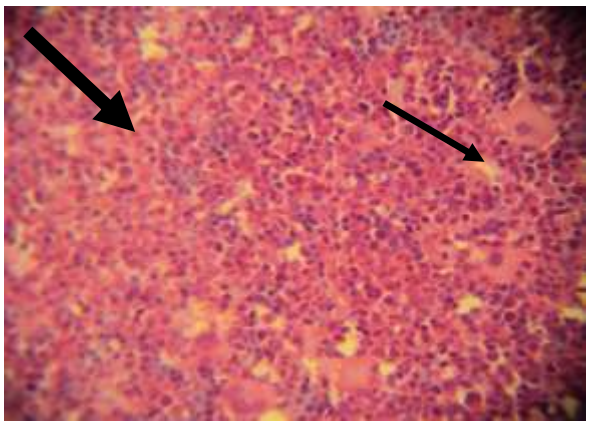


Fig (9):Histopathological section of Lung in tumor bearing female mice treated with ethanolic extract of *V. rosea* at dose (1g/kg b.w I/P) showing thickening of interalveolar septa due to mononuclear cells infiltration ( ) (H & E x400).

Fig (10): Histopathological section of Liver in tumor bearing female mice treated with ethanolic extract of *V. rosea* at dose (1g/kg b.w I/P) showing formation of early granuloma ( ) (H&E x400).



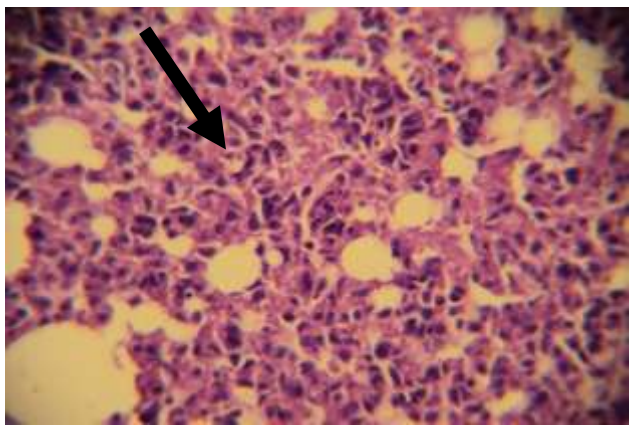
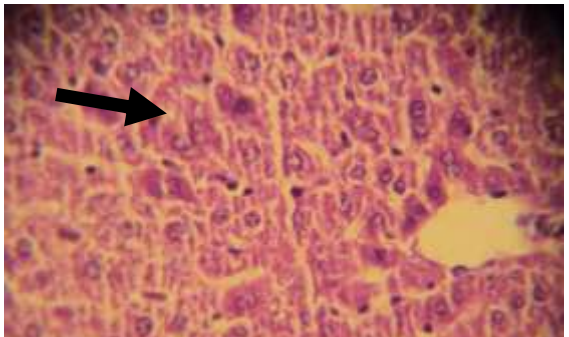


Fig (11):Histopathological section of bone marrow in tumor bearing female mice treated with ethanolic extract of *V. rosea* at dose (1g/kg b.w I/P) showing moderate hyperplasia of hemopoietic tissue ( ) with increased numbers of megakaryocytes ( ) (H&E x400).

Fig (12): Histopathological section of Lung in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w) showing large pneumonic area with infiltration of mononuclear cells in the interstitial tissue ( ) (H&E x400).



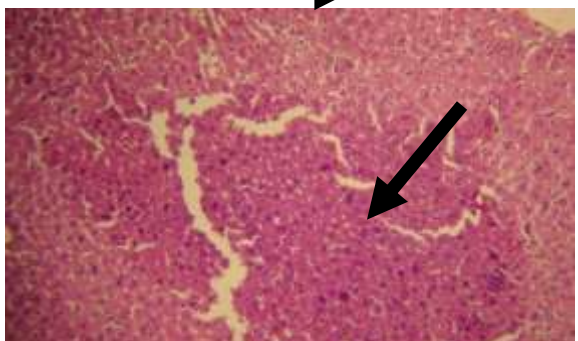
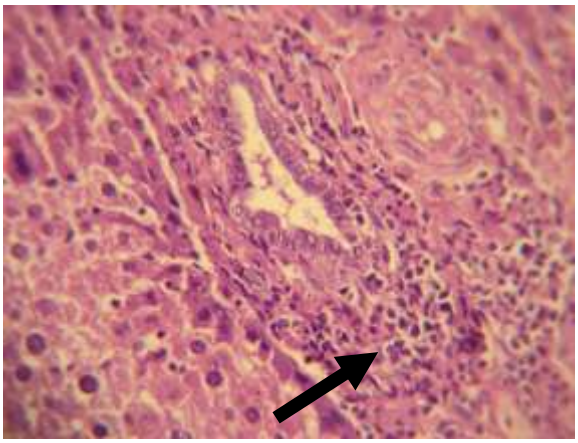


Fig (13): Histopathological section of Liver in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w) showing extensive coagulative necrosis of hepatocytes ( ) with increase in apoptosis ( ) (H&E x400).

Fig (14): Histopathological section of Liver in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w) showing hyperplastic nodule lacking the central vein ( ) (H&E x400).



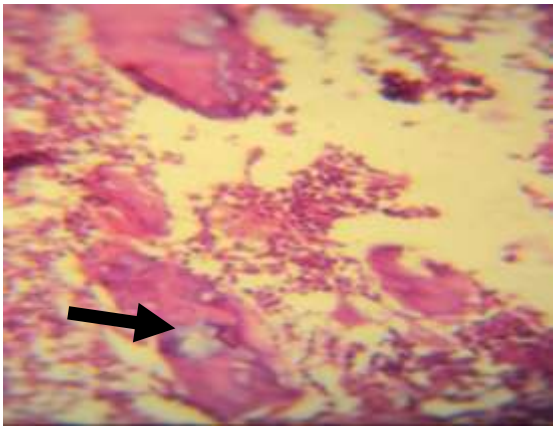
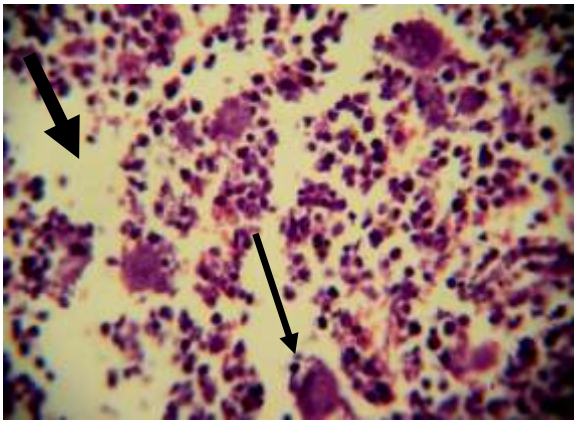


Fig (15):Histopathological section of Liver in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w) showing proliferation of fibrous connective tissue around the bile duct with infiltration of mononuclear cells ( ) (H&E x400).

Fig (16): Histopathological section of Bone in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w) showed bars of mineralized cartilage which result from impaired resorption of osteoclasts ( ) (H&E x400).



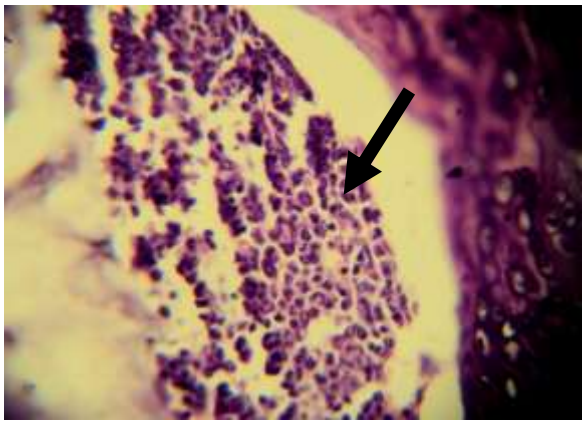


Fig (17): Histopathological section of Bone marrow in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w) marked depletion of hemopoietic tissue ( ) with increase in no. of megakaryocytes ( ) (H&E x400).

Fig (18): Histopathological section of Bone marrow in tumor bearing female mice treated with Vinblastine at dose (0.1mg/kg b.w) showing infiltration of neutrophils ( ).

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