**ABSTRACT**

Ascorbic acid (vitamin C) is a potent antioxidant which acts as a primary protective mode against aqueous radicals in blood. As compared to ascorbic acid or cisplatin alone, combination treatment of Dalton’s lymphoma bearing mice showed better therapeutic efficacy which is accompanied by increased uptake of platinum (drug) into tumor cells. Further, this study examines the involvement of ascorbic acid in cisplatin-mediated development of various hematotoxic features in mice bearing ascites Dalton’s lymphoma. Treatment of mice with cisplatin resulted in a decrease in blood hemoglobin, erythrocytes, packed cell volume and leukocytes along with the development of various morphological abnormalities in erythrocytes. However, combination treatment of mice with ascorbic acid plus cisplatin reduced various hematotoxocities. In conclusion, combination treatment with ascorbic acid plus cisplatin enhances cisplatin-mediated cytotoxicity against Dalton’s lymphoma and shows an improvement in different hematological toxicities induced by cisplatin which may also be imperative in improving survivability of the host.

**KEYWORDS:** Ascorbic acid, cisplatin, hematotoxicity.

**INTRODUCTION**

Ascorbic acid (vitamin C), a hydrophilic vitamin, is an important free radical scavenger antioxidant and an active reducing agent which is involved in various biological effects and plays an important role in the metabolism and detoxification of many endogenous and exogenous compounds.\(^1\) Mechanism of antioxidative action of ascorbic acid operates through direct scavenging and blocking of reactive oxygen species (ROS).\(^2\) Vitamin C
supplementation was found to significantly reduce the oxidation of guanine, a purine in DNA suggesting its antioxidant properties. Ascorbic acid in the form of dehydroxy ascorbic acid (DHA) is taken up and accumulated by cells through glucose transporters. Increased uptake and metabolism of glucose are characteristic of malignant transformation and over expression of glucose transporters is a common event in malignancies. Therefore, tumor cells often accumulate ascorbic acid greater to normal cells and may be better protected against the negative effects of ROS. Many studies have shown that vitamin C intake is inversely related to cancer with protective effects shown for various types of malignancies. In vitro and in vivo studies have reported that ascorbic acid increases extracellular superoxide and hydrogen peroxides that are selectively cytotoxic to transformed cells. While non-transformed cell lines are not impacted by ascorbic acid, cancer cell lines produce elevated levels of superoxide that make them susceptible to ascorbic acid’s cytotoxic effects. Ascorbic acid at a non-toxic concentration, in combination with certain pharmacological agents produces a synergistic or additive effect on the growth inhibition of tumor cells in in vitro and in vivo conditions. The protective role of ascorbic acid on cisplatin-induced nephrotoxicity and mutagenicity have also been reported in both humans and animal models.

Cis-diaminedichloroplatinum-(II), commonly known as cisplatin is a potent platinum-based cancer-chemotherapeutic drug widely used against a variety of malignancies. The anticancer activity of cisplatin mainly involves the formation of cisplatin-DNA adducts with inter and intra-strand DNA crosslink which account for about 90% of the total DNA damage induced by cisplatin to cause cytotoxic effects. In addition, cisplatin generates reactive oxygen species (ROS), which are known as one of the pathogenic intermediates following chemotherapy. Although cisplatin has been a mainstay for cancer therapy, its efficacy is often hampered by the development of dose-limiting side effects which include nephrotoxicity, myelosuppression, neurotoxicity, ototoxicity in the host and acquired resistance by cancer cells. Hence, to ameliorate the toxic effects of cisplatin without altering its antitumor effects, different experimental studies have been carried out using a combination of cisplatin with various modulating agents with varying degree of success.

Many studies found that dietary ascorbic acid in combination with various anticancer agents successfully reduced the drug-induced toxicity in the host. Our recent studies also reported the protective ability of ascorbic acid against cisplatin-induced mutagenic potentials.
and tissue toxicities in mice.\[31,32\] Besides the antimutagenic and cytotoxic properties, some contradictory role of ascorbic acid has also been suggested in enhancing carcinogenesis.\[33,34\] It has been demonstrated that treatment with ascorbic acid (vitamin C) significantly reduced the genotoxicity of well-known mutagens.\[35-37\] However, large amounts of ascorbic acid have been reported to cause some genotoxic effects in \textit{in vitro} test systems\[38,39\] because ascorbic acid has both antioxidant and pro-oxidant activities.\[40,3\]

Besides transport of oxygen, erythrocytes (RBCs) also function as conveyors of nutrients, participate in inter-organ communication, and serve as targets for drugs, environmental xenobiotics, and pathological factors.\[41\] Cisplatin-mediated hematological changes should be of vital importance as low blood cell counts are found to be one of the common side effects caused by chemotherapy,\[42\] and alterations in the rate of erythrocyte production following tumor growth have also been reported.\[43\]

Hematological parameters are routinely monitored for assessing the process of tumorigenesis and the chemopreventive effects of treatment employed.\[44\] The effects of drug-induced alterations on the status of hematological parameters during the process of carcinogenesis have not been well explored with very few reports as far as basic research is concerned.\[45,46,26\] In view of the undesirable side effects caused by anticarcinogenic agents and the possible protective implication of vitamin C in cancer chemotherapy, the present work was undertaken to assess the modulatory effect of dietary ascorbic acid on cisplatin-mediated cytotoxicity and hematological changes in mice bearing ascites Dalton’s lymphoma which has the significance in cancer treatment in general. The basic findings exhibit that the use of ascorbic acid with cisplatin could be very useful in decreasing cisplatin-mediated hematotoxicity in the host while showing enhanced antitumor activity and cytotoxicity against murine ascites Dalton’s lymphoma.

**MATERIALS AND METHODS**

**Chemicals**

Cisplatin solution (1 mg/ml of 0.9%, NaCl) was obtained from Biochem Pharmaceutical Industries, Mumbai, India. L-ascorbic acid (vitamin C) was purchased from HiMedia laboratories, Mumbai, India. Dichlorophenolindophenol (DCIP), RBC and WBC diluting fluids and other chemicals used in the experiments were of analytical grade and purchased from SRL Pvt. Ltd., Mumbai, India. Various solutions were always prepared in double glass-distilled water.
Tumor maintenance

Ascites Dalton’s lymphoma (DL) tumor is being maintained *in vivo* by serial intraperitoneal (i.p.) transplantation of viable tumor cells (1×10^7 cells in 0.25 mL phosphate-buffered saline (PBS), pH 7.4) in Swiss albino mice. Tumor transplanted hosts usually survive for 19-21 days. The maintenance and use of the mice and the experimental protocol of the present study was approved by the Institutional ethical committee (IEC) of North-Eastern Hill University, Shillong, India.

Drug treatment schedule

The treatment of tumor-bearing mice with cisplatin (10 mg kg\(^{-1}\) b.wt.) and ascorbic acid (1% in drinking water) have been used previously in our study\(^{[32]}\) and the same treatment schedule was followed in the present study also. Both sexes of tumor-transplanted mice of 12-14 weeks old were randomly divided into four groups consisting of 10 mice in each group. Mice in group-I, II, III and IV served as control, ascorbic acid (AA) alone treatment, cisplatin alone treatment and AA plus cisplatin treatment respectively as described earlier.\(^{[32]}\)

Antitumor activity and cell viability test

The antitumor efficacy was determined as percentage of average increase in life span (ILS) using the formula:

\[
\%\text{ILS} = (\frac{T}{C} \times 100) - 100, \quad \text{where, } T \text{ and } C \text{ are the mean survival days of treated and control group of mice respectively.}
\]

The pattern of changes in tumor volume was also recorded daily which may depict the changes in tumor growth. After 24, 48, 72 and 96 h of cisplatin treatment (i.e., on 11, 12, 13 and 14\(^{th}\) day of tumor transplantation) two animals were sacrificed by cervical dislocation and DL ascites tumor and blood samples were collected to be used for the various investigations. Ascites DL collected from the mice were centrifuged at (2000xg, 4\(^{\circ}\)C, 15 min) and the pellet was used as the DL cells and all the experiments were repeated thrice.

Cell viability of DL cells was checked using trypan blue exclusion test as described by Talwar et al\(^{[47]}\). Briefly, the DL cells (1x10^6 cells in 1 ml PBS) collected from mice were seeded in 24 well plates and by following the different treatment schedule the cells were treated with AA or cisplatin alone and/or combination of AA plus cisplatin treatment respectively. After incubation for 24, 48, 72 and 96 h, aliquot of the cell suspension was mixed with an equal volume of trypan blue (0.4% in PBS) and incubated for 2 min. Viable
(unstained cells) and dead cells (stained cells) were determined with a Neubauer haemocytometer under light microscope. The percentage of viability was calculated using the formula:

\[
\% \text{ Viability} = \frac{\text{total viable cells of treated}}{\text{total viable cells of control}} \times 100
\]

**Determination of platinum uptake**

The ascites tumor from different treatment groups of mice were collected from peritoneal cavity using a 1.0 ml disposable syringe and centrifuged at 5000xg for 10 min, washed once in cold PBS, pH 7.4. The cell pellets (0.5 g) were digested in 5.0 ml nitric acid and few drops of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) in a small clean conical flask with gentle heating to near dryness. 5.0 ml of perchloric acid was added to the digests and again heated to near dryness to remove excess nitric acid. The last step was repeated until a clear solution resulted. The digests were finally dissolved in distilled water maintaining the acidity of approximately 5% and the filtrates were stored in polypropylene bottles for platinum analysis in a labtam model 8440 m plasma lab ICP-OES emission spectrometer operated at PMT voltage 700 and wavelength 214.438 after calibrating the instruments with appropriate standard solutions.

**Ascorbic acid estimation**

The ascorbic acid concentration was estimated in serum using the method of Omaye et al\textsuperscript{[48]}

Serum collected from different treatment groups were deproteinized by addition of 1.0 ml of serum to 1.0 ml of ice cold 10% metaphosphoric acid and after thorough mixing, centrifuged (3000xg for 20 min) at room temperature. The precipitate was discarded and 0.6 ml aliquot of the supernatant was used for analysis. To each sample, 0.3 ml of citrate-acetate buffer (22 g of tri-sodium citrate dihydrate in 40 ml distilled water) and the total volume brought to 100 ml with distilled water, pH 4.15) was added and any turbid sample was centrifuged. Then 0.3ml of dichlorophenolindophenol (DCIP) solution (0.1 mg/ml in distilled water) was added to the sample, and after exactly 30 sec, it was read against distilled water at 520 nm. Few crystals of ascorbic acid were added to bleach the dye by reducing it completely and the absorbancy was measured again at 520 nm. This value serves as blank for the sample. The change in absorbance (\(\Delta A\)) due to the reaction of the dye by ascorbic acid is calculated from the following equation:

\[
\Delta A = (RB - RB_b) - (S - S_b)
\]

Where, \(RB\) = absorbance of reagent blank; \(RB_b\) = absorbance after bleaching
S = absorbance of the sample; \( S_b \) = absorbance after bleaching.

\( \Delta A \) is linearly related to ascorbic acid concentration and the concentration in the sample is obtained by comparison of \( \Delta A \) with the standard curve constructed with standards ranging between 0 and 20 μg of ascorbic acid per ml of 5% metaphosphoric acid.

**Hematological studies**

The blood samples for hematological studies were collected by puncturing the retro-orbital venous sinus using sterilized glass capillaries. EDTA was used as an anticoagulant to prevent the blood from clotting and the parameters were studied as soon as possible in order to avoid experimental errors.

Red blood cells (RBC) counts, white blood cells (WBC) counts, haemoglobin content and packed cell volume (PCV) were determined using the method of Dacie and Lewis\(^{[49]}\). The height of the column of red cells was taken as the packed cell volume (PCV, i.e. the volume occupied by the red cells expressed as a fraction of the total volume of the blood). Differential leucocytes counts (DLC) was carried out according to Swarup et al.\(^{[50]}\). The number of different types of white blood cells (neutrophils, basophils, monocytes, lymphocytes and eosinophils) were recorded and expressed in percentage.

**Scanning electron microscopy of abnormal RBCs**

Fresh blood collected from mice in different treatment groups was taken in an eppendorf tube and fixed in 2.5\% (v/v) glutaraldehyde at room temperature for 30 min. The fixed blood was then centrifuged (750xg at 4ºC) for 5 min and the pellets were washed twice in phosphate buffer and re-suspended in distilled water. A thin film of suspension was made on a clean coverslip for air drying and was affixed to a brass stub with double-stick tape, coated with gold in a fine coat ionic sputter (SCD-005, BAL-TEC Co.). The cells were thoroughly examined for abnormalities in red blood cells, counted and photographed under scanning electron microscope (JEOL JSM-6360) operated at 15 kV.

**Statistical analysis**

The values have been expressed as mean ± Standard Deviation (SD). One-way analysis of variance (ANOVA) was used to evaluate the difference among multiple groups followed by a post hoc test (Tukey test). P-value ≤ 0.05 was considered as statistically significant for all comparisons.
RESULTS

Antitumor activity and cell viability

An early sign of tumor growth was noted from 3rd to 4th day onwards following tumor transplantation. Tumor-bearing control mice survived for about 19–21 days. The mean survival time of mice treated with AA alone or cisplatin alone were significantly increased to about 34 days (ILS~79%) and 42 days (ILS~122%) respectively, while the host’s survivability was further increased to more than 46 days (ILS~142%) in combination treated group (Fig 1A). The increased survival of the hosts was accompanied with a significant reduction in tumor volume in mice in the combination treated group as compared to AA alone or cisplatin alone treated group (Fig 1B).

Fig 1: Survival pattern (A), and changes in the tumor volume (B) in DL-transplanted mice under different treatment conditions. Number of mice in each groups= 10, n= 5. Control= tumor-bearing mice without any treatment; AA= Ascorbic acid; ILS= Increase in life span.

Cell viability examination as determined by trypan blue exclusion test revealed a significant time dependent increase in the percentage of cell cytotoxicity at all the different treatment conditions. However, as compared to AA or cisplatin alone treatment, combination treatment of AA plus cisplatin (24-96 h) showed more cytotoxicity towards DL cells (Fig 2).
Cellular accumulation of platinum (drug uptake) in DL cells
Measurement of platinum (drug) in DL cells showed that the cellular accumulation of platinum was more initially at 24 h of treatment and decreased gradually during 48–96 h in both cisplatin alone and combination treated group. As compared to cisplatin alone treatment, platinum uptake was significantly more in tumor cells after combination treatment with AA plus cisplatin (Fig 3).

Ascorbic acid concentration
As expected, AA alone treatment caused a time-dependant significant increase in the level of ascorbic acid concentration in serum as compared to the corresponding control during 24-96 h of treatment. As compared to corresponding control, treatment with cisplatin alone showed
a time-dependant increase in ascorbic acid concentration in the serum during 72-96 h of treatment. As compared to cisplatin alone, combination treatment with AA plus cisplatin caused a significant increase in the concentration of ascorbic acid in the serum at corresponding time points (Fig 4).

![Graph showing changes in serum ascorbic acid concentration](image)

**Fig 4:** Changes in the serum ascorbic acid concentration (μg/ml wet wt.) in tumor-bearing mice at different treatment conditions. Results are expressed as mean ± SD. ANOVA, n= 3, *P≤ 0.05 as compared to the corresponding control; #P≤ 0.05 as compared to cisplatin. Control= Untreated tumor-bearing hosts; AA= Ascorbic acid; Cis= Cisplatin.

**Hematological findings**

As compared to tumor-bearing control, AA alone treatment caused a decrease in the hemoglobin (Hb) content (~16%), RBC (~24%), WBC counts (~47%) and in PCV (~19%). Further decrease in the Hb content (~31%), RBC (~39%), WBC counts (~66%) and in PCV (~36%) was noted following cisplatin alone treatment. As compared to cisplatin alone, combination treatment resulted in significant recovery in hemoglobin concentration, RBC and WBC counts and in PCV during 24-96 h of treatment conditions (Fig 5A). As compared to corresponding control, AA treatment showed a significant decrease in lymphocytes, eosinophils and basophils at initial period after treatment which then showed a recovery and increase at 96 h. A significant increase in monocytes was observed during 48-96 h. As compared to control, cisplatin treatment of tumor-bearing mice caused a significant decrease in lymphocytes, eosinophils and basophils while an increase was observed in monocytes and neutrophils. Combination treatment caused an increase in lymphocytes, eosinophils and a decrease in monocytes, neutrophils and basophils (Fig 5B).
Fig 5: Percent changes in hemoglobin (Hb) contents, RBC and WBC counts, PCV (A), and differential leukocytes counts (DLC) (B) in tumor-bearing mice at different treatment conditions. Results are expressed as mean ± SD, n= 3. AA= Ascorbic acid; Cis= Cisplatin.

Morphological abnormalities in RBCs
SEM observation revealed the development of various types of abnormalities in RBCs of tumor-bearing mice under different treatment conditions. Different morphological abnormal types in RBCs observed includes the normocytes (having smooth surface), microcytes (cells having smaller diameter than the normal erythrocytes), macrocytes (having larger diameter), echinocyes (presence of uniformly serrated projections), acanthocytes (spiculated or thorn-like cells), schistocytes (small fragmented spindle-shaped cells), spherocytes (sphere-shaped cell smaller than normal RBC), stomatocytes (elongated cells with presence of slot-like structure at the centre), ovalocytes (having oval-shaped cells) and elliptocytes (having elliptical-shaped cells) (Fig 6A a-j). The number of abnormalities in RBCs significantly increased under all treatment conditions as compared to control. However, combination
treatment of mice with AA plus cisplatin significantly decreased the frequency of abnormalities in the RBC of tumor-bearing mice as compared to cisplatin alone treatment (Fig 6B).

Fig 6: (A) Scanning electron micrographs (SEM) of RBCs from mice under different treatment conditions. Various morphological features/abnormalities (arrows) observed in RBCs are N= Normocytes (a), Ma= Macrocytes; Mi= Microcytes (b), Ec= Echinocytes (c), Ac= Acanthocytes (d), Sch= Schistocytes (e), Sp= Spherocytes (f), St= Stomatocytes (g), O= Ovalocytes (h), El= Elliptocytes. (B) Changes (mean %) in various morphological abnormalities in the RBCs of mice under different treatment conditions. Results are expressed as mean ± SD. ANOVA, n= 3, *P≤ 0.05 as compared to the corresponding control; #P≤ 0.05 as compared to cisplatin. Control= blood from untreated tumor-bearing hosts; AA= Ascorbic acid; Cis= Cisplatin.
DISCUSSION
The use of chemotherapy plays an important role in cancer treatment. Despite its success, treatment with some of the most effective anticancer drugs shows a number of symptoms of direct side effects in the patient. Cisplatin is one such highly effective chemotherapeutic agent displaying clinical usefulness against a wide variety of cancers. The high cytotoxicity of the drug explains its effectiveness against cancer cells, and also the cause of its side effects. Apart from inducing mutagenicity and carcinogenicity, cisplatin-mediated therapy has also been reported to cause hematotoxicity. Hence, a large number of combinations have been used with cisplatin to limit its toxicity. It has been reported that ascorbic acid (AA) may also be used as an adjuvant in order to enhance therapeutic efficacy of cisplatin in the host. Findings from the present studies showed that the tumor-bearing mice treated with combination of AA plus cisplatin caused an increase in survival time by about 20% more than cisplatin alone treatment (Fig 1A). The increase in survivability of the hosts was also accompanied by a decrease in tumor volume as compared to the group of mice treated with either agent alone (Fig 1B) which may suggest that as compared to AA or cisplatin alone, combination treatment with AA plus cisplatin could be a better therapeutic strategy against murine ascites Dalton’s lymphoma. The synergistic antioxidant function of ascorbate with vitamin E has also shown to protect membranes against peroxidation. Combined vitamin C and K3 treatment in vitro and in vivo have been found to produce tumor growth inhibition and increased the lifespan of tumor-bearing mice. Vitamin C has been found to significantly increase apoptosis along with up-regulation of p53 during cisplatin treatment in human colon cancer cells. The analysis of DL cells viability under different treatment conditions also revealed that the cell death was more in mice treated with combination of AA plus cisplatin as compared to either treatment alone, thus clearly indicating the increased cytotoxicity of DL cells under combined treatment conditions (Fig 2).

The cytotoxic activity of cisplatin may also be correlated with the amount of platinum bound to DNA. In the present study, accumulation of platinum (drug) in DL cells was noted to be more during 24 h which gradually decreased during 48-96 h of different treatment conditions (Fig 3). This may be due to its export from the cells and may also be correlated with the recovery of glutathione levels during the later period as reported in our earlier studies. Elevation of glutathione levels has been shown to increase the resistance of many cancer cells. In fact changes in the intracellular glutathione levels could also affect the
drug uptake by DL cells. In a study using freshly isolated peripheral blood mononuclear cell, it has been reported that the increased intracellular glutathione concentration is correlated with decreased platinum-DNA binding.\cite{64} The availability of more drugs during initial stage of treatment may be thought to give rise to various metabolic dysfunctions directly or indirectly related to cisplatin cytotoxicity which may be partially repaired or retained within the DL cells, leading to tumor regression. The anti-proliferative effects of ascorbic acid on tumor cells have been well studied.\cite{65,66} Fromberg et al\cite{67} reported that ascorbate exerts anti-proliferative effects by inducing cell cycle arrests and sensitizes tumor cells towards cytostatic drugs. The amount of platinum (drug) in DL cells significantly increased after treatment with AA plus cisplatin (Fig 3) which could be an important contributory aspect in resulting better cytotoxic effects during combination treatment against murine ascites Dalton’s lymphoma. It has been suggested that ascorbic acid increases tumor cell membrane permeability to chemotherapeutic drugs and helps to increase the uptake of chemotherapy drugs into cancer cells.\cite{68} Hence, the enhancement of cisplatin-induced tumor inhibition may be due to modulation of permeability of tumor cell membrane by ascorbic acid, causing an increase in the uptake of cisplatin into tumor cells and making the DNA repair machinery less efficient due to more adduct formation in the DNA leading to tumor cell regression, thereby increasing hosts survivability (Fig 1).

It has been illustrated that ascorbic acid prevents proliferation and metastasis of cancer cells.\cite{69} Clinical trials indicate that ascorbic acid may confer protection on various normal tissues without attenuating antitumor response. The mechanism of protection is based on physiological differences between the tissue types and on differential uptake of ascorbic acid. Pauling et al\cite{70} reported that large quantities of dietary ascorbic acid decreased the incidence of and delayed the first appearance of spontaneous mammary tumors in RIII/Imr in mice. The protective role of ascorbic acid has been demonstrated with high ascorbic acid and glutathione levels in liver.\cite{71} As was expected, in the present study ascorbic acid alone treatment increased serum ascorbic acid concentration in tumor-bearing mice as compared to their corresponding controls (Fig 4). Kurbacher et al\cite{72} reported that vitamin C could increase the anti-neoplastic activity of cisplatin and some other anticancer agents. The possible anti-carcinogenic effects of ascorbic acid may be accounted for by its ability to detoxify carcinogens as well as its ability to block carcinogenic processes through its antioxidant activity. In present study, significant increase in ascorbic acid concentration in the serum was noted after the combined administration of AA plus cisplatin as compared to cisplatin alone.
Combination treatment of vitamin C and low-dose methotrexate has shown to exert a strong anticancer effect in Hep3B cells.\textsuperscript{73} It has also been reported that combination treatment of cysteine plus cisplatin increased blood glutathione level as opposed to its decrease when treated with cisplatin alone.\textsuperscript{74} Therefore, the overall increase in the ascorbic acid level in serum during combination treatment may play an important role in attenuating cisplatin-mediated hematological toxicities in the host (Fig 5). Cisplatin-induced anemia (decrease RBC count, PCV% and Hb concentration)\textsuperscript{75,76} is a well-known side-effect. Regardless of the cause, anemia in cancer has a complex and generally negative impact. The results obtained in present study revealed that cisplatin alone treatment caused significant reduction in RBC counts with subsequent decline in Hb concentration and PCV (Fig 5A). Ascorbic acid alone treatment also showed slight decrease in the hematological values. In a study by Ray and Chowdhury,\textsuperscript{77} increase in tumor growth resulted in marked reduction in the RBCs in mice bearing a wide spectrum of experimental tumors. Thus, the observed decrease in the number of RBCs accompanied by a decreased Hb contents in present study seems to appear that cisplatin (drug) exposure as well as the process of tumorigenesis might have caused the lysis of RBCs. The decreased life span of RBCs and anemia may also be correlated with decreased blood antioxidant capacity. This suggestion of decreased antioxidant capacity and increased fragility in RBC may be supported by the observation of various types of cisplatin-induced morphological abnormalities in erythrocytes (Fig 6A) in the present study. Sirag\textsuperscript{78} reported that the disturbances in RBCs could reflect an imbalance between its production and loss and that non-regenerative anemia arises from reduced production of erythrocytes. However, combination treatment with ascorbic acid plus cisplatin caused significant recovery in these hematological values (Fig 5A) and significant decrease in the frequency of abnormal RBCs (Fig 6B) as compared to cisplatin alone treatment. This observation suggests that ascorbic acid has a potential of enhancing hemoglobin contents through increasing/restoring RBCs count thereby, suggesting the ameliorating effect of ascorbic acid against cisplatin-induced hematotoxicity which could also be assisted by the increased ascorbic acid concentration in the serum as observed in present study (Fig 4).

A high WBC count usually means that the body is fighting an infection whereas a very low WBC count can be caused by problems with the bone marrow, a condition called leukopenia which means that organism is less able to fight off infections. In the present study, WBC increased during tumor growth progression (Fig 5A). Therapeutic doses of cisplatin are evidently toxic to bone marrow cells and probably can trigger apoptosis and affect cell cycle
causing anemia and a decrease in leukocyte count. As compared to control, ascorbic acid (AA) or cisplatin alone treatment significantly reduced the leukocyte counts which may reflect alterations in the immune functions. However, pre-treatment with ascorbic acid in combination treated group caused an improvement in WBC counts suggesting the ameliorative effects of ascorbic acid through its antioxidant activity. Sindhu and Kuttan in their study in cisplatin-induced renal failure in mice reported that WBC count was significantly elevated when treated with carotenoid lutein, a non-toxic antioxidant. Leukocytosis is a pathological condition often encountered in a clinical setting, usually caused by an increase in the number of blood leukocytes mainly neutrophils, affecting the WBC that frequently rises as a reaction to infection, chronic inflammation and cancer. Generally, neutrophils are mobilized to sites of tissue injury under the influence of chemokines and represent the hallmark of inflammation and tissue damage. Our present findings also support this view as an increase in neutrophil counts was observed when tumor-bearing mice were treated with cisplatin alone as compared to control animals (Fig 5B). Lymphocytes are of fundamental importance in the immune system and play a key role in maintaining antitumor immunity providing an important opportunity for the immunotherapy of cancer. In an experimental investigation, elimination of about 30% lymphocyte population was observed in female Balb/c mice upon transplantation of Ehrlich carcinoma in comparison with animals without tumor and hypothesized that the eliminated population can block the tumor growth. In the present study, combination of AA plus cisplatin caused betterment in the counts of the lymphocytes, eosinophils and basophils in tumor-bearing mice as compared to cisplatin alone (Fig 5B) which may be of significance in the antitumor activity as it may play a role in enhancing hosts' immunity.

**CONCLUSION**

Present findings demonstrate that combination chemotherapy with dietary ascorbic acid and cisplatin could be very useful in augmenting cisplatin-mediated therapeutic efficacy against murine ascites Dalton's lymphoma in the hosts. This may involve enhancement of platinum uptake by tumor cells in presence of ascorbic acid (in combination therapy). At the same time, combination treatment with AA plus cisplatin in tumor-bearing mice could be helpful in developing suitable condition in the hosts such as improved hematological values, which may be involved in decreasing cisplatin-induced hematotoxicity and strengthening hosts’ immunity thereby potentiating cisplatin's antitumor efficacy and host survivability.
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