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# Ameliorative effects of Gentisic acid on carboplatin induced hematological toxicities in Wistar Rats

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**Abstract :** Hematological toxicity is a frequent and severe adverse effect of carboplatin chemotherapy, limiting its clinical use despite being one of the most potent anticancer agents. The present study was designed to evaluate the protective effects of a naturally occurring plant phenolic acids i.e. gentisic acid (2,5 dihyroxybenzoic acid) against carboplatin induced hematological toxicities in wistar rats. Exposure to carboplatin at a dose of 35 mg/kg caused significant decrease in hematological parameters of blood such as red blood cells, total leucocytes, platelets, neutrophills, basophills, lymphocytes and moncytes counts whereas increase in eosiphill counts rat blood indicating severe pancytopenia. Administration of gentisic acid at 10, 30 and 100 for 14 days resulted in a significant amelioration of altered blood parameters in a dose dependent manner indicating its potential as a protective agent for the prevention and amelioration of caboplatin induced hematological toxicities.
**Key words :** Gentisic acid; 2,5-Dihyroxybenzoic acid; Hematological toxicities; Carboplatin; Cancer chemotherapy; myelosuppression.

## Introduction:

Cancer chemotherapy is associated with wide a range of side effects, adversely affecting patient health and quality of life. Myelosuppression remains the major toxicity encountered in the cytotoxic chemotherapy today leading to massive hypocellularity of the bone marrow by injuring hematopoitic progenitor stem cells resulting in depletion of red blood cells, white blood cells (leucopenia) and platelets (thrombocytopenia) thereby increasing the risk of anaemia, potentially fatal opportunistic infections, and uncontrolled hemorrhage, respectively, which proves to be life threatening to the patients.[1–3] This in turn requires limiting the dosage and frequency of the drug to overcome these complications, decreased its efficacy.[4]

Carboplatin (cis-diammine [1,1-cyclobutanedicarboxylato]-platinum [II]), a new platinum complex is currently used in oncology clinics for the treatment of a variety of cancers such as ovarian cancer, lung cancer and head and neck cancer. The clinical use of carboplatin (CP) has been associated with various toxicities including nephrotoxicity, neurotoxicity and ototoxicity. One of the dose-limiting toxicities in patients receiving



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CP has been reported as myelosuppression, mainly in the form of anaemia, thrombocytopenia and leucopenia.[5-11] These hematological toxicities are manifested through different mechanism involving depression of hemopoiesis and enhanced lysis of blood corpuscles.[12,13]

Current scenario to overcome chemotherapy-induced myelosuppression includes few treatment modalities such as administration of growth factors, bone marrow transplantation, blood transfusion, but the procedures tend to be costly, complicated and painful with limited effectiveness in reversing the serious complications of myelosuppression. Hence alternative treatment with newer therapies with fewer side-effects and better efficacy is the need of the day.[14,15]

Use of active principles of medicinal plants for reducing the toxicities of cancer chemotherapeutic agents has been reported extensively. Most of the potent drugs used today in the treatment of major ailments are obtained from the plants in the form purely isolated or structurally modified compounds.[16]

Plant phenolic acids, secondary plant products are found in many fruits, nuts and vegetables are reported for their protective potential against cancer chemotherapy induced toxicities.[17,18] Gentisic acid (GA) is a phenolic acid present in some plants such as olives, gentian, sesame, artichokes and various fruits including kiwi fruit, apple, bitter melon, blackberries, grapes, pears, and in aloe vera and mushrooms.[19] It has been documented for its potential analgesic, anti-inflammatory, antimutagenic, antirheumatic, antiparkinsonian, antiarthritic, cytostatic, siderophoric and iron chelating activities. GA also possesses capability to inhibit low-density lipoprotein oxidation in human plasma documented in several studies. It has also been claimed to have an effective role in the anticarcinogenetic activity of China-rose hibiscus (Hibiscus rosa-sinensis) extract.[20-22] Recently gentisic acid has shown its inhibitory activity against Fibroblast Growth Factor (FGF) and protective activity against cyclophospamide induced gentotoxicity and hepatototxicity.[23,24]

The present investigation was carried out to evaluate the protective activity of GA against CP induced hematological toxicities in wistar rats.

## Materials and methods

### Chemicals and kits

Gentisic acid was purchased from Sigma- Aldrich Chemicals Co., St. Louis, MO, USA. Carboplatin (Paraplatin) was purchased from GlaxoSmithKline Pharmaceuticals Ltd. Other chemicals and solvents used were of analytical grade and purchased from commercial suppliers. Heamcytometer, Sahli's heamoglobinometer for estimations of blood parameters were purchased from Kiran Enterprises, Pune, India.

### Experimental Animals

Swiss albino mice weighing 25-30 g and wistar albino rats weighing (200-250g) were used. They were caged in a room under standard laboratory conditions (temperature 23±10 $^0$C, relative humidity 55±5% and lighting 08:00-20:00 h).The rats were fed on a pelleted diet (Nutrivet Lifesciences, Pune, India) and water ad libitum. The rats were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during day (08:00- 16:00 h).

### Ethical clearance

All the studies were carried out in accordance with the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India) and the Institutional Animal Ethical Committee (IAEC) approved the study (Approval No.: 1036/RE/S/2007/CPCSEA/16-17/F-6).

### Preliminary acute oral toxicity testing

Healthy adult albino mice were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD-2000). The mice were

observed continuously for 2 h for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days.[25]

**Treatment protocol**

Animals were divided in six groups, each containing six animals (n=6).

Group I (Normal Control): The animals were given distilled water (2 ml/kg b.w./day), parallel to the drug treated groups, throughout the course of the study of 15 days.

Group II (CP control group): The animals first received distilled water (1 ml/kg b.w./day) by oral route for 9 days, and subsequently received CP (35 mg in 1ml normal saline per kg b.w.) once daily by intraperitoneal injection in association with normal saline for additional 5 days.

Group III (CP + GA 3): The animals first received GA alone at a dose of (3 mg/kg b.w./day) by oral intubation for 9 days, and subsequently received CP (35 mg/kg b.w./day) by intraperitoneal injection in association with GA (3 mg/kg b.w./day) for additional 5 days.

Group IV (CP + GA 10): The animals first received GA alone at a dose of (10 mg/kg b.w./day) by oral intubation for 9 days, and subsequently received CP (35 mg/kg b.w./day) by intraperitoneal injection in association with GA (10 mg/kg b.w./day) for additional 5 days.

Group V (CP + GA 30): The animals first received GA alone at a dose of (30 mg/kg b.w./day) by oral intubation for 9 days, and subsequently received CP (35 mg/kg b.w./day) by intraperitoneal injection in association with GA (30 mg/kg b.w./day) for additional 5 days.

Group VI (CP + GA 100): The animals first received GA alone at a dose of (100 mg/kg b.w./day) by oral intubation for 9 days, and subsequently received CP (35 mg/kg b.w./day) by intraperitoneal injection in association with GA (100 mg/kg b.w./day) for additional 5 days.

**Blood Sample collection**

At the end of the experimental period, blood was withdrawn from retro orbital plexus and transferred into a sterile EDTA container.

**Estimation of hematological parameters**

Estimation of red blood cell (RBC) counts

The RBC counts were determined following the method of Raghuramulu, Madhavan, & Kalyansundaram, 1983, using Neubaeurs chamber.[26]

Estimation of total leukocyte counts (TLC)

The TLC counts were determined following the method of Raghuramulu, Madhavan, & Kalyansundaram, 1983 using Neubaeurs chamber.[21]

Estimation of differential leucocyte counts (DLC)

The DLC counts were determined following the method of Dacie & Lewis, 1984 using Neubaeurs chamber.[27]

Estimation of hemoglobin content

The RBC contents were determined following the method of Varley, 2005 using Sahli Helige's hemoglobinometer.[28]

Estimation of platelet count

The platelet counts were determined following the method of Samuel, 1986 using Neubaeurs chamber.[29]

### Statistical analysis

The results were expressed as mean ± SEM. Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test *,#-P<0.05, **,##-P<0.01, ***,###-P<0.001; *-Normal Control group with CP induction control group; #- GA treated groups against carboplatin induction control group.

## Results

### Estimation of RBC counts

Treatment with CP produced a significant (P<0.001) decrease in the RBC counts of CP control group as compared to normal control group, CP groups co-treated with GA (10, 30 and 100 mg/kg) showed significantly (P<0.05, P<0.01 and P<0.001) increased the RBC counts in a dose dependent manner in comparison with CP control group rats. The dose 3 mg/kg was found to be ineffective in this regard (Table 1).

### Estimation of total leukocyte counts (TLC)

TLC counts of CP control group were significantly (P<0.001) lowered as compared to normal control group. Co-treatment of GA (10, 30 and 100 mg/kg) with CP produced a significant (P<0.05, P<0.01 and P<0.001) and dose dependant increase in the TLC counts in comparison with CP control group rats. The dose 3 mg/kg was found to be ineffective in this regard (Table 1).

### Estimation of differential leucocyte counts (DLC)

There was significant (P<0.001) increase in the eosinophil counts whereas significant decrease in basophils (P<0.01), neutrophils (P<0.001), lymphocytes (P<0.001) and monocytes (P<0.01) counts in carboplatin induced control group animals as compared normal control group animals. Co-treatment of GA (10, 30 and 100 mg/kg) with CP produced significant (P<0.05, P<0.01 and P<0.001) reduction in eosinophil count and significantly (P<0.05, P<0.01 and P<0.001) increased the basophils, neutrophils, lymphocytes and monocytes in a dose dependant manner as compared to CP control group. The dose 3 mg/kg was found to be ineffective in this regard (Table 1).

### Estimation of hemoglobin (Hb) content

Treatment with CP produced a significant (P<0.001) decrease in the Hb contents of CP control group as compared to normal control group, CP groups co-treated with GA (10, 30 and 100 mg/kg) showed significantly (P<0.05, P<0.01 and P<0.001) increased the Hb contents in a dose dependent manner in comparison with CP control group rats. The dose 3 mg/kg was found to be ineffective in this regard (Table 1).

### Estimation of platelet counts

Platelet counts of CP control group were significantly (P<0.001) lowered as compared to normal control group. Co-treatment of GA (10, 30 and 100 mg/kg) with CP produced a significant (P<0.05, P<0.01 and P<0.001) and dose dependant increase in the TLC counts in comparison with CP control group rats. The dose 3 mg/kg was found to be ineffective in this regard (Table 1).

**Table 1: Effect of CP and GA on hematological parameters**

| Groups | RBCs (Million/$mm^3$) | TLC (/$mm^3$) | Eosinophills (%) | Basophills (%) | Neutrophills (%) | Lymphocytes (%) | Monocytes (%) | Hb count (g%) | Platelet (Lakhs/ $mm^3$) |
|---|---|---|---|---|---|---|---|---|---|
| **Normal Control** | 6.63±0.08 | 7100±22.80 | 1.08±0.02 | 2.97±0.18 | 61.76 ± 5.34 | 32.90 ±4.11 | 7.01±2.05 | 14.8 ±0.17 | 3.57±0.05 |
| **CP Control** | 3.23±0.05*** | 2750±9.87*** | 6.76±0.25*** | 1.16±0.02** | 31.65±6.35*** | 15.44±6.21*** | 3.45±1.56** | 7.51±0.15*** | 1.45±0.03*** |
| **GA 3 + CP** | 3.66±0.07 | 2820±11.28 | 6.56±0.18 | 1.09±0.04 | 39.43±5.67 | 19.45±5.11 | 3.77±1.52 | 6.98±0.12 | 1.36±0.01 |
| **GA 10 + CP** | 4.85±0.06# | 4800±10.25# | 3.98±0.08# | 1.92±0.02# | 42.67±4.21# | 25.37±4.89# | 4.97±2.33# | 10.03±0.16# | 2.55±0.05# |
| **GA 30 + CP** | 6.01±0.06## | 5803±12.39## | 3.04±0.04## | 2.55±0.01## | 55.11±5.72## | 31.25±4.33## | 5.80±2.66## | 11.20±0.19## | 2.89±0.03## |
| **GA 100+CP** | 6.55±0.08### | 7021±13.21### | 1.55±0.02### | 3.45±0.04### | 62.23±6.45### | 33.27±2.48### | 7.67± 3.12## | 13.85±0.20### | 4.01±0.087### |

The results were expressed as mean ± SEM. Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Tukey's Kramer Multiple Comparison test *,#-P<0.05, **,##-P<0.01, ***,###-P<0.001; *-Normal Control group with CP induction control group; #- GA treated groups against CP induction control group.

## Discussion

Cancer chemotherapy induced hematological disorders have been reports to span almost the entire spectrum of hematology, affecting red cells, white cells, platelets, and the coagulation system. These hematologic abnormalities induced by chemotherapeutic agents are mediated by different mechanisms, including inhibition of hematopoiesis due to mild to moderate suppression of bone marrow, immunological effects and altered enzymatic pathways.[30]

Carboplatin, a platinum complex has shown prominent antitumour activity against a number of human cancers but the major dose-limiting toxicity of this analog is reported to be hematological toxicities.[31] The present investigation was aimed to assess the alterations produced by the CP on hematological parameters in rats and effect of a plant phenolic acid GA on these alterations taking in consideration its wide range of pharmacological activities documented previously.[19-24]

It has been reported in the earlier clinical studies that patients treated with platinum compounds have a higher chances of severe anemia.[32] In the present study the administration of CP resulted into significant decrease in the RBC counts as well as heamoglobin content of blood in the CP control rats as compared to normal rats indicated severe anemic condition. These results were in accordance with the previous findings. The co-treatment with GA at the doses 10, 30 and 100 mg/kg showed a significant protection against these alterations as compared to CP control group indicating anti-anemic activity of GA against CP induced anemia. GA has been reported for its iron chelating properties which have been proved to be useful in the treatment of thalassemic patients.[33,34] It has also been reported for being an endogenous siderophore whose deficiency may lead to anemia and splenic overload in mice.[35] These reports may be correlated in this study wherein the CP induced anemia has been successfully ameliorated which can be attributed to the iron chelating properties of GA. Huang et al., 2011 in their study documented the association between the grade of platinum-based chemotherapy induced leukopenia and the clinical outcome of patients with metastatic non-small cell lung cancer patients amongst which neutropenia and monocytopenia was found to be more prominent.[36] Few other studies reported the lymphocytopenia and basocytopenia associated with platinum based chemotherapeutic agents.[37,38] Chemotherapy induced neutropenia is a life-threatening and serious adverse effect that may lead to a high risk of developing serious complications such as pyrexia associated with severe or life-threatening infections. It found more frequently in immunocompromised and vulnerable elderly patients resulting in the fatal outcome.[39,40] Basopenia (or basocytopenia), characterized by a deficiency of basophils is one of the major hematological adverse effects of antineoplastic drugs increasing the vulnerability of patients towards severe infections.[41] A recently evidenced study showed that the chemotherapy-induced leucocytosis results into severe lymphopenia that may modify the immune milieu of a patient by depleting regulatory cells and endogenous cells that compete for activating cytokines.[42] Platinum based chemotherapy induced monocytopenia is a form of leukocytopenia associated with a deficiency of monocytes resulting from apoptosis induced by cross linking of monocyte DNA.[43] In an investigation by Kondo et al., 1999 identified that monocytopenia after chemotherapy proves to be a risk factor for neutropenia and associated consequences.[44]

In our study CP treatment for 5 days induced significant decrease in the nuetrophil, basophill, lymphocyte and monocyte count indicating severe leucocytoenia induced by CP and the results were found to be in accordance with previous reports. Co-treatment with GA at doses of 10, 30 and 100 mg/kg exhibited a significant attenuation of neutropenia, basocytopenia, lymphocytopenia and monocytopenia caused by CP by significantly increasing their counts respectively; indicating protective effect of GA against CP induced leucocytopenia.

Although all the leukocytes counts were found to be decreased but there was significant increased in the eosinophill count indicative of allergic manifestations induced by CP which was in agreement with the earlier studies documented.[45] GA (10, 30 and 100 mg/kg) was successful in normalizing the eosinophill counts showing its capability to treat the CP induced hypersensitivity reactions. Thrombocytopenia induced by cancer chemotherapy is a major complication which can cause delay or reduction in subsequent courses of chemotherapy. As reported in literature thrombocytopenia occurred in 82% of those receiving only carboplatin leading to severe hemorrhage.[46,47] The thrombocyte counts in CP control group were found to be significantly reduced as compared to the normal control group indicating CP induced thrombocytopenia in similarity to the previous reports. Co-treatment with GA (10, 30 and 100 mg/kg) significantly prevented the decrease in thrombocyte counts and showed normalized counts as compared to CP control group indicating its potential

protective action against thrombocytopenia. Siddik et al., 1987 in their studies found that the dose-limiting toxicity, anaemia caused by carboplatin appears to be a secondary effect of thrombocytopenia.[13] Hence the anti-anemic effect of GA can also be attributed to its ameliorative effect against CP induced thrombocytopenia. In the whole study 3 mg/kg of GA was found to be ineffective in treating CP induced hematological toxicities.

## Conclusion

In conclusion GA may be considered as a potential natural phenolic agent that can be used to protect and ameliorate the hematological adverse effects, such as anemia, leucocytopenia and thrombocytopenia induced by CP. These protective effects can be attributed to attenuation of myelosupression. Further studies are required to reveal the possible molecular mechanisms involved in protective effects of GA.

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