RESEARCH ARTICLE

Influence of Platelet-rich Plasma and Ascorbic Acid on Carboplatin-induced Hematotoxicity in Male Albino Rats

Intidhar I. Yaseen,* Hanady S. Al-Shmgani, Ahmed Hafidh

Department of Biology, College of Education for Pure Science, Ibin Al-Haitham, University of Baghdad, Baghdad, Iraq

Received: 19th June, 2021; Revised: 31st July, 2021; Accepted: 17th August, 2021; Available Online: 25th September, 2021

ABSTRACT

There has been inclusive concern to platelet-rich plasma (PRP) treatment in degenerative medicine in recent years due to its valuable properties. This study aimed to assess the effect of PRP alone or in combination with ascorbic acid on carboplatin-induced hematotoxicity. Thirty male albino rats were divided into five equal groups; The first group served as control. The second group was injected with a single dose of carboplatin (60 mg/kg, i.p.) on day 1. The third group was injected with AA (100 mg/kg, i.p.) on days (0–7) and carboplatin (60 mg/kg, i.p.) on day1. The fourth group was injected with PRP extract (150 µL/Rat, s.c.) on days (0, 2, 4 and 6) and carboplatin (60 mg/kg, i.p.) on day1. The fifth Group was injected with AA (100 mg/kg, i.p.) on days (0–7) in combination with PRP extract (150 µL/rat,s.c.) on days (0, 2, 4, and 6) and carboplatin (60 mg/kg, i.p.) on day1. The results showed that carboplatin treatment indicated a significant decrease in body weight. Blood analysis showed a significant decrease in white blood cell (WBCs), red blood cell (RBCs), hemoglobin (Hb), and packed cell volume (PCV) compared to the control group. Treatment with PRP or AA or AA+PRP showed improvement in rat weight and some blood parameters.

Keywords: Ascorbic Acid, Carboplatin, Hematotoxicity, Platelet-Rich plasma.

International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.26

How to cite this article: Yaseen II, Al-Shmgani HS, Hafidh A. Influence of Platelet-rich Plasma and Ascorbic Acid on Carboplatin-induced Hematotoxicity in Male Albino Rats. International Journal of Drug Delivery Technology. 2021;11(3):807-811.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The use of PRP treatment has been effective in degenerative cellular diseases. The high concentrrtion of platelet in a small volume of plasma is essential to mediate the release of several growth factors and cytokines, including transforming growth factor (TGF-β), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF), these growth factors are necessary for the regenerative and healing process.[1] Therefore, and due to PRP properties, several studies indicate that PRP has anti-angiogenesis and anti-inflammatory effects; thus, it has been used in laparoscopic surgery, chronic diseases, neuropathies, arthritis, and tendinopathies.[2,3] It is also used to treat alopecia and perforated corneal ulcers.[4,5] EI-Tahawy *et al*. found that PRP in diabetic rats regenerates pancreatic islet, and new lobules were formed.[6] PRP or platelet gel is a therapeutic strategy used for the restoration of surgical and nonsurgical wounds. The efficacy of this strategy lies in the launch of a wide variety of proteins from platelets concerned in renewed growth, angiogenesis, immune control, trapping circulating stem cells, and tissue redesigning hemostasis.[7] PRP can consist of exclusive portions of plasma, white blood cells, erythrocytes, and platelets according to the machine and approach used.[8]

Ascorbic acid (vitamin C) is a water-soluble vitamin required for multiple biological functions. It is fundamental for average growth and development and is a necessary enzyme cofactor for several enzymes.[9] Ascorbic acid is a powerful reducing agent and scavenger of free radicals, functioning as a scavenger of oxidizing free radicals and adverse reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radical, and singlet oxygen.[10]

Carboplatin is a second-generation platinum agent, has been used as a chemotherapeutic agent.[11] For the treatment of several types of cancer, including ovarian, cervical, head-neck, and non-small small cell lung cancers.[12] Unfortunately, the clinical use of carboplatin has been limited due to its side effects, especially myelosuppression. Several mechanisms have been postulated to give an explanation for the mechanisms underlying the carboplatin-induced toxicities such as manufacturing of ROS.[13] The present study was carried out to investigate the effect of ascorbic acid and platelet-rich plasma on hematotoxicity induced by carboplatin.

MATERIALS AND METHODS

Chemicals

Carboplatin purchased from (Fresenius Kabi, India). Ascorbic acid was obtained from (Bayer, Germany). All

*Author for Correspondence: ahmedhafidh8@gmail.com

other reagents and chemicals, unless otherwise stated, were obtained from Sigma Aldrich (USA) and (Chemical point, Germany).

Blood Collection and Platelet-rich Plasma (PRP) Preparation

The PRP was prepared using double centrifugation protocol as described by Mohsen and Hafidh.[14] Nine male albino rats were anesthetized with dietyl-ether, and 9–10 mL blood was collected from each rat by cardiac puncture. 0.5 Ll of each blood sample was placed in ethylenediaminetetraacetic acid (EDTA) tubes to measure the initial count of platelets and the rest was collected into acid citrate dextrose (ACD) PRP tube and then centrifuged at 2000 rpm for 5 minutes. Supernatant was transferred and centrifuged for 15 minutes at 4000 rpm, 2/3 of the top layer, which represented the platelet-poor layer PPP was removed and the remaining 1/3 which represents the PRP, was well mixed. 0.75 µL of PRP was separated for final platelet count.

The remaining PRP was activated by adding 10% calcium chloride (8 mL PRP: 2 mL $CaCl_2$), left for 1 hour at room temperature, and then centrifuged at 4000 rpm for 5 minutes at 4°C. The supernatant was then filtered using 0.22 µm millipore filter and kept at –20°C until used.

Experimental Animals

Thirty male albino rats weighing 150–277 g were acclimatized for two weeks at a temperature of 24 ± 5 and accessed libitum to tap water and rodent pellets.

Rats were divided into five groups (6 in each)

- *Group I:* Rats served as control.
- *Group 2:* Rats injected intraperitoneally with a single dose of 60 mg/kg carboplatin (C) on day 1.
- *Group 3:* Rats injected intraperitoneally with 100 mg/kg ascorbic acid (AA) daily on days (0–7) and injected intraperitoneally with a single dose of 60 mg/kg carboplatin on day 1
- *Group 4:* Rats injected subcutaneously with 150 µL/rat PRP extract on days (0, 2, 4, and 6) and injected intraperitoneally with a single dose 60 mg/kg carboplatin on day 1
- *Group 5:* Rats injected intraperitoneally with 100 mg/kg AA on days (0–7) and intraperitoneally with 150 µL/rat PRP extract on days (0, 2, 4, and 6) and injected intraperitoneally with a single dose of 60 mg/kg carboplatin on day 1.

Hematological Parameters

At the end of the experiment, rats were anesthetized with diethyl ether, and blood samples were collected into an EDTA tube by heart puncture to determine some hematological parameters. WBC, RBC, Hb, and PCV were estimated by Mindary BC-5000 automated analyzer, China.

Statistical Analysis

Data were processed and analyzed using Graph Pad Prism 6 was using in Statistical analysis. Data are expressed as means ± standard errors (SE). One-way analysis of variance (ANOVA) followed by Tukey's test, $p \leq 0.05$, was considered statistically significant.

RESULTS

Platelet Yield

Final Platelet concentration in PRP was 3.7 fold over the baseline concentration, as shown in Figure 1.

Clinical Features and Body Weight Changes: All the control rats were normal, healthy, and active during the treatment. However, rats treated with carboplatin appeared weak and less active. The present study results revealed that the administration of single intraperitoneal dose of carboplatin (60 mg/kg) induced a significant reduction ($p \leq 0.05$) in body weight compared to the initial weight. However, the reduction in body weight in the other treatment groups was not significant, as shown in Figure 2.

Hematological Studies

The results of the hematological analysis revealed a highly significant decrease ($p \leq 0.05$) in WBC count in carboplatin-treated rats. Treatment with AA or PRP or AA+PRP significantly elevated ($p \leq 0.05$) WBC count compared to Carboplatin group as shown in Figure 3.

RBC count is significantly decreased ($p \leq 0.05$) in the carboplatin group compared to the control group.

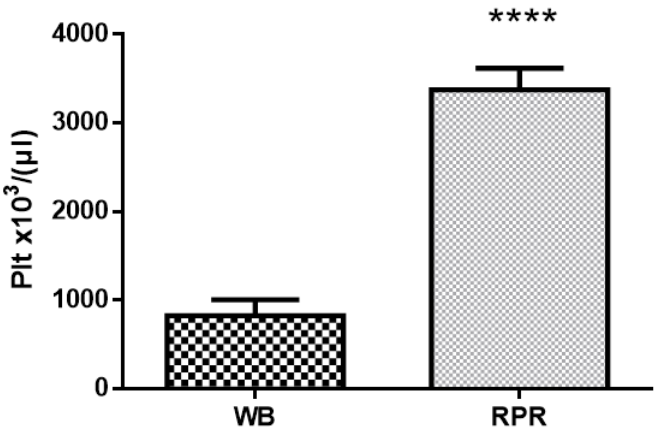

Figure 1: Platelet count in whole blood (WB) and Platelet Rich Plasma (PRP**).** Data represent mean ± standard error (SE). **** Indicates significant differences at ($p \leq 0.05$)

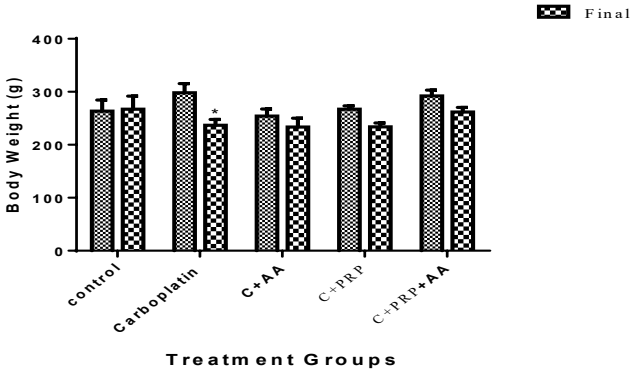

Figure 2: Effect of ascorbic acid (AA) and platelet-rich plasma (PRP) on body weight in carboplatin (C)-treated rats. Data represent mean ± standard error (SE). * Indicates significant differences at ($p \leq 0.05$) in final body weight compared to initial body weight

The administration of AA or PRP or AA+PRP could not reduce such change compared to the carboplatin group as shown in Figure 4.

The results exhibited a significant decrease ($p \leq 0.05$) in Hb amount and PCV in carboplatin group compared to the control group. The above changes were significantly reversed ($p \leq 0.05$) in the C+AA group, while there were no significant changes in Hb amount and PCV in C+PRP or C+PRP+AA groups compared with the carboplatin group (Figures 5 and 6).

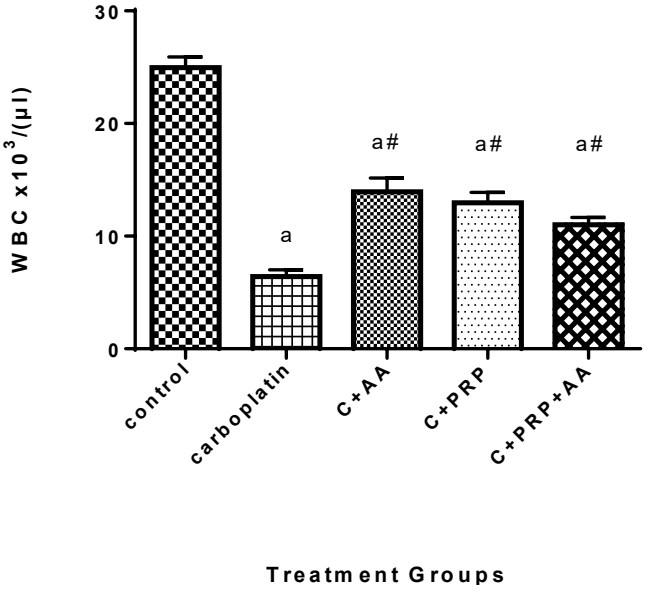

Figure 3: Effect of ascorbic acid (AA) and platelet-rich plasma (PRP) on WBC count in carboplatin (C)-treated rats. Data represent mean ± standard error (SE). **a** Indicates significant differences at ($p \leq 0.05$) with the control group. # indicates significant differences compared to the carboplatin group.

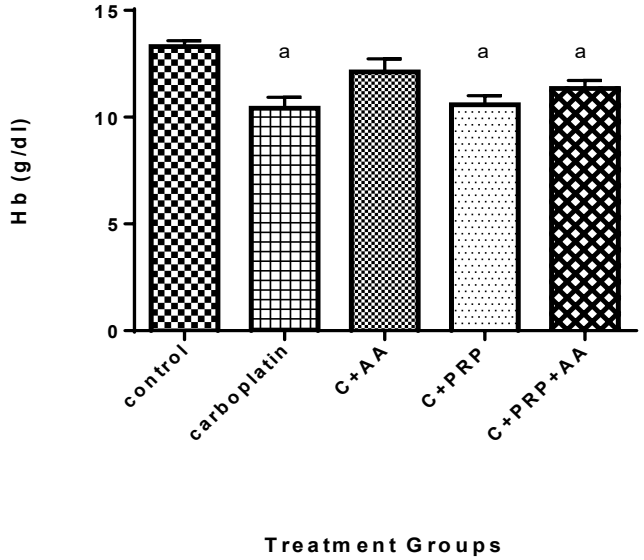

Figure 5: Effect of ascorbic acid (AA) and platelet-rich plasma (PRP) on Hb in carboplatin (C)-treated rats. Data represent mean ± standard error (SE). **a** Indicates significant differences at ($p \leq 0.05$) with the control group.

DISCUSSION

Carboplatin is an anti-cancer drug with side effects such as intestinal toxicity and causes intestinal mucosal damage, diarrhea, infiltration of inflammatory cells, and increased expression of inflammatory cytokines in the intestine.[15] Symptoms of carboplatin-induced gastrointestinal mucositis include ulceration, inflammation, nausea, flatulence, abdominal pain, and diarrhea.[16] These factors may cause the previously mentioned symptoms and weight loss in the rat treated with carboplatin. On the other hand, groups injected with ascorbic acid or PRP, or both with carboplatin, improved their activity and appetite. A standard clinical appearance may be attributed to the ability of each of them to protect the rat from

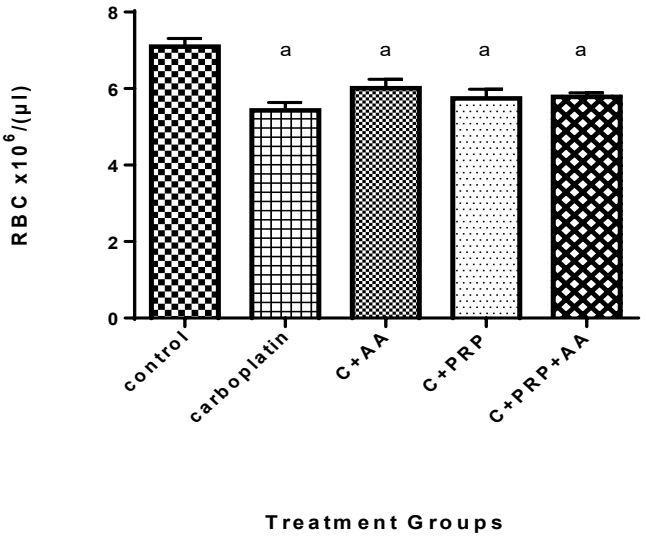

Figure 4: Effect of ascorbic acid (AA) and platelet-rich plasma (PRP) on RBC count in carboplatin (C)-treated rats. Data represent mean ± standard error (SE). **a** Indicates significant differences at ($p \leq 0.05$) with the control group.

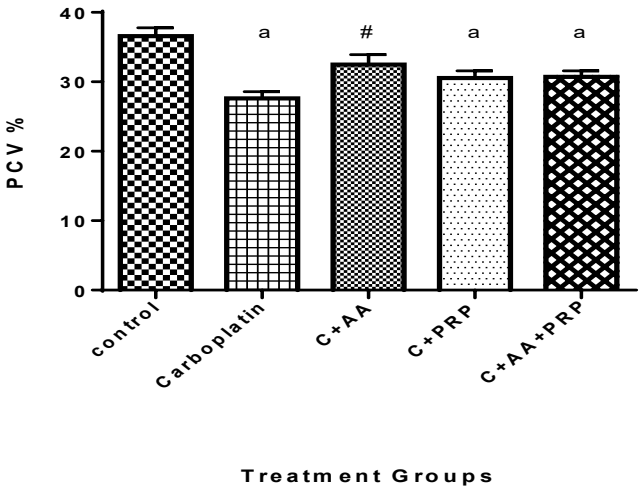

Figure 6: Effect of ascorbic acid (AA) and platelet-rich plasma (PRP) on PCV in carboplatin (C)-treated rats. Data represent mean ± standard error (SE). a Indicates significant differences at ($p \leq 0.05$) with the control group. # indicates significant differences compared to carboplatin group.

the oxidative stress caused by the treatment with carboplatin. Injections of both ascorbic acid and PRP protected the rat from the effects of carboplatin on their weight.

Carboplatin has been reported to cause dose-related myelosuppression resulting in anemia, which may manifest through a mechanism involving either depression or erythropoietic activity, internal hemorrhaging as a result of thrombocytopenia, and Red cell lysis.[17]

A single dose of carboplatin caused a significant decrease in WBC count, RBC count, Hb, and PCV in rats. These results are consistent with other studies.[18-20] which reported a marked decrease in the number of WBC cells due to injury or inflammation during treatment with carboplatin in myelotoxicity due to its ability to bind to cell DNA and reduced ability of cells to replicate. Also, changing the permeability of the red blood cell membrane and increasing mechanical fragility and iron metabolism.[21] Another suggested mechanism explaining carboplatin damage is induction to oxidative stress; reactive oxygen species (ROS) are toxic to bone marrow cells and stimulate apoptosis.[22] Moreover, due to the phosphide-rich membranes of red blood cells, they oxidize quickly and lead to a decrease in RBC count, and an increase in MDA also may cause oxidation of red blood cell membranes, osmotic hemolysis, decrease in the level of thiols in the cell membrane and cause oxidation of RBC membranes, changes in ionic permeability. Solubility or increased lipid fluidity can activate another chain reaction that leads to reduced survival for a more extended period and removal of RBC from the system by the necrosis process.[23] Carboplatin causes a decrease in PCV, and this is in agreement with several researches.[24-26]

Several antioxidants have been investigated to obstruct free radicals' formation and prevent or reduce the adverse effects caused by carboplatin. Treatment with AA or PRP or AA+PRP showed a noticeable improvement in clinical features, body weight, and WBC count, while the treatment of AA alone showed a significant increment in Hb and PCV compared to animals injected with carboplatin alone. Reversal of some abnormalities to a normal status may reflect the causal association of antioxidant/antiradical properties of ascorbic acid and platelet-rich plasma.[8,10]

REFERENCES

1. Sampson S, Gerhard M, Mandelbaum B. Platelet-rich plasma injection grafts for musculoskeletal injuries: a review. Current Reviews in Musculoskeletal Medicine. 2008;1(3):165-174.
2. AndiaI,Maffulli N. Platelet-rich plasma for managing pain and inflammation in osteoarthritis. Nature Reviews Rheumatology. 2013;9(12):721-730.
3. Andia I, Latorre PM, Gomez MC, Burgos-Alonso N, Abate M, Maffulli N. Platelet-rich plasma in the conservative treatment of painful tendinopathy: a systematic review and meta-analysis of controlled studies. British medical bulletin. 2014;110(1):99-115.
4. Alio JL, Rodriguez AE, Martinez LM, Rio AL. Autologous Fibrin Membrane Combined With Solid Platelet-Rich Plasma in the Management of Perforated Corneal Ulcers. JAMA Ophthalmology. 2013;131(6):745-751.
5. Cervelli V, Garcovich S, Bielli A, Cervelli G, Curcio BC, Scioli MG, Orlandi A, Gentile P. The Effect of Autologous Activated Platelet Rich Plasma (AA-PRP) Injection on Pattern Hair Loss: Clinical and Histomorphometric Evaluation. BioMed Research International. 2014;1-9.
6. El-Tahawy NF, Rifaai RA, Saber EA, Saied SR, Ibrahim RA. The effect of Platelet Rich Plasma (PRP) Injection on the Endocrine Pancreas of the Experimentally Induced Diabetes in Male Albino Rats: A Histological and Immunohistochemical Study. J Diabetes Metab. 2017;8:730.
7. Chandra RK, Handorf C, West M, Kruger EA, Jackson S. Histologic effects of autologous platelet gel in skin flap healing. Archives of facial plastic surgery. 2007;9(4): 260-263.
8. Brass L. Understanding and evaluating platelet function. Hematology American Society of Hematology Education Program. 2010;1:387-396.
9. Nishikimi M, Koshizaka T, Ozawa T, Yagi K. Occurrence in humans and guinea pigs of the gene related to their missing enzyme l-gulono-γ-lactone oxidase. Archives of biochemistry and biophysics. 1988;267(2):842-846.
10. Ramanathan K, Balakumar BS, Panneerselvam C. Effects of ascorbic acid and a-tocopherol on arsenic-induced oxidative stress. Human & experimental toxicology. 2002;21(12):675-680.
11. Tanley SW, Diederichs K, Kroon-Batenburg LM, Levy C, Schreurs AM, Helliwell JR. Carboplatin binding to histidine. ActaCrystallographica Section F:Structural Biology Communications. 2014;70(9):1135-1142.
12. Chen X, WangJ, Fu Z, Zhu B, Wang J, Guan S, Hua Z. Curcumin activates DNA repair pathway in bone marrow to improve carboplatin-induced myelosuppression. Scientific Reports. 2017;7(1):17724.
13. Xu W, Liu LZ, Loizidou M, Ahmed M, Charles IG. The role of nitric oxide in cancer. Cell Research. 2002;12(5):311-320.
14. Mohsen HJ, Hafidh A. Influence of Erythropoietin and Platelet-Rich Plasma on Cyclophosphamide-Induced Hepatotoxicity in Male Albino Rats. Journal of Global Pharma Technology. 2019;11:670-677.
15. Yu C, Zhou B, Xia X, Chen S, Deng Y, Wang Y, Wu L, Tian Y, Zhao B, Xu H, Yang L. Prevotella copri is associated with carboplatin-induced gut toxicity. Cell Death & Disease. 2019;10(10):714.
16. Touchefeu Y, Montassier E, Nieman K, Gastinne T, Potel G, Bruley des Varannes S, Le Vacon F, de La Cochetière MF. Systematic review: the role of the gut microbiota in chemotherapy-or radiation-induced gastrointestinal mucositis-current evidence and potential clinical applications. Alimentary Pharmacology & Therapeutics. 2014;40:409-421.
17. Dallalio G, Means RT. Effects of oxidative stress on human erythroid colony formation: modulation by γ-interferon. Journal of Laboratory and Clinical Medicine. 2003;141(6): 395-400.
18. Bhalchandra W, Alqadhi YA, Ninawe AS. Ameliorative role of bee honey and royal jelly against cisplatin induced alteration in hematological parameters in male wistar albino rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2018;10(4):110-114.
19. Hassan I, Chibber S, Naseem I. Ameliorative effect of riboflavin on the cisplatin induced nephrotoxicity and hepatotoxicity under photoillumination. Food and Chemical Toxicology. 2010;48(8):2052-2058.
20. Ashry K,Elkady AA. Royal jelly modulates hepatotoxic effect of rats treated with cisplatin. AJSE. 2014;47(1):172-180.

21. Markovic SD, Zizic JB, Djacic DS, Obradovic AD, Curcic MG,Cvetkovic DM.Alteration of oxidative stress parameters in red blood cells of rats after chronic in vivo treatment with cisplatin and selenium arch. Biol. Sci. Belgrade. 2011;63:991-999.
22. Cazzola M. Mechanisms of anemia in patients with malignancy: implications for the clinical use of recombinant human erythropoietin. Medical Oncology (Northwood, London, England.2000;17 Suppl 1:S11-6.
23. Tolooei M, Mirzaei A. Effects of Pistacia Atlantica Extract on Erythrocyte Membrane Rigidity, Oxidative Stress, and Hepatotoxicity Induced by CCl4 in Rats. Global Journal of Health Science. 2015;7:32-38.
24. Nasr AY. Protective effect of aged garlic extract against the oxidative stress induced by cisplatin on blood cells parameters and hepatic antioxidant enzymes in rats. Toxicology Reports. 2014;1:682-691.
25. Maheswari R, ManohariS. Syzygium cumini (L.) seeds extract ameliorates cisplatin-induced hepatotoxicity in male Wistar rats. Int J Pharm Sci. Res. 2015;6:444-50.
26. Geyikoğlu F, Çolak S, Türkez H, Bakır M, Koç K, Hosseini-gouzdagani MK, Çerig S, Sönmez M. Oleuropein Ameliorates Cisplatin-induced Hematological Damages via Restraining Oxidative Stress and DNA Injury. Indian Journal of Hematology and Blood Transfusion. 2017;33(3):348-354.