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5-Flurouracil induced hematological alterations in wistar rats and its amelioration with naringenin

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Abstract
The present study was aimed to evaluate protective effect of NARENGENIN (NG) on 5-Flurouracil (5-FU) induced hematological alterations in Wistar rats. A total of forty eight (48) adult male abino Wistar rats were divided into 4 groups each. The group 1 (control), group 2 treated with 5-FU (@ 20 mg/kg b. wt., IP) for 5 days, group 3 administered with NG (@ 100 mg/kg b.wt, P/O) for 28 days and group 4 rats provided with 5-FU (@ 20 mg/kg b. wt) +NG (@ 100 mg/kg b. wt). The experiment was carried out for a duration of 28 days and each group were sacrificed on 14th and 28th day of the experiment. Hematological parameters were significantly reduced in group 2 expect TLC and significant improvement of all parameters was noticed in ameliorative group (group 3).

Keywords: 5-Flurouracil, hematology, naringenin, wistar rats

1. Introduction
5-FU is an antimetabolite, fluropyrimidine analogue, broad-spectrum anti-cancer drug used clinically since 1957's (Heidelberger *et al.*, 1957) [1]. It is the third most commonly used chemotherapeutic agent in treatment of solid malignancies across the World and second most common drug associated with cardiotoxicity after anthracyclines (Sorrentino *et al.*, 2012; Polk *et al.*, 2014 and Yuan *et al.*, 2019) [2-4]. It incorporates its metabolites into DNA and RNA and interferes with nucleoside metabolism which inhibits thymidylate synthase (TS) which interfere DNA replication leads to cytotoxic cell death and apoptosis (Longley *et al.*, 2003 and Miura *et al.*, 2010) [5, 6].
5-FU produces some adverse effects like nausea, vomiting, stomatitis, mucositis, diarrhoea, leucopenia, hemolytic anemia, thrombocytopenia and neurotoxicity (Eskandari *et al.*, 2014 and Yousef and Aboelwafa, 2017) [7, 8]. Anti cancerous drugs, heavy metals and pesticides induce haemotoxicity apart from damaging other organs viz: kidneys, liver, heart, lungs, retina and bones in humans and experimental animals [9, 10]. 5-FU also decreases in the activity of antioxidant enzymes results in cardiac (Sengul *et al.*, 2021) [11], pulmonary (Gedikli and Erbas, 2021) [12], hematological (Gelen and Sengul, 2018) [13] through oxidative stress, inflammation and apoptosis (Gelen *et al.*, 2018) [14].
Nowadays, the main focus is on using natural products like Quercetin, Rutin and Resveratrol (RES) that have rich sources of phyto chemicals, provide a pool of anti-oxidants. Naringenin (NG) is a flavonoid found in grapefruit, citrus, orange and tomato (Igual *et al.*, 2013) [15]. It has protective effects against cardiotoxicity (Rajadurai and Prince, 2007) [16], pulmonary (Gedikli and Erbas, 2021) [12], hepatotoxicity, nephrotoxicity (Gelen *et al.*,2018) [14] and hematological toxicities (Gelen and Sengul, 2018) [13] through anti-oxidant, immune-modulatory and anti-inflammatory properties (Nie *et al.*, 2012) [17].

2. Materials and Methods
All chemicals were of analytical grade and they are obtained from Qualigens Pvt. Ltd., Mumbai and SRL Pvt. Ltd., Mumbai, India. 5-FU was procured from Celon laboratories private limited, Hyderabad.

2.1 Experimental Animals
A total 48 healthy male albino Wistar rats of 3 months of age, weighing between 180-220 g, were obtained from Jeeva Life Science (ISO 9001:2015 certified company), Hyderabad. The Experiment was carried out according to the guidelines and prior approval of Institutional

Animal Ethics Committee (No.9/24/C.V.Sc., Hyd. IAEC-Rats/ 12.06.2021). Before beginning of experiment, all animals were kept one week for acclimatization under the same environmental conditions of temperature 20-22 ^{0}C, 12 h light/dark cycle. Animals were placed on commercial standard bedding material and provided with standard pellet feed and *ad libitum* water through out the experimental period.

2.2 Experimental design

Table 1: Experimental design with group wise treatment protocol

Group	No. of rats	Treatment
Group 1	12	Control (Saline @ 1 mL/ rat/ single dose/ Oral)
Group 2	12	5-Flurouracil (5-FU @ 20 mg/ kg body wt/ Intra-peritoneal route of administration (ROA) for 5 days
Group 3	12	Naringenin powder (NG @ 100mg/rat once daily orally for 28 days
Group 4	12	5-Flurouracil (5-FU @ 20 mg/ kg body wt/ Intra-peritoneal ROA + Naringenin powder (@ 1 mL/rat once daily orally for 28 days).

2.3 Sample collection and analysis

Six (6) rats from each group were sacrificed on 14th and 28th day of experiment. On the day of sacrifice, 2-3 mL of blood was collected from retro-orbital plexus with the help of capillary tube in an anticoagulant coated vaccutainers {K3-EDTA tube, 13 mm x 75 mm, 4 mL (Rapid Diagnostics Pvt. Ltd., Delhi)} to carry out all hematological parameters. Prior to blood collection, the selected experimental rats were put to fast for 12 hours. All the blood samples were used for estimation of Total Erythrocyte Count (TEC-millions/µL), Total Leukocyte Count (TLC-thousands/µL), Hemoglobin (Hb-g%) concentration, Packed Cell Volume (PCV), platelets by using automatic whole blood analyzer (Humacount, med source ozone biomedical Pvt. Ltd., Faridabad, Haryana) and results were tabulated for statistical analysis.

2.4 Statistical analysis

Data obtained were subjected to statistical analysis by applying one-way ANOVA using statistical package for social sciences (SPSS) version 16.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at $P< 0.05$ (Snedecor and Cochran, 1994) [18].

3. Results

Haematological studies revealed that in group 2 there were significantly reduced mean values of total erythrocyte count (TEC), haemoglobin (Hb) concentration, packed cell volume (PCV), platelets and significantly elevated mean values of total leucocyte count (TLC) when compared to group 4 on 14th and 28th day of experiment. Significantly ($P< 0.05$) increased mean values of TEC, Hb, PCV, platelets and decreased TLC values were recorded in group 4 when compared to group 2 rats on day 14th and 28th day of the experiment. Statistically, no significant difference were observed between the groups 1 and 3.

Table 2: Effect of 5-FU and NG on haematological parameters

Groups	Group 1		Group 2		Group 3		Group 4	
Days	14th	28th	14th	28th	14th	28th	14th	28th
TEC (mil/µL)	8.78±.17^a	9.48±.36^a	5.54±.15^c	4.78±.08^c	8.91±.08^a	9.38±.04^a	7.51±.3^b	5.99±.06^b
Hg (g%)	16.43±.716^a	16.60±.16^a	9.64±.08^c	8.81±.09^c	16.18±.04^a	16.48±.14^a	11.67±.17^b	12.51±.17^b
TLC (thou/µL)	9.36±.17^c	9.12±.10^c	15.26±.10^a	17.01±.23^a	8.89±.09^c	9.01±.07^c	12.75±.15^b	13.23±.10^b
PCV (%)	44.46±.20^a	49.44±1.92^a	37.50±.65^c	32.06±.4^c	43.52±.5^a	47.16±.60^a	39.16±.31^b	41.95±.51^b
Platelets (Lakhs/µL)	8.31±.41^a	9.23±.23^a	3.89±.14^c	4.12±0.39^c	8.12±.53^a	9.13±.25^a	5.23±.27^b	7.13±.24^b

Values are Mean±SE (n=6); One-way ANOVA

Means with different superscripts in a column differ significantly at ($P<0.05$)

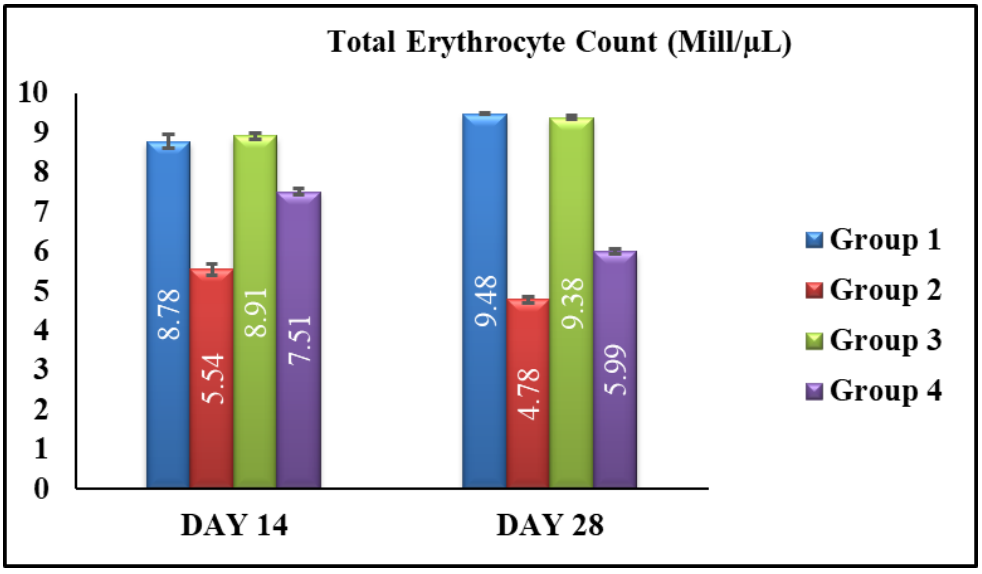

Fig 1: Total Erythrocyte Count: (TEC-Mill/µL) in different groups

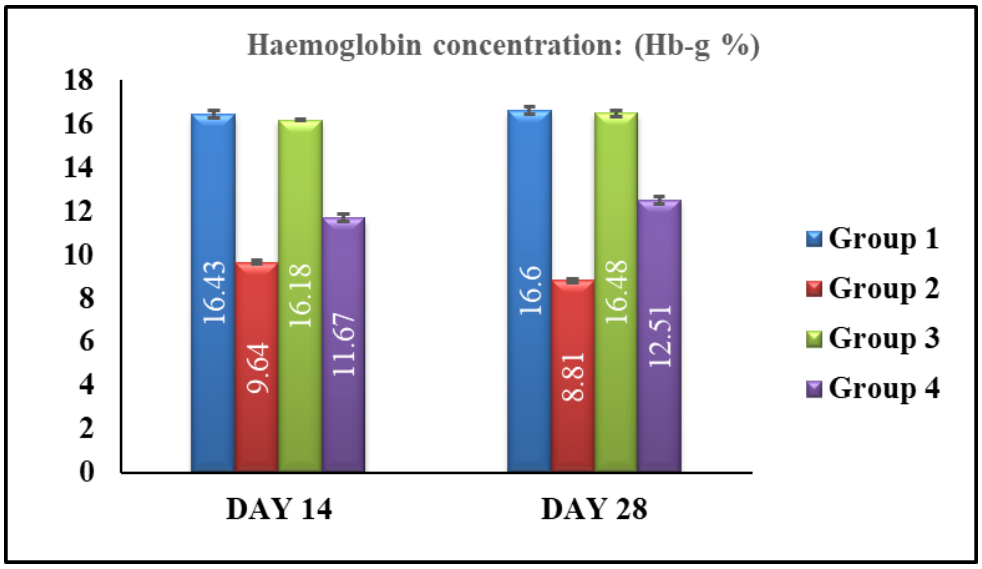

Fig 2: Haemoglobin concentration: (Hb-g %) in different groups

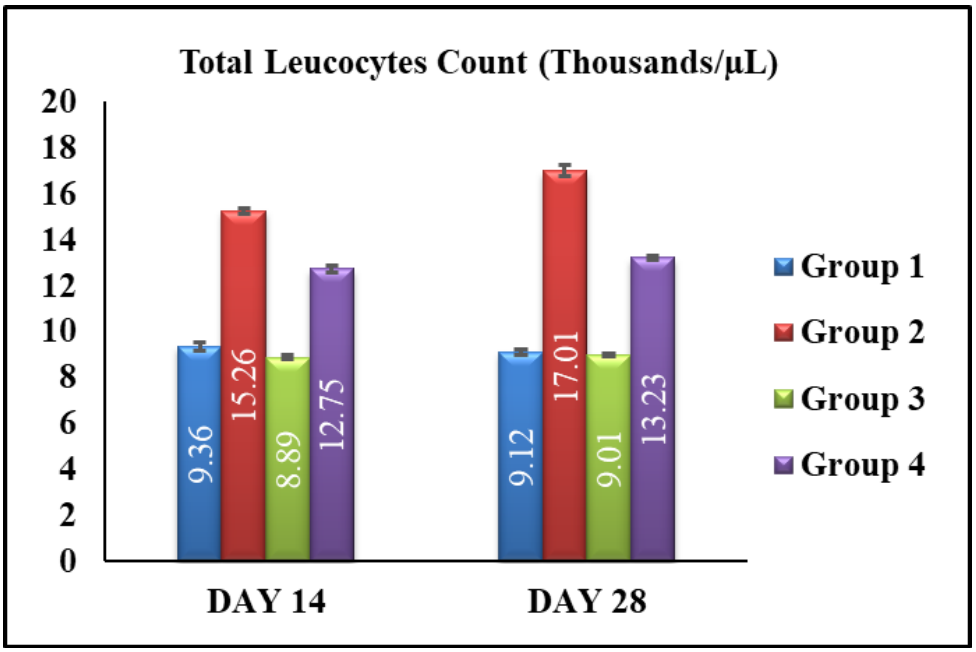

Fig 3: Total Leucocytes Count: (TLC-Thousands/µL) in different groups

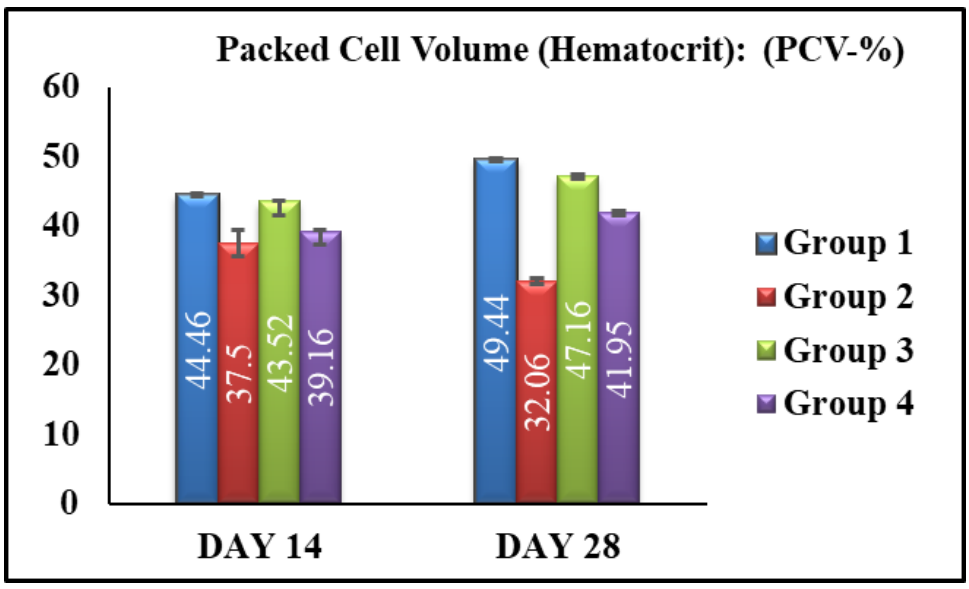

Fig 4: Packed Cell Volume (Hematocrit): (PCV-%) in different groups

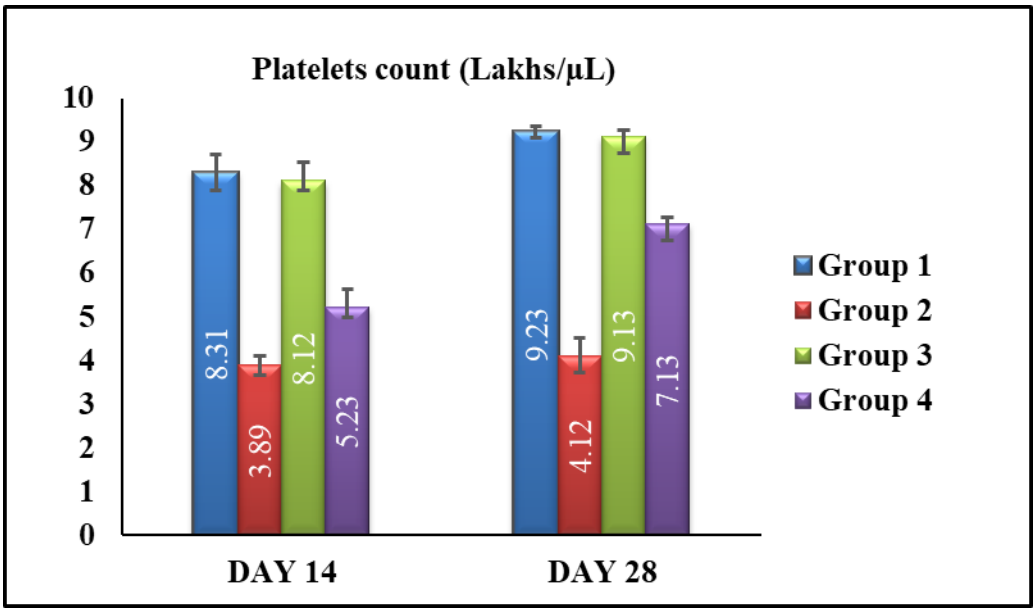

Fig 5: Platelets count (Lakhs/µL) in different groups

4. Discussion

Haematological examination assess the harmful effects of chemotherapeutic drugs on living organisms and provides the opportunity to clinically investigate the presence of metabolites, other constituents in the body of animals and it plays a vital role in the physiological, nutritional and pathological status of an animal (Lijuv*et al.*, 2013) [19]. In the present study, significantly ($P<0.05$) decreased mean values of various haematological parameters *viz*., TEC, Hb concentration, PCV, platelets and numerically elevated mean values of TLC were observed in the present study among group 2 rats when compared with group 1 rats on 14th and 28th day of experiment. A significant change in hematological parameters may be due to destruction of bone marrow cells or enhancement of osmotic fragility and brittleness of RBCs, disrupts erythropoiesis, altered RBCs membrane permeability and increased LPO and MDA levels of erythrocytes. There is also change in RBC morphology due to deoxygenation of Hb along with impaired iron metabolism which lead to anemia and thereby decreased in Hb concentration. Decrease in the PCV per cent may be due to the increased rate of breakdown of RBCs. A significant ($P<0.05$) increase in mean values of TLC could be due to generation of ROS production is associated with intense inflammatory process that leads to influx of inflammatory cells by activation of animal's defence mechanism. These findings are in agreement with Spasojevic *et al.* (2009; Zhao *et al.* 2014; Gelen and Sengul 2018 and Mao *et al.* 2019 [13, 20-22].

In group 4 rats, a significant increase in erythrocyte indices were observed in comparison to group 2. Hypothetically, NG might have helped in promoting hematopoiesis through secretion of erythropoietin which stimulate proliferation and differentiation of myeloid progenitor cells and protect cells against oxidative damage (Mahmoud, 2013; Karale and Kamath, 2016 and Gelen and Sengul, 2018) [13, 23, 24].

5. Conclusion

In conclusion, 5-FU caused significant reduction TEC, Hb, PCV and platelets by the formation of free radicals and increased TLC due to immune response. However, naringenin supplementation to treated rats exhibited comparatively reduced adverse effects indicating its protective antioxidant, anti-inflammatory property. Thus, present investigation confirmed the moderate protective role of NG against 5-Flurouracil toxicity.

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7. References

1. Heidelberger C, Chaudhuri NK, Danneberg P, Mooren D, Griesbach L, Duschinsky Rx *et al*. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. Nature. 1957;179(4561):663-666.
2. Sorrentino MF, Kim J, Foderaro AE, Truesdell AG. 5-Fluorouracil induced cardiotoxicity: Review of the literature. Cardiology Journal. 2012;19(5):453-457.
3. Polk A, Vistisen K, Vaage-Nilsen M, Nielsen DL. A systematic review of the pathophysiology of 5-Fluorouracil induced cardiotoxicity. BMC Pharmacology and Toxicology. 2014;15(1):1-11.
4. Yuan C, Parekh H, Allegra C, George TJ, Starr JS. 5-FU induced cardiotoxicity: case series and review of the literature. Cardio-Oncology. 2019;5(1):1-7.
5. Longley DB, Harkin DP, Johnston PG.5-Fluorouracil: Mechanisms of action and clinical strategies. Nature Reviews Cancer. 2003;3(5):330-338.
6. Miura K, Kinouchi M, Ishida K, Fujibuchi W, Naitoh T, Ogawa H, *et al*. 5-FU metabolism in cancer and orally-administrable 5-FU drugs. Cancers. 2010;2(3):1717-1730.
7. Eskandari MR, Moghaddam F, Shahraki J, Pourahmad J. A comparison of cardiomyocyte cytotoxic mechanisms for 5-Fluorouracil and its pro-drug capecitabine. Xenobiotica. 2014;1(3):79-87.
8. Yousef HN, Aboelwafa HR. The potential protective role of taurine against 5-Fluorouracil-induced nephrotoxicity in adult male rats. Experimental and Toxicologic Pathology. 2017;69:265-274.
9. Yadala Ravikumar D, Madhuri M, Lakshman A, Gopala Reddy, Kalakumar B. Haematological Alterations Induced by Cadmium (Cd) and Chlorpyrifos (CPF) in

Male Wistar albino Rats. International Journal of Current Microbiology and Applied Sciences. 2019;8(08):480-485.
10. Ravikumar Y, Madhuri D, Lakshman M, Reddy AG, Kalakumar B. Changes in Biochemical Parameters, Antioxidative Enzymes and Histopathology of Liver Induced by Cadmium (Cd) and Chlorpyrifos (CPF) in Wistar Rats. Indian Journal of Animal Research 2021;10:1-5.
11. Sengul E, Gelen V, Gedikli S. Cardioprotective activities of Quercetin and Rutin in Sprague Dawley rats treated with 5-Fluorouracil. Journal of Animal and Plant Sciences. 2021;31(2):423-431.
12. Gedikli S, Erbas E. Protective Effects of Naringin on 5-Fluorouracil induced lung Toxicity in Rats. Kocatepe Veterinary Journal. 2021;14(1):16-25.
13. Gelen V, Sengul E. Hematoprotective effect of naringin on 5-FU toxicity in rats. Chemical Resource of Journal. 2018;3(1):127-130.
14. Gelen V, Sengul E, Yildirim S, Atila G. The protective effects of naringin against 5-Fluorouracil-induced hepatotoxicity and nephrotoxicity in rats. Iranian Journal of Basic Medical Sceinces. 2018;21(4):404-410.
15. Igual M, Garcia-Martinez E, Camacho MM, Martinez-Navarrete N. Jam processing and storage effects on β-carotene and flavonoids content in grape fruit. Journal of Functional Foods. 2013;5(2):736-744.
16. Rajadurai M, Prince PSM. Preventive effect of naringin on isoproterenol-induced cardiotoxicity in Wistar rats: An *in vivo* and *in vitro* study. Toxicology. 2007;232(3):216-225.
17. Nie YC, Wu H, Li PB, Luo YL, Long K, Xie LM *et al*. Anti-inflammatory effects of naringin in chronic pulmonary neutrophilic inflammation in cigarette smoke-exposed rats. Journal of Medicinal Food. 2012;15(10):894-897.
18. Snedecor GW, Cochran G. *Statistical methods*, 8th Edition, IOWA State University Press, America, USA 1994, 64-67.
19. Lijuv B, Jeena K, Kuttan R. Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma longa* L). Food and Chemical Toxicology. 2013;53:52-61.
20. Spasojevic I, Jeli CS, Zakrzewska J, Bacic G. Decreased oxygen transfer capacity of erythrocytes as a cause of 5-fluorouracil related ischemia. Molecules. 2009;14(1):53-67.
21. Zhao T, Mao G, Zhang M, Zou Y, Feng W, Gu X *et al*. 2014. Enhanced antitumor and reduced toxicity effect of Schisanreae polysaccharide in 5-Fu treated Heps-bearing mice. International Journal of Biological Macromolecules 2014;63(4):114-118.
22. Mahmoud AM. Hematological alterations in diabetic rats-role of adipocytokines and effect of citrus flavonoids. Journal Experimental and Clinical Sciences Internatinal Journal. 2013;12(4):647-657.
23. Mao GH, Zhang ZH, Fei F, Ding YY, Zhang WJ, Chen H. *et al*. Effect of Grifolafrondosa polysaccharide on anti-tumor activity in combination with 5-FU in Heps-bearing mice. International Journal of Biological Macromolecules. 2019;121(7):930-935.
24. Karale S, Kamath JV. Protective effect of Naringinflavonoid on cisplatin induced hematotoxicity and hepatotoxicity in Wistar rats. Toxicology International. 2016;23(2):189-195.