Influence of Some Flavoring Substances on the Hematological Parameters of Rats

Duca Alina[1], Mihele Dana[2]

[1] Department of Clinical Laboratory and Alimentation Hygiene, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] "Prof. Dr. Scarlat Longhin" Clinical Hospital of Dermatovenerology, Bucharest, Romania

Introduction: Given the risk of side effects of flavoring substances used in the food industry, their cytotoxic effect on mouse fibroblast cell cultures and the risk of malignant degeneration, in this paper we observed eventual changes to hematological parameters of rats under the influence of flavorings: (±) - limonene p-methyl-1,8-diene (orange flavoring substance), (±) -3,7-dimethyl-6-octenal (lemon flavoring substance), ethyl format (rum flavoring substance) and 4-hydroxy-3-methoxybenzaldehyde (vanilla flavoring substance).
Materials and methods: The changes were followed in red blood cell counts, red cell indices, hemoglobin in erythrocytes, leukocytes, differential blood count, platelet count and platelet indices. For measurements we used white Wistar rats, weighing 240±10g. The flavoring substances were administered in doses of 25 mg/kg orally for 7 days compared to a control group treated with saline solution at a dose of 10 mg/kg orally. Blood was collected after 7 days of treatment in tubes with EDTA-Na 1 mg/2 ml blood and measurements were made with an automated hematology analyzer.
Results: After 7 days of treatment we found the orange and lemon flavoring substances determined a significant decrease in the number of erythrocytes, hematocrit and hemoglobin values, mean corpuscular volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, but the number of leukocytes and platelet count did not change significantly. The vanilla and rum flavoring produced no statistically significant changes in hematological parameters
Conclusions: The orange and lemon flavoring substances studied after statistical processing of experimental data by Student t-tests and ANOVA has modified significant the hematological parameters (number of erythrocytes, hemoglobin and hematocrit value).

Keywords: flavoring, hematological parameters

Received: 13 April 2012 / Accepted: 7 September 2012

Introduction

Currently it is estimated that over 3000 food additives are used, their consumption per inhabitant being of 2.6 kg annually. Given the risk of their side effects and even the risk of malignant degeneration using flavoring substances, there is a global tendency to use them in accordance with the principles of permitted substances after toxicological examinations. Depending on the obtained results the acceptable daily intake should be established [1,2]. Recent studies have demonstrated the cytotoxicity of flavoring substances on cell cultures of mice fibroblasts and the histopathological changes they induce in the liver and kidney tissue fragments of rats [3,4].

The aim of our study was to observe the eventual changes to hematological parameters of rats under the influence of several flavoring substances: (±) - limonene p-methyl-1,8-diene (orange flavoring substance), (±) -3,7-dimethyl-6-octenal (lemon flavoring substance), ethyl format (rum flavoring substance) and 4-hydroxy-3-methoxybenzaldehyde (vanilla flavoring substance).

Material and method

For measurements we used white, male Wistar rats, weighing 240±10 g. For two days before the experiment, the animals were left in the loft to adjust to their new habitat. The animals were kept in mobile cages at constant temperature and humidity conditions. Food was administered twice daily and water ad libitum in bottles. The experiment complies with European Council Directive 1986 (86/609 EEC) and the Ordinance 37 of February 2, 2002 the Romanian Government. The animals were divided into five groups of 10 rats each and were treated with flavoring substances, as follows: batch no 1 – control group (reference) treated with saline 1 ml/100 g, batch no 2 – treated with orange flavoring substance at a dose of 25 mg/kg orally, batch no 3 – treated with lemon flavoring substance at a dose of 25 mg/kg orally, batch no 4 – treated with vanilla flavoring substance at a dose of 25 mg/kg orally and batch no 5 – treated with rum flavoring substance at a dose of 25 mg/kg orally. After 7 days of treatment, two hours after the last administration, the animals were anesthetized with chloroform and slaughtered. Blood was collected into tubes with EDTA Na 1 mg/2 mL blood. Measurements were made with automated hematology analyzer VITROS 950 [5]. Statistical evaluation of the results was performed by Student t test and ANOVA using Microsoft Excel 2003.

Results

After 7 days of treatment we found that the orange and lemon flavoring substances induced a statistically significant decrease in the number of erythrocytes (RBC) ($p<0.001$), hematocrit value (HCT) ($p<0.001$), and hemoglobin values (HGB) ($p<0.001$), as presented in Figures 1–3. They also induced a statistically significant decrease in the me-

Correspondence to Alina Duca
Email: alina_duca_79@yahoo.com

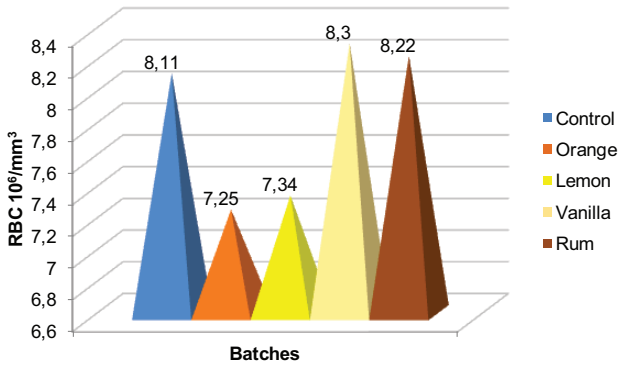

Fig. 1. Treatment effect on red blood count

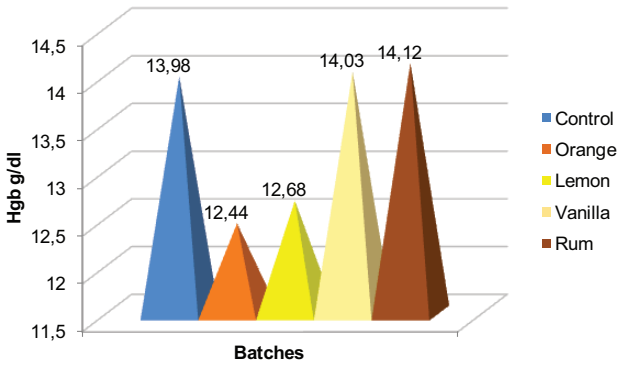

Fig. 2. Treatment effect on hemoglobin

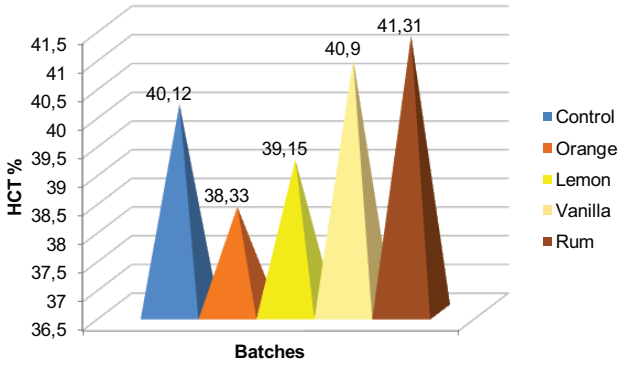

Fig. 3. Treatment effect on hematocrit

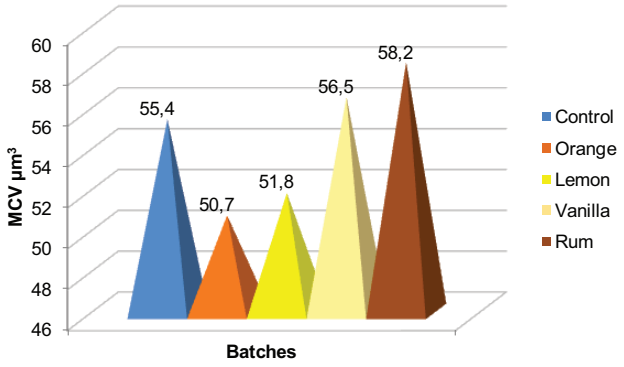

Fig. 4. Treatment effect on medium corpuscular volume

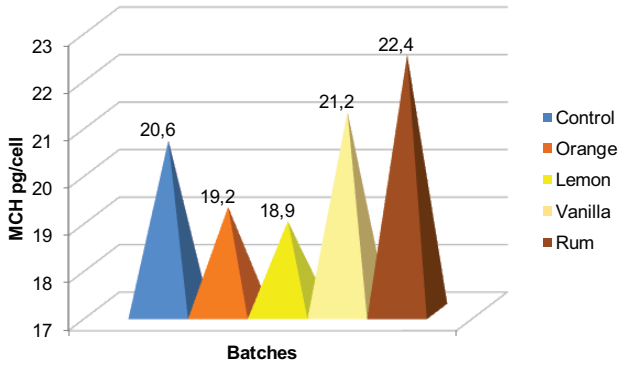

Fig. 5. Treatment effect on mean erythrocyte hemoglobin

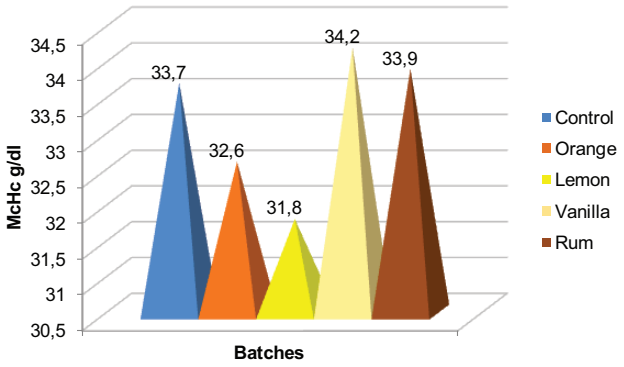

Fig. 6. Treatment effect on mean erythrocyte hemoglobin concentration

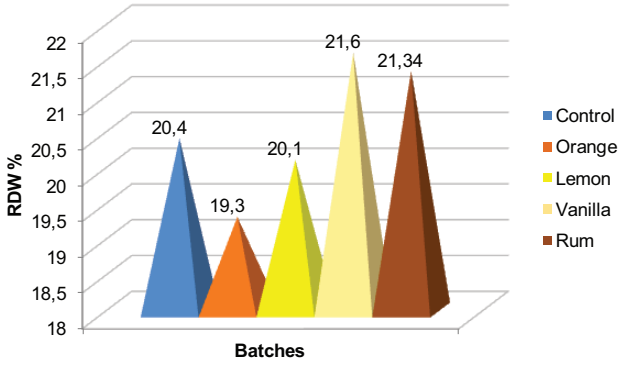

Fig. 7. Treatment effect on red cell distribution

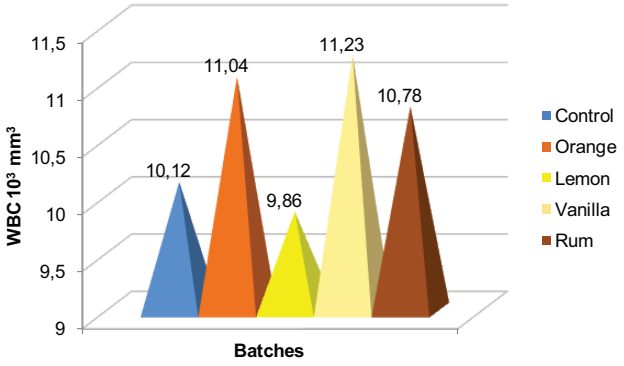

Fig. 8. Treatment effect on leukocyte count

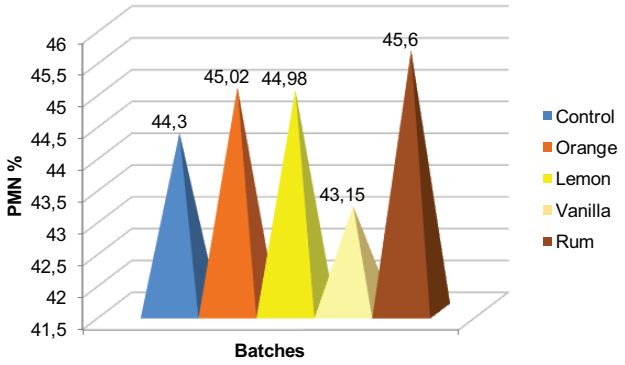

Fig. 9. Treatment effect on polymorphonuclear granulocytes

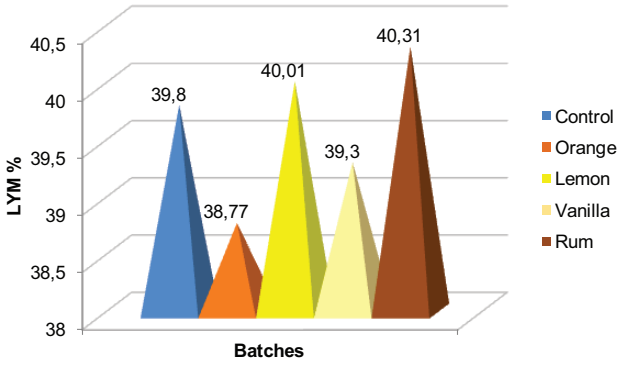

Fig. 10. Treatment effect on lymphocytes

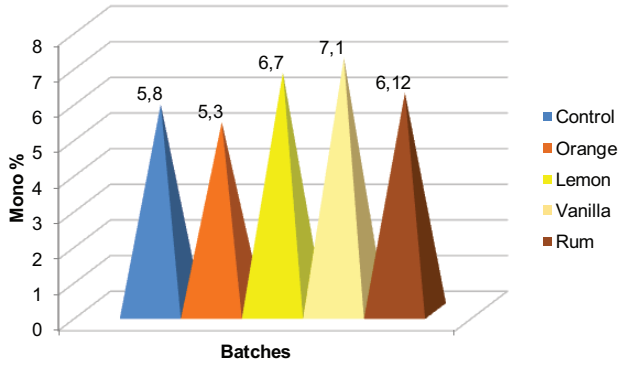

Fig. 11. Treatment effect on monocytes

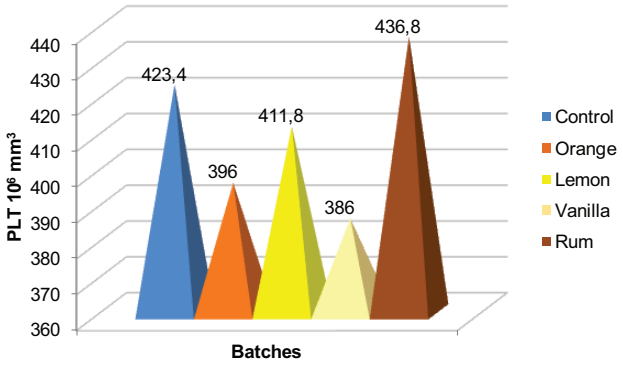

Fig. 12. Treatment effect on the number of platelets

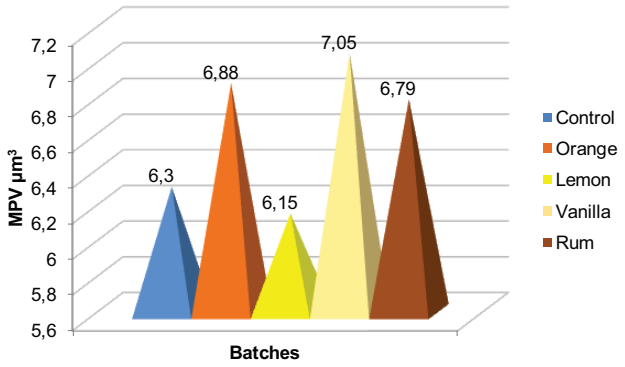

Fig. 13. Treatment effect on average platelet volume

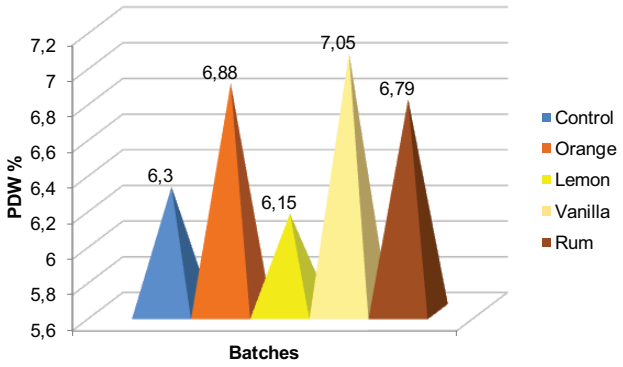

Fig. 14. Treatment effect on platelet distribution

dium corpuscular volume (MCV) ($p<0.001$) and mean erythrocyte hemoglobin (MCH) ($p<0.001$) (Figures 4, 5), and a statistically significant decrease in the mean erythrocyte hemoglobin concentration (McHc) ($p<0.001$). At the same time while red cell distribution (RDW) values did not change significantly (Figures 6 and 7).

There were no statistically significant changes in leukocyte count (WBC), polymorphonuclear granulocytes (PMN), lymphocytes (LYM), and monocytes (Mono) (Figures 8–11).

Also, no statistically significant changes were observed regarding the number of platelets (PLT), the average platelet volume (MPV) and platelet distribution (PDW) (Figures 12–14).

Discussion

The erythrocyte count is a test based on evaluating erythropoiesis. Low blood cell count causes anemia. In combination with hematocrit and hemoglobin concentration, erythrocyte count is useful in the detection and monitoring of anemia and erythrocytosis/polycythemia [6–10]. If the corpuscular volume is below average suggests iron deficiency anemia or thalassemia [11,12].

To our knowledge there are no other studies regarding the influence of these flavoring substances on the hematological parameters of rats. The changes on these parameters following the treatment with orange and lemon flavoring substances confirm the cytotoxicity of these substances on mouse fibroblast cell cultures and histo-

pathological changes occurred in rats following this treatment [3,4].

Conclusions

It is shown that the treatment of animals with orange and lemon flavoring substances reduces significantly the number of erythrocytes and hemoglobin, the hematocrit and hemoglobin concentration mean erythrocyte.

References

1. Cummings JH, Branch WJ. Postulated mechanisms whereby fiber may protect against large bowel cancer. In: Dietary Fiber in Health and Diseases, Vahouny & Kritchevky (eds), Plenum Press: New York, 1982; 313-325.
2. Nichols GA, Gomez-Caminero A. Weight changes following the initiation of new anti-hyperglycemic therapies. Diabetes Obese Metab. 2007;9:96-102.
3. Duca A, Manda G, Mihele D. The cytotoxic potential effect of some flavorings agents on a mouse fibroblast cell culture. Farmacia. 2012;60(1):58-63.
4. Mihele D, Duca A, Vrabie CD, Dogaru E. Influence of food additives on biochemical parameters in rats. Rev Med Chir Soc Med Nat Iasi. 2009; 113(2) Supplement 4:259-262.
5. Mihele D. Clinical Biochemistry. Laboratory Methods, ed. III. Medical Publishing House, Bucharest, 2007, 346.
6. DeMott W, Tilzer L. Hematology. In Laboratory Test Handbook, 3rd Edition, Hudson (Cleveland), 1994, 517-617.
7. Fischbach F. Blood Studies: Hematology and Coagulation; Appendix J: Effects of the Most Commonly Used Drugs on Frequently Ordered Laboratory Tests. Manual of Laboratory and Diagnostic Tests, 8th ed., Philadelphia, Lippincott Williams & Wilkins, 2009, 67-110, 1227-1247.
8. Glader B. Anemia: General Considerations. In Wintrobe's Clinical Hematology, Philadelphia ed., 2004, 948-975.
9. Means R. Erythrocytosis. In Wintrobe's Clinical Hematology. Philadelphia, 2004, 1495-1505.
10. Perkins S. Examination of the Blood and Bone Marrow. In Wintrobe's Clinical Hematology, Philadelphia, 2004, 3-21.
11. Thomas L. Hematology. In Thomas L, ed Clinical Laboratory Diagnostics, Frankfurt am Main: TH-Books, 1988, 463-547.
12. Wallach J. Hematologic Diseases. In Interpretation of Diagnostic Tests. Philadelphia, 1996, 293-316.