GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Macrocytic anemia induced via oral administration of toxic dose of acetaminophen

Adaobi Linda Okerulu and Osah Martins Onwuka *

Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Gregory University Uturu, Abia State, Nigeria.

GSC Biological and Pharmaceutical Sciences, 2022, 19(01), 178–184

Publication history: Received on 06March 2022; revised on 11 April 2022; accepted on 13 April 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.19.1.0141

Abstract

The abuse of Acetaminophen (over-the-counter drug) is rapidly increasing globally, thus inducing physiological alterations and toxicity. In this study, it was hypothesized that overdose of acetaminophen may induce certain type of anemia; hence the type of anemia induced by oral exposure to toxic dose of acetaminophen was evaluated. Fifteen (15) Wistar rats weighing approximately 160 g were grouped into three (3) groups; Group 1 (control) received 2ml of distilled water, Group 2 received 200 mg/kg of acetaminophen and Group 3 received 700 mg/kg of acetaminophen for 14 days. After administration, the rats were dissected on anesthesia (chloroform); blood was collected via cardiac puncture. The samples collected were assayed for Red Blood cell (RBC) Count, Hemoglobin concentration (HB), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC). The data was statistically analyzed using Graph Pad Prism (version 8). Statistical significance was considered at $P < 0.05$. Results showed that acetaminophen (200 mg/kg and 700 mg/kg) significantly increased MCV, MCH and decreased RBC and HB in a dose dependent manner with no significant effect on MCHC when compared to group 1 (control) ($P<0.05$). In conclusion, Acetaminophen (700 mg/kg) decreases RBC, HB and increases MCV, MCH and MCHC which suggests that toxic dose of acetaminophen can cause macrocytic anemia in Wistar rats.

Keywords: Acetaminophen; Anemia; Toxic dose; Macrocytic anemia; Red blood cell; Over-the-counter drug

1. Introduction

Acetaminophen despite having minor disadvantageous consequences when taken at curative doses, can be used to relieve pain and reduce fever [1]. It has been proven all over the world as one of the most available over-the counter drug [2]. The abuse of this drug could lead to suicide since it is common and easily available [3]. Its toxicity could cause adverse effects on liver, kidney, red blood cells. [4, 5]

Studies have suggested that acetaminophen causes depletion of erythrocytes by causing alteration of red blood cell counts, hemoglobin and hematocrit or PCV [6]. In this study, we hypothesize that acetaminophen may cause a certain type of anemia, hence the type of anemia found in toxic dose of acetaminophen was evaluated. Related physiological parameters such as; Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume(MCV), Mean Corpuscular Hemoglobin Concentration(MCHC), Hemoglobin(HB) concentration, Red blood cell count (RBC) were evaluated.

The various parameters aids in interpretation of various types of anemia; Iron deficiency anemia and macrocytic anemia are as a result of low and high MCH figures respectively [7]. MCV determines the average red cell size whether it is microcytic as a result of low MCV, normocytic as a result of normal MCV and macrocytic as a result of high MCV [8]. Iron deficiency anemia, anemia of chronic disease, macrocytic anemia, and hemolytic anemia is as a result of low and high

* Corresponding author: Osah Martins Onwuka; Email: osahmartinz@gmail.com
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical and Health Sciences, Gregory University Uturu, Abia State, Nigeria.

MCHC figures respectively [9]. Iron deficiency anemia, kidney disease, macrocytic anemia, lung disease result from low hemoglobin concentration, while burns result from high hemoglobin concentration [10]. Anemia and Polycythemia Vera are as a result of low RBC and high RBC respectively [11].

2. Material and methods

2.1. Ethical approval

All procedures performed in this research were in accordance with the ethical standards of the institution Gregory University Uturu, Abia State where the study was conducted. All rules applying to animal safety and care were observed, all applicable international, national and institutional guidelines for the care and use of animals were followed.

2.2. Experimental design

Fifteen Wistar rats weighing about 150-200 g were obtained from the animal farm of the College of Medicine, Gregory University Uturu, Abia State. The animals were acclimatized for two weeks with standard laboratory conditions. The animals were housed in clean well-ventilated, standard wire mesh cages which were cleaned daily. They were also fed with normal rat chow and clean tap water.

2.3. Experimental procedure

15 rats were grouped into 3 groups of 5 rats each

- Group 1: Control group, received 2ml of distilled water orally for 14 days.
- Group 2: Acetaminophen 200 mg/kg daily for 14 days.
- Group 3: Acetaminophen 700 mg/kg daily for 14 days.

2.4. Experimental drug and reagents

Acetaminophen (500 mg of Emzor Paracetamol) and other reagents used in this experiment were purchased from a pharmacy store at Uturu Abia State.

2.5. Collection of blood samples

After administration, the animals were anaesthetized with chloroform. Blood was collected via cardiac puncture using sterile syringes with needles and put in Ethylene Diamine Tetra-acetic Acid (EDTA) tubes for the measurement of RBC, HB, MCV, MCH, and MCHC.

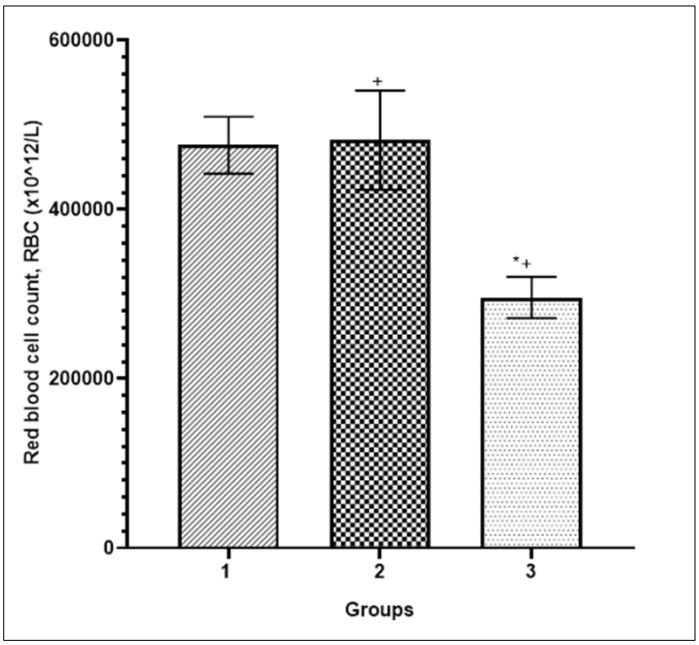
2.6. Determination of Hematological parameters

Hematological parameters were measured using hematological analyzer as described by [12]. Data on RBC, HB, MCV, MCH, and MCHC were obtained.

2.7. Statistical analysis

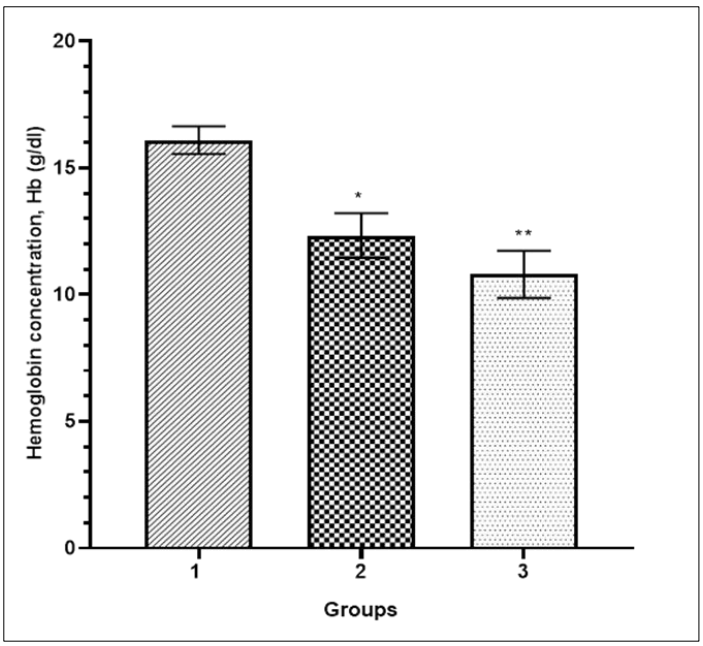
Graph Pad Prism (version 8) was used for data analysis. All data were expressed as mean ± SEM (standard error of mean). One-way analysis of variance (ANOVA) was used to determine the difference between the means of various groups. Value of $P < 0.05$ was considered significant.

3. Results



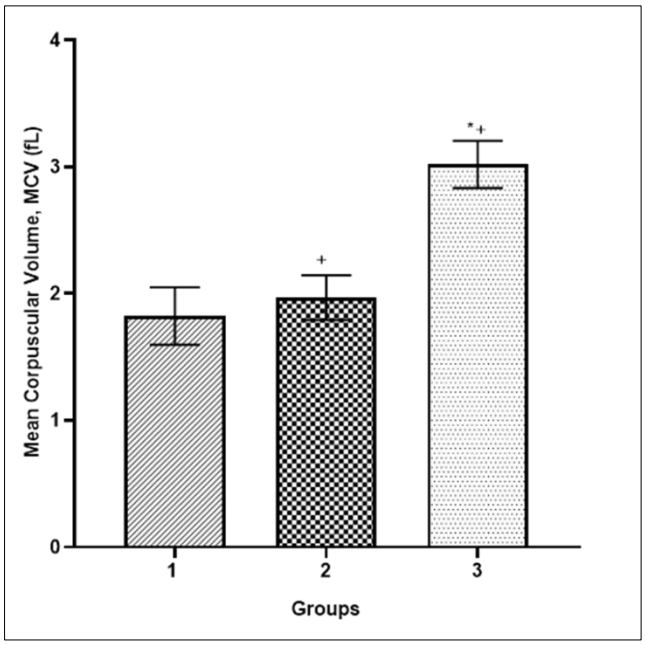
RBC (x10/L) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05). + indicates values that are significantly different on comparison between Group 2 and 3. Group 1= control (2ml of distilled water); Group 2 = 200 mg/kg of Acetaminophen; Group 3 = 700 mg/kg of Acetaminophen

Figure 1 Red blood cell count, (RBC) in all experimental groups administered Acetaminophen



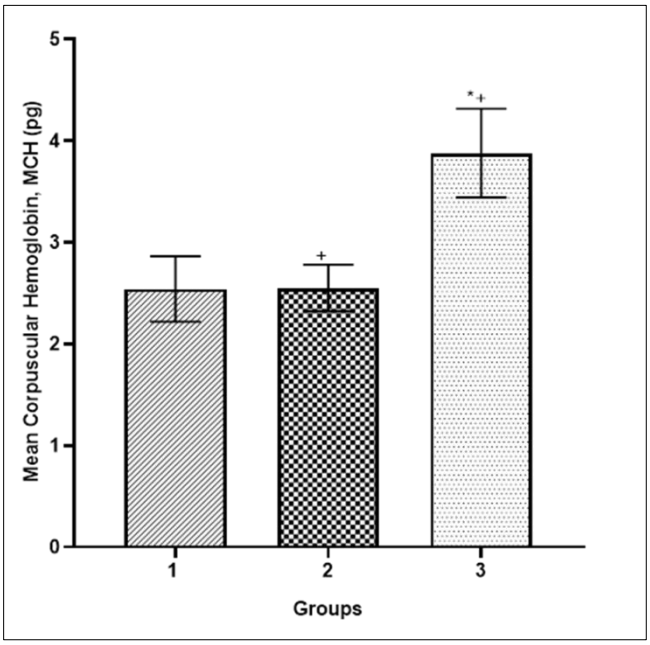
Hemoglobin Concentration, Hb (g/dl) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05, **P<0.01). + indicates values that are significantly different on comparison between Group 2 and 3. Group 1= control (2ml of distilled water); Group 2 = 200 mg/kg of Acetaminophen; Group 3 = 700 mg/kg of Acetaminophen

Figure 2 Hemoglobin concentration, hb (g/dl) in all experimental groups (acetaminophen treated and non-treated)



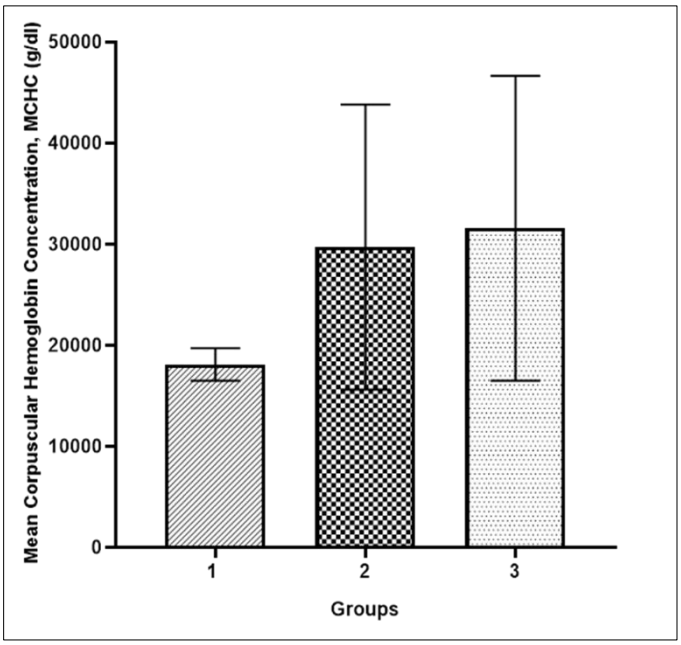
Mean Corpuscular Volume (fL) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05). + indicates values that are significantly different on comparison between Group 2 and 3. Group 1= control (2ml of distilled water); Group 2 = 200 mg/kg of Acetaminophen; Group 3 = 700 mg/kg of Acetaminophen

Figure 3 Mean Corpuscular Volume, (MCV) in all experimental groups administered Acetaminophen



Mean Corpuscular Hemoglobin (pg) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05). + indicates values that are significantly different on comparison between Group 2 and 3. Group 1= control (2ml of distilled water); Group 2 = 200 mg/kg of Acetaminophen; Group 3 = 700 mg/kg of Acetaminophen

Figure 4 Mean Corpuscular Hemoglobin, (MCH) in all experimental groups administered Acetaminophen



Mean Corpuscular Hemoglobin concentration (g/dl) in all groups; Values are Mean ± SEM. * indicate values that are significantly different from control (*P < 0.05). + indicates values that are significantly different on comparison between Group 2 and 3. Group 1= control (2ml of distilled water); Group 2 = 200 mg/kg of Acetaminophen; Group 3 = 700 mg/kg of Acetaminophen.

Figure 5 Mean Corpuscular Hemoglobin concentrations, (MCHC) in all experimental groups administered Acetaminophen

4. Discussion

Acetaminophen (paracetamol), benefits include its use in the treatment of mild pain, such as headache and pain in joints and muscles, and to reduce fever. Acetaminophen is the major metabolite of acetanilide and phenacetins, which were commonly, used as drugs for their analgesic (pain-relieving) effects. Acetaminophen is among the common over-the-counter drugs; thus it is easily abused [13-15].

Toxic dose of acetaminophen has been reported to have adverse effects such as hepatotoxicity, nephrotoxicity, [5] loss of appetite, nausea, vomiting etc. Toxic dose of acetaminophen has been shown to affect red blood cell and induce hemolytic anemia, hematogenic malignancies [16, 17].

Red blood cell (RBC) indices are individual components of a routine blood test called the complete blood count (CBC). It measures the quantity and physical characteristics of different types of cells found in the blood. RBCs are normally all the same color, size, and shape. However, certain conditions can cause variations that impair their ability to function properly which is also expressed in its physical characteristics. (18-20)

The RBC indices measure the size, shape, and physical characteristics of the RBCs. Medical doctors can use RBC indices to help diagnose the cause of anemia. Anemia is a common blood disorder in which you have too few, misshapen, or poorly functional RBCs. Thus, RBC indices, RBC, HB are used to diagnose different types of anemia. Low RBC count or abnormal RBC indices indicates some form of anemia. Anemia can occur if: too few RBCs are created (aplastic anemia); RBCs are destroyed prematurely (hemolytic anemia) and a significant blood loss occurs (hemorrhage). [20, 21]

In this study, evidence of macrocytic anemia induced by toxic dose of acetaminophen was evaluated. The result suggested that toxic dose of acetaminophen could induce anemia, since 700 mg/kg of acetaminophen significantly decreased the Red blood cell counts, Hemoglobin concentration in the Wistar rats compared to control ($P<0.05$) (Figure 1 and 2). This is because reduction in red blood cell count and hemoglobin concentration has been known to be a pointer to anemia [22].

Further result suggested that there is an alteration in the red blood cell indices which is also an indicator for the type of anemia. 700 mg/kg of acetaminophen significantly increased MCV (Figure 3), MCH (Figure 4) and showed non-significant increase in MCHC (Figure 5) compared to control ($P<0.05$). This suggests that toxic dose of acetaminophen can cause macrocytic anemia since when MCV is higher than normal indicates that red blood cells are larger than normal

and it is referred to as macrocytic anemia. While MCV will be lower than normal when red blood cells are too small and the condition is called microcytic anemia [23, 24].

High MCH scores are commonly a sign of macrocytic anemia. This condition occurs when the blood cells are too big [25]. Thus the increased MCH (Figure 4) caused by 700 mg/kg of acetaminophen was as a result of macrocytic anemia as was seen in the MCV (Figure 3).

This study showed a non-significant increase in MCHC (figure 5) which suggest that 14 days' administration of 700 mg/kg may not affect MCHC. High MCHC means that the relative hemoglobin concentration per red blood cell is high. Low MCHC means that the relative hemoglobin concentration per red blood cell is low [26].

Conditions that can cause low MCHC also result to low MCV and MCH and vice versa. Anemia in which both MCV and MCHC are low are called microcytic, hypochromic anemia. While when both MCV and MCHC are high is called macrocytic Anemia [24, 25, 26].

5. Conclusion

In conclusion, acetaminophen (700 mg/kg) decreased RBC count, Hemoglobin concentration and increased MCV, MCH and MCHC which suggested that toxic dose of acetaminophen can cause macrocytic anemia in Wistar rats.

Compliance with ethical standards

Acknowledgments

I am grateful to Almighty God for giving me the inspiration, grace and strength to carry out this work. I sincerely appreciate Department of Human Physiology, Gregory University, Uturu, Abia State, Nigeria for giving me the privilege of using their facilities for this study. My gratitude also goes to my co-author, family, friends, colleagues and others who one way or the other contributed to this study I appreciate all of you. Thanks!

Disclosure of conflict of interest

There was no conflict by the authors.

Statement of ethical approval

All procedures performed in this research were in accordance with the ethical standards of the institution Gregory University Uturu, Abia State where the study was conducted. All rules applying to animal safety and care were observed, all applicable international, national and institutional guidelines for the care and use of animals were followed.

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