Vitamin-C Treated Haematology Assessment of Swiss Albino Mice (Mus musculus)

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Received: 11.07.2020; Revised: 27.08.2020; Accepted: 30.08.2020; Published: 1.09.2020

Abstract: Vitamin C is an essential dietary supplement that plays a vital role in battling health conditions. The antioxidant has positive effects on the functioning of the body. The purpose of this study is to investigate the outcome of varying concentrations of Vitamin C on the hematological parameters of Swiss Albino mice. Four albino mice were separated and accommodated in similar caging conditions. Three of them were orally administered with 250mg/kg, 500mg/kg, and 1000mg/kg Vitamin C powder, along with their diet. The fourth one was kept as control, in order to provide a standard value for future comparison. After 30 days of administration of the Vitamin C, the red blood cell count, the white blood cell count, the hemoglobin concentration, the platelet cell count, and the packed cell volume of the blood samples were tested.

Keywords: vitamin C; haematology; albino mice.

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1. Introduction

Vitamin C is considered to be in all products with an evident ascorbic acid constituent. It doesn’t only involve the acid but also its isomers as they are interchangeable [1]. Vitamin C is said to be an excellent antioxidant with its eminent take in electron transfer, immune response, cell metabolism, enzymatic reactions [2], and oxidation-reduction balance in the body. The vitamin has a vast therapeutic effect, it has been tested for disease prevention, and its influence has been observed for the treatment of health conditions as extreme as cancer [3]. Prevention of inactivation of other antioxidants is also checked upon by Vitamin C [4]. Promotion of absorption of iron in the small intestine[5] and oxidative damage diminishing in vascular walls [6] and are also important reasons why the compound has been the subject of the study. Favorable dosing is important for the proper mediation of studies that involve Vitamin C [7]. A mathematical model had also been devised to express the consistent effectiveness of Vitamin C when used for treatment for medical purposes [8]. In cases of infection, the Vitamin C requirement of the patient’s body increases; hence external administration is also encouraged [9]. The experiment uses Swiss Albino mice as the animal model because of its evolutionary connectivity and similar physiology to that of human beings [10]. The genetic correspondence between the two species has been illustrated by various genomic studies [11]. Mice have been used as the experimental subject for disease and research, going as far as gnotobiology to as familiar as physiology[12]. Vitamin C and other antioxidants have been tested for their possible effect on the lifespan of the model organisms, thus making it an excellent choice for this hematological study [13]. Since mice models are used by
pharmaceutical and drug research laboratories [14] to determine the effect of their production, the mice with prolonged health can be used to find the long term effect of any drug and their chronic side effects, if any. The hematological analysis also helps in the establishment of the physical health status [15-18]. The comparison to the control group is essential to ascertain if the animal is responding differently to the feed being provided. Vitamin C has also been researched for its efficiency as a possible treatment for COVID-19, because of its inexpensiveness and positive effects on the treatment of infections, in critically ill or ventilated patients [19]. The study aims to classify if the addition of vitamin C to the diet of the mice will indicate better health in it.

2. Materials and Methods

Forty-four-week old adult Swiss Albino mice between the weights of 44-55g were procured from the Animal House at the Department of Pharmacology, Centurion University of Technology and Management, Odisha, India. Approval of Institutional Animal Ethics Committee (IAEC) Registration No-2024/PO/Rc/S/18/CPCSEA dated 07/08/2018. The experiment was conducted at the laboratory of the Department of Zoology at the university. The forty mice were randomly divided into four groups for the experiment work. The animals were housed for 60 days. They were kept in conventional laboratory cages with the ball bearing water bottle to place water at their disposal for consumption on-demand [20]. They were fed three times a day with a mixture of various flower seeds, lentils, vegetables, and fruits. They were all fed similar diets, with the only difference being the Vitamin C addition to their diet at different doses (250mg/kg, 500mg/kg, and 1000mg/kg). One group was fed with no vitamin C and was to become the control group. Another group was fed with 250mg/kg dose of Vitamin C with no other addition to their diet. The process was similar for the 500mg/kg and the 1000mg/kg dose as well.

2.1. Hematological analysis.

After the commencement of 60 days of dosing, the blood samples were extracted from the animal, from the orbital sinus of the mice [21-22] with the help of a capillary tube, into an EDTA vial. The total red blood cell count and the total white blood cell count were done with the help of a hemocytometer. The differential counts of lymphocytes were done by staining it with Giemsa’s stain. The hemoglobin was calculated with the help of Sahli’s haemoglobinometer, and the Packed Cell Volume was determined by the Wintrobe tube.

2.2. Statistical analysis.

The Mean Corpuscular Volume, The Mean Corpuscular Haemoglobin, and the Mean Corpuscular Haemoglobin Concentration were calculated from their respective formulas. The data was presented after statistical analysis in the form of Mean ± Standard Deviation, which was evaluated using MS Excel.

3. Results and Discussion

The results obtained after carrying out the experiment had the above-mentioned parameters in them. The RBC count for the control group was determined to be 4.03±0.208 (Table 1). The second group for the experiment, which had 250mg/kg of the dosage, has a little elevated RBC count of 4.50±0.293 (Table 1). The third group, with the 500mg/kg dose had its
RBC count further elevated to be 4.680±0.190 (Table 1). Lastly, the group with the highest dosage, 1000mg/kg, also followed suit with the elevation and had a count of 4.84±0.243 (Table 1), which is higher than all the other groups tested. The WBC count was next in line, and the lowest count was observed in the highest dosage, and the observed value was 7.76±0.44 (Table 1). The next was the level of the 500mg/kg dosage, which was higher than the level of the 1000mg/kg dosage and was 7.80±0.41 (Table 1). The 250mg/kg dosage had the WBC count a bit higher, and that was 8.06±0.39 (Table 1). The highest level was observed in the control group and its was 8.16±0.39 (Table 1).

The Haemoglobin count or Hb in the control group was observed to be 12.52±0.7g/dl (Table 1). The Hb level in the treatment group of 250mg/kg was higher than that of the control group, and it was 12.87±0.41g/dl (Table 1). The 500mg/kg dosage had the Hb count a bit higher, and the value was 13.14±0.57g/dl (Table 1). The highest level was observed in the group with 1000mg/kg dose of Vitamin C, and it was 13.40±0.47g/dl (Table 1).

The differential count of the blood samples had been done, and the values for the control group had Neutrophils at 17±3% (Table 1), Eosinophils at 0.2±0.1% (Table 1), 0% Basophils (Table 1), Lymphocytes at 80±3% (Table 1) and no Monocytes. The differential count values for the 250mg/kg group were somewhat the same with Neutrophils at 17±3% (Table 1), Eosinophils at 0.1±0.1% (Table 1), 0% Basophils (Table 1), Lymphocytes at 81±3% (Table 1), and no Monocytes. A slight elevation was observed in the values of the 500mg/kg group the Neutrophils were at 19±3% (Table 1), no Eosinophils, 0 Basophils (Table 1), Lymphocytes at 83±3% (Table 1), and no Monocytes. The group with 1000mg/kg treatment has the highest values and the Neutrophils at 20±3% (Table 1), Eosinophils at 0% (Table 1), 0 Basophils, Lymphocytes at 83±3% (Table 1), and no Monocytes. The Packed Cell Volume of the test subjects differed as well. The lowest observation was taken in the control group with the 50.9±1.6% PCV (Table 1). The volume in the second group with 250mg/kg dosage was 51.6±1.6% (Table 1). The third group had the PCV at 53.0±1.5% (Table 1), and the group with the highest dosage had the highest level, and it was 53.3±1.6% (Table 1). The Mean Cell Volume of the test subjects differed as well. The highest observation was taken in the control group with the 128±4.6% MCV. The volume in the second group with 250mg/kg dosage was 117±5.0fl (Table 1). The third group had the MCV at 110±3.7fl (Table 1), and the group with the highest dosage had the highest level, and it was 114±2.3fl (Table 1). The Mean Corpuscular Haemoglobin was next in line.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Mean±SD)</th>
<th>250mg/kg (Mean±SD)</th>
<th>500mg/kg (Mean±SD)</th>
<th>1000mg/kg (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC(×10^6/mm³)</td>
<td>4.03±0.208</td>
<td>4.50±0.293</td>
<td>4.680±0.190</td>
<td>4.84±0.243</td>
</tr>
<tr>
<td>WBC(×10³/mm³)</td>
<td>8.16±0.39</td>
<td>8.06±0.39</td>
<td>7.80±0.41</td>
<td>7.76±0.44</td>
</tr>
<tr>
<td>Hb(g/dl)</td>
<td>12.52±0.7</td>
<td>12.87±0.41</td>
<td>13.14±0.57</td>
<td>13.40±0.47</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>17±3</td>
<td>17±3</td>
<td>19±3</td>
<td>20±3</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.2±0.1</td>
<td>0.1±0.1</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>80±3</td>
<td>81±3</td>
<td>83±3</td>
<td>83±3</td>
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<tr>
<td>Monocyte (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>50.9±1.6</td>
<td>51.6±1.6</td>
<td>53.0±1.5</td>
<td>53.3±1.6</td>
</tr>
<tr>
<td>MCV(fl)</td>
<td>128±4.6</td>
<td>117±5.0</td>
<td>110±3.7</td>
<td>114±2.3</td>
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<tr>
<td>MCH(pg)</td>
<td>28.1±1.0</td>
<td>27.5±1.44</td>
<td>28.7±0.4</td>
<td>32.25±0.77</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>24.1±0.76</td>
<td>24.73±0.65</td>
<td>25.13±0.37</td>
<td>25.33±0.57</td>
</tr>
</tbody>
</table>

Abbreviations: RBC- Red Blood Cell, WBC- White Blood Cell, Hb- Haemoglobin, PCV- Packed cell Volume, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration.

Table 1. Hematological parameters and their mean values ± standard deviation.

https://doi.org/10.33263/BRIAC112.90449050
https://biointerfaceresearch.com/
The observation of the control group was 28.11±1.0/pg (Table 1). The volume in the second group with 250mg/kg dosage was 27.51±1.44pg (Table 1). The third group had the MCH at 28.71±0.4pg (Table 1), and the group with the highest dosage had the highest level, and it was 32.25±0.77pg (Table 1).

Figure 1. Graph showing the different parameters (RBC, WBC, and DLC count) in Mean±SD.

The control group of the experiment displayed results that resembled the observations made by certain researchers in the past. According to work done [23] to obtain the reference values for mice hematology, the Haemoglobin value (12.52±0.7) of the present study was less than the one obtained in their experiment (13.63±1.4), the similar could be said about the Neutrophils percentage, which was 22.64±6.4. The Haemoglobin value was nearly similar to the control mice's hemoglobin value in research conducted [24]. The lymphocyte percentage was 75.17±6.6, which was in agreement with the values obtained in the control group of the present study. The MCH of the mentioned research work was 26.53±4.8, which was similar to that obtained in the present work. In an experiment by [25], the lymphocyte count of the control mice was 78.7±6.7, nearly similar to the control of the present study, and the same goes for the Haemoglobin control value, which was also nearly similar to the control value of the mentioned experiment, 11.5±0.40. The RBC value elevated along with the Vitamin C treatment, and an identical course was followed by the hemoglobin value, which also elevated (Figure 1) with the higher dose. The WBC experienced the opposite effect; the WBC values were decreasing (Figure 1) with the administration of the Vitamin C. In the differential count of WBC, the Neutrophils percentage was similar (Table 1) in the control and the 250mg/kg and
then it increased with the treatment. The eosinophil percentage was negligibly low (Table 1) and in control, and there were no eosinophils observed on the treated mice. The lymphocyte had a small elevation (Figure 1) with the treatment values. The basophil count was not available, and the monocyte count was also zero (Table 1), indicating the absence of those cells in the blood of the test subjects. This was in agreement with the findings of [26]. The PCV value of the control group was 50.9±1.6 (Table 1), which is nearly similar to the control PCV value (49.32±3.21) of the work done, and the MCHC value was found to be in ascending with the increase in the concentration of the dosage (Figure 2), and the control value was close to the control value in the research work of [27]. The PCV values elevated as well, which is in agreement with the work done, and the MCH followed an increasing pattern, as in the case of [28] research work with a small dip in the value of the 250mg/kg concentration (Figure 2) where there was a hike in the PVC value (Figure 2) after treatment with Vitamin C, in comparison to the control. The MCV followed a decreasing pattern, with a small dip in the value of the 500mg/kg concentration. The decline in the MCV value (Figure 2) could likely be because of the rise in the RBC count [29] on Vitamin C treatment.

4. Conclusions

The treatment of the mice with Vitamin C indicates an overall positive effect on the various parameters taken into consideration, so it is evident that the administration of Vitamin C can be helpful for research on toxicity in drugs and to study the long term impact of pharmaceuticals. The study also indicates that a higher Vitamin C intake could have beneficial results at a hematological level.

Funding

This research received no external funding.

Acknowledgments

Both the authors acknowledged the Head, Department of Pharmacology, Centurion University of Technology and Management, Odisha, India, for the experiment by using the animal house.

Conflicts of Interest

The authors declare no conflict of interest.

References


