EFFECT OF CIMETIDINE ON SOME HEMATOLOGICAL INDICES OF WISTAR RATS: MODULATORY ROLE OF VITAMIN C

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ABSTRACT

Introduction: Cimetidine is a drug used in the treatment of dyspepsia, a clinical condition with widespread distribution. Many studies have reported cimetidine to cause many unwanted effects. However, in most of those studies cimetidine was administered at higher than therapeutic doses.

Aim: The aim of this study was, therefore, to evaluate the effect of chronic cimetidine treatment at therapeutic dose on some hematological indices and the possible modulatory role of vitamin C on any such effect.

Experimental design and Animal grouping: Forty adult male Wistar rats were divided into four groups (n = 10) and treated orally for 60 days with distilled water (control); cimetidine (30 mg kg^{-1}); cimetidine (30 mg kg^{-1}) + vitamin C (25 mg kg^{-1}) and cimetidine (30 mg kg^{-1}) + vitamin C (50 mg kg^{-1}). At the end of the study blood was collected for analysis.

Results: Total white blood cell (WBC) count (5.99 ± 0.20 x $10^3/mm^3$) of the cimetidine only-treated group was significantly lower than that of the control (7.95 ± 0.29 x $10^3/mm^3$).However, the valuesof red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration (MCHC), and color index of the experimental groups were not significantly different from that of the control. Treatment with vitamin C modulated the cimetidine-induced decrease in total WBC count.

Conclusion: It was concluded that chronic cimetidine administration at therapeutic dose caused a significant decrease in WBC count and this was modulated by vitamin C.

Key words: Cimetidine, WBC count, RBC count, hemoglobin and vitamin C.

INTRODUCTION

Cimetidine, the prototype histamine H_2-receptor antagonist is among the most commonly prescribed drugs in the world (Al-Nailey, 2010) but it is also available without prescription (Pino and Azer, 2020).Although antagonists of the histamine H_2-receptor block the actions of histamine at all H_2 receptors (Ronald and Ashley, 2003; Luangpirom and Komnont, 2011), in the parietal cells of the stomach it prevents the production of acid (Rang *et al*.2012). Thus, it promotes the healing of gastric and duodenal ulcers (Hamid *et al*.2010; Aprioku *et al*.2014), both of which are among the commonest causes of dyspepsia.

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The prevalence rates of dyspepsia are reported to be 30.4% in India (Shah *et al*., 2001), 32% in the U.S. (Shaib and El-Serag, 2004), 38-41% in the U.K. (Penston and Pounder, 1996; Moayyedi *et al*. 2000) and 45% in Nigeria (Ihezue *et al*.1996). Tijjani and Umar (2008) reported that it accounted for one third of the patient who were referred for upper gastrointestinal endoscopy in Kano, Nigeria. Also, it is documented by the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK, 1999)that about 60 million Americans experience dyspepsia at least once a month and about 15 million have it daily. These facts necessitate the widespread usage of cimetidine globally. Also, H_2 receptors are present inneutrophils, eosinophils, basophils, mast cells and lymphocytes (Hill *et al*. 1997; Maguire and Davenport, 2016). Cimetidine has been reported to have immune-modulatory properties due to its ability to reduce regulatory/suppressor T cell-mediated immunosuppression (Jafarzadeh *et al*. 2019).

Vitamin C is a water soluble vitamin that has been reported to play an important role in a number of physiological processes in the body. One of such function is that it facilitates the dietary absorption of iron(Lynch and Cook, 1980) and iron plays an important role in the formation of hemoglobin. A study by Finkelstein and colleagues(2011)reported that hemoglobin levels were positively correlated with plasma vitamin C levels. In addition, it is a potent antioxidant which has been reported to protect the immune cells against intracellular reactive oxygen species (ROS) production (Chambial *et al*. 2013).

Many studies have reported cimetidine to cause many unwanted effects. However, in most of those studies cimetidine was administered at higher than therapeutic doses. The aim of this study was, therefore, to evaluate the effect of chronic cimetidine treatment at therapeutic dose on some hematological indices and the possible modulatory role of vitamin C on any such effect. The difference may be due to the high doses of cimetidine used in those studies.

MATERIALS AND METHODS

Animals and Conditions

A total of forty adult male Wistar rats weighing 120± 10 grams, were used for the study. They were housed in spacious plastic cages in a well-ventilated room, and were allowed access to standard laboratory rat chow and tap water *ad libitum* throughout the duration of the study. Experimental protocols were in accordance with the guidelines for animal research, as stated in the Guide for the Care and Use of Laboratory Animals (National Research council of the National Academies, U.S.A. 2011).

Preparation of Administered Drugs

Five tablets (1000mg) of cimetidine (Gasrol, Sam Pharmaceuticals, Nigeria) were crushed into powder using pestle and mortar and dissolved in 100mls of distilled water to make a stock solution containing cimetidine at a concentration of 10 mg ml^{-1}. This was stored at room temperature, protected from light and used up within 3 days. Similarly, a stock solution containing vitamin C (Pure grade vitamin C powder, Bulk supplements, Nevada, U.S.A.) at a concentration of 10 mg ml^{-1} was prepared and stored.

Experimental Design and Grouping

Rats were randomly assigned into 4 groups of 10 rats each. Each animal was administered either drug or distilled water orally using metallic canula at 10:00am daily for 60 days because treatment with cimetidine is for 2 months and above even if symptoms of dyspepsia have subsided. Group 1 (Control)-received distilled water; group 2- Cimetidine at a therapeutic dose of 30 mg kg^{-1}; group 3-Cimetidine (30 mg kg^{-1})+ vitamin C (25 mg kg^{-1}); group 4-Cimetidine 30 mg kg^{-1} + vitamin C (50 mg kg^{-1}).

Animals Sacrifice and Samples Collection

At the end of the experiment each rat was anesthetized by chloroform inhalation in a gas chamber. The chest was then opened and blood samples aspirated by heart puncture using 10ml syringe. Blood collected was quickly discharged into EDTA and plain bottles for determination of hematological parameters.

Determination of Hematological parameters

The following hematological parameters: Red blood cell (RBC) count, White blood cells (WBC) count, packed cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and color index were determined.

WBC count was done as described by Udombon (2013). Blood was drawn upto the 0.5 mark on an WBC pipette and diluted with WBC diluting fluid (Turks solution) using a dilution factor of 1:20, it was then mixed by gently rolling the pipette in between the thumb and index finger. The pipette was then allowed to stay for 5 – 10 minutes for proper mixing. One drop of diluted blood was expelled to remove undiluted blood along the stem. The diluted blood was then introduced into the improved Neubauer counting chamber and the cells counted at the four corners of the top and bottom large squares using a microscope (Olympus Microscope, Japan) at a magnification of x 40. The number of cells counted was multiplied by 50.

Blood for RBC count was drawn to the 0.5 mark on an RBC pipette and diluted with RBC diluting fluid (Hayem`s solution) using a dilution factor of 1:200, it was then mixed by gently rolling the pipette in between the thumb and index finger. It was then allowed to stay for 5 – 10 minutes for proper mixing. One drop of diluted blood was expelled to remove undiluted blood along the stem. The diluted blood was then introduced into the improved Neubauer counting chamber and the cells counted in each of 80 small squares in a selected diagonal of the 5 large squares using a microscope (Olympus Microscope, Japan) at magnification x 40. The number of cells counted was multiplied by 10,000 (Udombon, 2013).

Blood was drawn into heparinised capillary tubes (John Poulten Limited, England), to determine PCV. The end of the tube where the blood was introduced was carefully sealed by using cristaseal. The sealed tubes were then placed in a microhaematocrit centrifuge (Hawksley, England) with the sealed end facing the edge of the centrifuge and the other open end facing the spindle (centre). The centrifuge was then covered tightly and the tubes spun for 5 minutes at 12,000 revolutions per minute after which, the tubes were removed and the PCV value was read using a haematocrit reader (Hawksley, England).

For Hb estimation,4ml of 0.04% ammonia solution was placed in a test tube. Blood was then drawn into a Hb pipette up to mark 40mm and then added to the ammonia solution and allowed to stand for 5 minutes in other to convert Hb into oxyhemoglobin. Another 4 ml of ammonia solution was added to make a total volume of 8ml. The Hb solution was then poured into a cuvette. The spectrophotometer (Bechman Coulter, Model BU520, Austria) was then standardized using ammonia solution as blank. Subsequently, the cuvette containing the Hb solution was placed in the spectrophotometer and the optical density was read at a wavelength of 520nm against a reagent blank. The final haemoglobin result was calculated using the following formula:

(Reading of test / Reading of standard) x Hemoglobin content per 100ml standard

MCV, MCH, MCHC and color index were calculated using the following formula (Udombon, 2013)

Mean corpuscular volume = (Packed cell volume / Red blood cell count) x 10

Mean corpuscular hemoglobin = (Hemoglobin in g/dl / Red blood cell count) x 10

Mean corpuscular hemoglobin concentration = (Hemoglobin in g/dl / PCV)x 100

Color index = Hemoglobin% / Red blood cell count %

Statistical Analysis

All data were presented as mean ± SEM and were analyzed using SPSS version 20.0 (SPSS Inc, Chicago, United States). One-way ANOVA and Tukey post-hoc tests were used to compare means. Values of $P \leq 0.05$ were considered significant.

RESULTS

Figure 1 shows the WBC and RBC count of rats treated with distilled water, cimetidine and vitamin C. Total WBC count of the animals in group 2 (5.99 ± 0.20 x 10^3/mm^3) was significantly lower compared to control group (7.95 ± 0.29 x 10^3/mm^3) (P = 0.001). This shows that cimetidine decreased total WBC count in the treated rats. Total WBC count of animals in group 3 (8.01 ± 0.19 x 10^3/mm^3) was similar to that of animals in control group (7.95 ± 0.29 x 10^3/mm^3) (P = 0.998), indicating that treatment with 25 mg kg^{-1} vitamin C has restored the decrease in WBC count caused by cimetidine. Total WBC count of the rats in group 4 (6.82 ± 0.18 x 10^3/mm^3) was significantly lower compared to that of control group (7.95 ± 0.29 x 10^3/mm^3) (P = 0.004), suggesting that treatment with 50 mg kg^{-1} vitamin C has not restored the decrease in total WBC count caused by cimetidine.

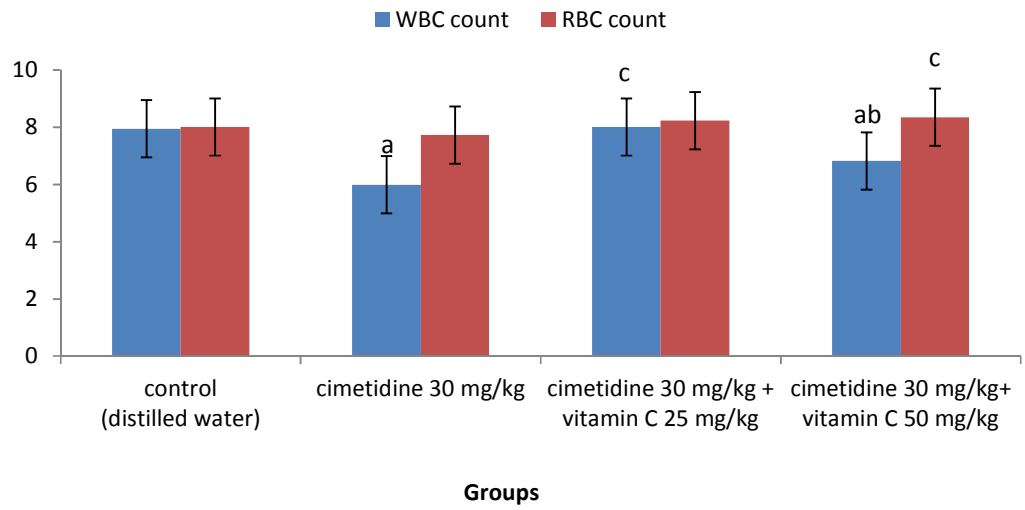

Figure 1: White blood cell count (x10^3/mm^3) and Red blood cell count (x10^6/mm^3) of rats following 60 days of treatments (Mean ± S.E.M., n=10)

[a] = significant compared to control group

[b] = significant compared to cimetidine 30 mg/kg + vitamin C 25mg/kg group

[c] = significant compared to cimetidine 30 mg/kg group

Furthermore, total WBC count of animals in group 4 (6.82 ± 0.18 x 10^3/mm^3) was significantly lower when compared to that of group 3 (8.01 ± 0.19 x 10^3/mm^3) (P = 0.003) indicating dose dependence of vitamin C effect, as treatment with 25 mg kg^{-1} vitamin C was more effective than 50 mg kg^{-1} vitamin C in reversing the decrease in total WBC count caused by cimetidine. Total WBC count of animals in group 3(8.01 ± 0.19 x 10^3/mm^3) was significantly higher compared to group 2 (5.99 ± 0.20 x 10^3/mm^3) (P = 0.001), indicating that 25 mg kg^{-1} vitamin C reversed the decrease in WBC count caused by cimetidine. There was no significant difference in total WBC count of animals in group 4 (6.82 ± 0.18 x 10^3/mm^3) compared to those in group 2 (5.99 ± 0.20 x 10^3/mm^3) (P = 0.055) indicating that 50 mg kg^{-1} vitamin C did not significantly modulate the decrease in WBC count caused by cimetidine.

RBC count of the animals in group 2 (7.73 ± 0.18 x 10^6/mm^3), group 3 (8.23 ± 0.18 x 10^6/mm^3) and group 4 (8.35 ± 0.08 x 10^6/mm^3) was not significantly different compared to that in control (8.01 ± 0.12 x 10^6/mm^3) (P = 0.540, P = 0.709 and P = 0.362), respectively.

This indicates that treatment with 30 mg kg^{-1} cimetidine, 25 mg kg^{-1} vitamin C and 50 mg kg^{-1} vitamin C did not significantly affect RBC count. The RBC count of animals in group 4 (8.35 ± 0.08 x $10^6/mm^3$) and group 3 (8.23 ± 0.18 x $10^6/mm^3$) was statistically the same (P = 0.936). This shows that there was no dose dependence of vitamin C effect on this parameter. There was no significant difference in RBC count of animals in group 3 (8.23 ± 0.18 x $10^6/mm^3$) when compared to group 2 (7.73 ± 0.18 x $10^6/mm^3$) (P = 0.092). RBC count was significantly increased in group 4 (8.35 ± 0.08 x $10^6/mm^3$)when compared to group 2 (7.73 ± 0.18 x $10^6/mm^3$) (P = 0.024).

Figure 2 shows the PCV and Hb concentration of the animals following treatments. There was no significant difference between the groups in PCV (P = 0.170) andhemoglobin concentration (P = 0.289). This indicates that treatment with cimetidine did not have significant effect on these parameters.

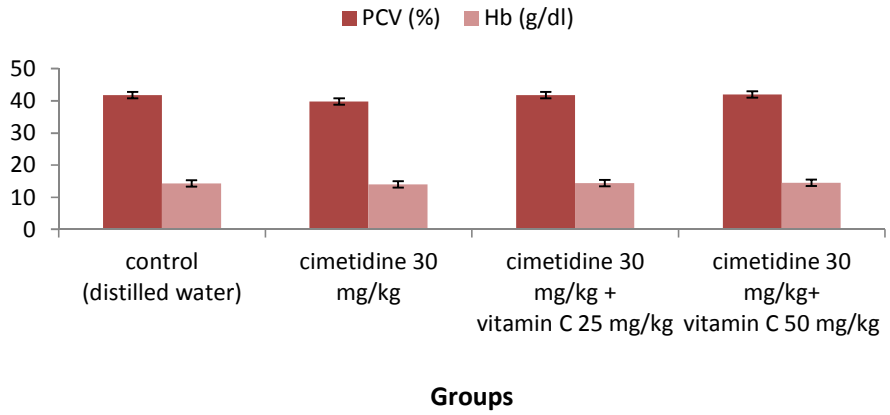

Figure 2: PCV and Hb concentration of rats following 60 days of treatments (Mean ± S.E.M., n=10)

Figure 3 shows the MCV, MCH, MCHC and C.I of the animals following treatments. There was no significant difference between the groups in MCV (P = 0.790), MCH (P = 0.512), MCHC (P = 0.841) and color index (P = 0.518). This indicates that treatment with cimetidine did not have significant effect on these parameters.

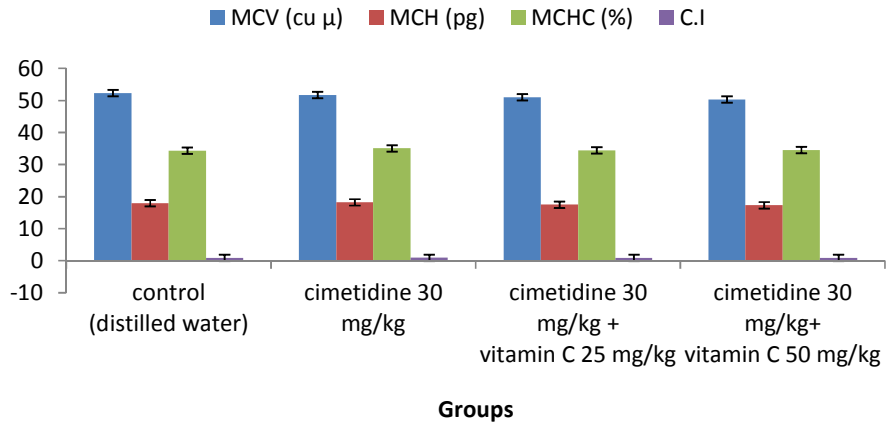

Figure 3: MCV, MCH, MCHC and Color Index (C.I) of rats following 60 days of treatment (Mean ± S.E.M., n=10)

DISCUSSION

Cimetidine has caused a significant reduction in total WBC count of the treated rats. The mechanism of this effect may be due to blockage of H_2 - receptors present in pluripotent hematopoietic stem cells of bone marrow (Bryon, 1977;Bryon, 1980) resulting in inhibition of histamine – induced initiation of DNA synthesis of cell cycle (specifically G_0 to S phase) in bone marrow stem cells (Posnett *et al*. 1979). This detrimental effect may predispose the animals to increased risk of infections. The finding of this study is in agreement with that of Byron (1977), Al – Kawas *et al*. (1979), de Galocsy and de Strihou (1979), Chang and Morrison (1979), Byron (1980) as well as Strom *et al*. (1995). This finding however, disagreed with that of Hast *et al*. (1989) who reported an increase in WBC count suggesting that cimetidine has a stimulatory action on the immune system by possibly blocking receptors on subsets of T–lymphocytes and inhibiting histamine–induced immune suppression which is possibly why it has been used to successfully restore immune functions in patients with malignant disorders, hypogamma-globulinemia and AIDS–related complexes. Asakage *et al*. (2005) reported leukocytosis and together with Lefranc *et al*. (2006) described cimetidine as a modulator of cellular immunity due to increase in neutrophils count as well as $CD3^+$ T–lymphocytes and subsets of $CD4^+$ cells among them following use of the drug.

Co-treatment with vitamin C at 25 mg kg^1 modulated the cimetidine-induced leucopenia. This effect may be due to both membrane-stabilizing and antioxidant properties of vitamin C (Jariwalla and Harakeh, 1996). During antioxidant reaction, α-tocopherol is converted to α-tocopherol radical by donating labile hydrogen to a lipid or lipid peroxyl radical. Vitamin C together with vitamin E helps to regenerate α-tocopherol (Young and Woodside, 2001) from α-tocopherol radicals in membranes and lipoproteins. α-tocopherol is the most active form of vitamin E in humans, a powerful biological antioxidant considered to be the major membrane bound antioxidant employed by the cell (Flora, 2002). It was reported to preserve cell membrane function including ion transport, membrane fluidity, prevent the release of Fe^{2+} and Mg^{2+} from their binding proteins, decrease the rate of lipid peroxidation (Abubakar *et al*. 2004) and prevent cell injury by maintaining the sulfhydryl groups of membrane proteins as well as quench free radicals (Basu and Dickerson, 1996), thus maintaining the functional and structural integrity of important immune cells (Arita *et al*. 1995). In addition, vitamin C through its antioxidant functions has been shown to stimulate both the production and function of leukocytes (LPI, 2015) as well as protect leukocytes from self-inflicted oxidative damage (Jariwalla and Harakeh, 1997). This protective effect of vitamin C at 25 mg kg^{-1} may be exploited as a combination therapy in chronic cimetidine usage especially in subjects at risk of leucopenia, such as those on chemotherapy.

However, treatment with vitamin C at 50 mg kg^{-1} failed to modulate cimetidine-induced leucopenia. This effect may probably be due to the pro-oxidant effect of vitamin C as described by Podmore *et al*. (1998), Lee *et al*. (2001) and Yarube *et al*. (2014). It may also be due to significant blockage of histamine – induced initiation of DNA synthesis in bone marrow stem cells by cimetidine (Posnett*et al*. 1979) thereby dampening the ability of vitamin C to effectively stimulate the production of leucocytes.

Cimetidine has caused an insignificant reduction in RBC count. This may be due to mild suppressive effects of cimetidine on colony-forming unit-erythrocytes (CFU-E) of bone marrow (Byron, 1980). Also, there reports that heme biosynthesis can be inhibited by cimetidine (Pino and Azer, 2020). The finding of this study is in agreement with that of Calzado *et al*. (1982) and disagrees with those of others (Posnett*et al*. 1979; de Galoscy and de Strihou, 1979) who reported a significant decrease in all blood cell counts with use of cimetidine.

The difference may be due to the high doses of cimetidine used in those studies. Treatment with both doses of vitamin C resulted in an insignificant increase in RBC count, though not statistically significant, as similarly reported by Cook and Reddy(2001). This might be because Vitamin C, which is the only dietary constituent other than animal tissue, was shown to repeatedly augment the absorption of non-heme iron (Lynch and Cook, 1980). Iron, in turn, is essential RBC production (Dudrick*et al*. 1985). Food iron is absorbed by the intestinal mucosa from two separate pools of heme and non-heme iron. Heme iron, delivered from hemoglobin and myoglobin is well absorbed and relatively unaffected by other foods eaten in the same meal (Lynch and Cook, 1980). On the other hand, absorption of non-heme iron the major dietary pool found in food products such as grains, nuts, seeds, vegetables and iron-fortified food is greatly influenced by meal composition (Campbell, 2015). Ascorbic acid acts as a powerful enhancer of non-heme iron absorption by forming a chelate with ferric iron at acid pH that remains soluble at the alkaline pH of the duodenum and upper jejunum where it is predominantly absorbed and can reverse the inhibiting effect of substances such as phytates, tannins and antacids (Lynch and Cook, 1980; Muir and Hopfer, 1985; Hallberg *et al*. 1989; Conrad and Umbreit, 1993).

In addition, vitamin C has a pronounced enhancing effect on the absorption of dietary non-heme iron only when assessed by feeding single meals in contrast to the negligible effect on iron balance of long-term supplementation (Cook and Reddy, 2001), which may be a possible reason for the insignificant increase in RBC count.

Although, packed cell volume and hemoglobin concentration were reduced by cimetidine, these changes were statistically insignificant. This shows that cimetidine did not put the rats at the risk of anemia. However, this effect may be due to mild suppression of the bone marrow colony forming unit-erythrocyte by cimetidine (Byron, 1980) with resultant slight reduction of red blood cells production, packed cell volume and hemoglobin concentration.

The mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and color index were statistically similar in all groups, indicating further the low risk of anemia caused by cimetidine.

CONCLUSION

Chronic intake of therapeutic dose of cimetidine significantly decreased WBC count in rats and this was modulated by vitamin C.

RECOMMENDATIONS

Caution should be exercised regarding chronic treatment with cimetidine due to its potential to cause leukopenia.Vitamin C at 25 mg kg^{-1} may be recommended as co-treatment with cimetidine especially in chronic conditions.

CONFLICT OF INERREST

None declared.

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