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Effects of vitamin C as a rescue agent against ciprofloxacin (Ciprocin®) induced toxicities in mice

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Abstract

Background

Ciprofloxacin is a common and widely used antibiotic in both human and animal health, But indiscriminate, overdose and long term used may cause toxicity The research work was designed to examine the changes in hematological parameters: Total Erythrocyte count (TEC), Hemoglobin (Hb), Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC) and biochemical parameters: Total Cholesterol (T), Triglyceride (TG), High Density Lipoprotein (HDL) and Serum Creatinine (SC) in mice treated with ciprofloxacin and its interaction with vitamin C.

Methods

A total of 20 male Swiss Albino mice aging one month and weighing of 22.5±2.3 gm were taken, and were allotted into four equal groups (n=5). After acclimatization, Group D was kept as control and was fed with normal mice pellet and water only. Mice of group A, B, and C were maintained as treatment groups and fed with ciprofloxacin (Ciprocin®)@ 2 mg/kg body weight (bwt), 1 mg/kg bwt and 0.5 mg/kg bwt in normal saline orally respectively throughout the experimental period of 14 days. Besides 2 mg Vitamin C @ 2mg /kg bwt was supplied in water for all the treated mice.

Results

The results indicated that the values of RBC and Hb in the treated groups were decreased significantly (P<0.01) as compared to the control group. White blood cell (WBC) counts in group B and C were almost similar to those of control group, whereas it was lowered significantly (P<0.05) in group A. However, lymphocyte, monocyte levels significantly (P<0.01) increased in all the treated groups as compared to the control, whereas neutrophil and eosinophil counts in group B and C were lowered (P<0.01) as compared to the control. The TG values were recorded as lower (P<0.01) in group C than in group A. Cholesterol level was found highest (P<0.01) in control mice (group D) as compared to treated groups A, B and C. HDL was decreased and SC was increased significantly (P<0.01) in the treated groups as compared to the control group.

Conclusions

It could be concluded that treatment mice with vitamin C concomitantly with ciprofloxacin not exert restoring effect significantly on hematological parameters of TEC and Hb but exert effect significantly on total WBC count, lymphocyte, monocyte and lipid profile.

Keywords: Vitamin C, Hematological parameters, biochemical parameters, lipid profile.

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Introduction

Ciprofloxacin has a good bioavailability after oral administration, good to excellent tissue penetration and is relatively safe (Ball and Tillotson, 1995; Papich, 1998). It is very active against wide variety of pathogenic bacteria including some gram-positive and gram-negative organisms. But, ciprofloxacin in mice inhibits IgG forming cells, delayed type of hypersensitivity responses and proliferation of lymphocytes in response to lipopolysaccharide or concanavalin A (Valera *et al*. 1995). High concentrations of ciprofloxacin (150 mg/l) inhibit lymphocyte proliferation (Shalit, 1991). Hepatotoxicity associated with ciprofloxacin has been reported in experimental animals. Moreover, Nordmann et al. (1989) evaluated the cytotoxicity and uptake of ciprofloxacin in primary cultures of rat's hepatocytes. They also reported that ciprofloxacin at 200 mg/l was found to be hepatotoxic to rats. In addition, ciprofloxacin produced significant enhancement of lipid peroxidation and alteration of glutathione redox status in hepatic tissues in rats (Hincal & Taskin, 1995).

Vitamins protect us against a variety of diseases such as heart disease, Alzheimer's diseases, respiratory diseases and infectious diseases by boosting our immunity (Akhilender, 2003). Vitamin C strengthens and protects the immune system by stimulating the activity of antibodies and immune system cells. It significantly decreases the low density lipid (LDL) and triglyceride (TG) (Afkhami & Shojaoddiny, 2007). Previous study suggested that Vitamin C is an important nutrient in maintaining cell membrane integrity (Priyadharshini, 2013). Currently, the most important benefit claimed for Vitamin C is its role as antioxidant, which is a scavenger of particles known as oxygen free radicals (or oxidants). Consuming 3-6mg of β carotene daily will maintain plasma beta-carotene blood levels in the range associated with a lower risk of chronic diseases.

Only few researchers (Priyadharshini, 2013) showed and examined that the therapeutic effect of Vitamin C against antibiotics, particularly Ciprofloxacin toxicities. In Bangladesh there is no available data in this context. So, in our study we like to determine the rescue effects of Vitamin C in altering blood parameters, lipid profile and serum creatinine as a consequences of Ciprofloxacin induced toxicities in mice.

Materials and Methods

One month old, 20 male Swiss Albino mice (*Rattusnor vigicus*) were purchased from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, weighing 22.5±2.3 gm, they were housed in wooden cages under room temperature and lighting (12 hours' light/ 12 hours' dark). The mice were randomly divided into 4 equal groups (n=5)-A, B, C and D. Group D was kept as control and was fed with normal mice pellet and water only. Mice of group A, B, and C were maintained as treated groups and fed with ciprofloxacin @ 2 mg/kg bwt, 1 mg/kg bwt and 0.5 mg/kg bwt in normal saline orally respectively throughout the experimental period of 14 days. Besides 2 mg Vitamin C /kg bwt was supplied in water for all the treated mice.

At the end of the experiment (14 days), blood samples collected directly from heart and half of blood sample were placed in tubes which contain anticoagulant (EDTA) and rest blood samples were collected into clean dry tubes for serum separation. The tubes were then incubated overnight in the refrigerator (4°C). The serum samples were separated and centrifuged at 1500 at rpm for 15 minutes to get rid of unwanted blood cells if necessary.

Hemoglobin concentration was determined by Acid hematin / Shahli method and result was expressed in gm/dl (Lambert & Ritheistein, 1977). Red and White blood cells count were counted per cubic millimeter of blood by Hemocytometer and result was expressed in million/cumm and thousand/cumm respectively. Differential leukocytes count (DLC), thin blood film was prepared and stained with modified wright stain (Lambert & Ritheistein, 1977)

The serum biochemical parameters determination like Total Serum cholesterol, TG, HDL and

serum creatinine were performed (Dr. Mohammad Hossain Central Laboratory) central laboratory of Bangladesh Agricultural University, Mymensingh.

Statistical Analysis

All recorded data were calculated and analyzed using statistical SPSS program by one-way analysis of variance (ANOVA) followed by Duncan (Bruning & Kintz ,1977) to assess the differences among the treatment means at 5% level of significance.

Results and Discussion

Effect on hematological parameters

Administration of mice with three different doses of ciprofloxacin during the experimental period (14 days) produced a dose dependent significant decrease ($P<0.05$) in hemoglobin concentration, RBC count and WBC count as compared with the control group (Table 1 & Fig-1). Treatment mice with vitamin C concomitantly with ciprofloxacin during experimental period caused a significant increase ($P<0.05$) in white blood cells count but treatment with vitamin C did not affect significantly in red blood cells count, Hb compared with control group.

The values of TLC in group B and group C were similar and statistically non-significant than that of control group but in higher doses treated group, the value of TLC was significantly ($P<0.05$) lower than that of control as well as other treated groups.

A significant reduction in RBC count and Hb concentration were seen after ciprofloxacin administration. This may be happened due to intoxication of ciprofloxacin on hemopoietic organs. Co-administration of vitamin C was not effective in preventing the drug induced adverse reaction in hemopoietic organs although Vitamin C acts as antioxidant. Channa and Janjua (2003) reported that Ciprofloxacin causes severe liver damage, marked dilation of sinusoids and pyknotic nuclei within the liver. This liver effects may be responsible for the reduction of blood parameters. The result obtained in the present study is similar to the study of (Priyadharshini, 2013) who reported that vitamin A and E were more effective in preventing the ciprofloxacin induced adverse reaction on blood parameter than vitamin C. Thus we can conclude that rescue effect of vitamin C is not prominent as the antibiotic has severe adverse effect on different systems of the body.

Like the RBC counts, the WBC counts were also decreased in all treated groups but the values of group B & C were more or less similar at normal level except the higher ciprofloxacin induced group. This may be the stimulating effects of vitamin C on the body immune system. Previous study (Hardie **et al**. 1991) suggested that vitamin C may be act as immune stimulant. In addition, Vitamin C, commonly associated with immune health. It may be no surprise that vitamin C enables our body to synthesize white blood cell. Our result also supported by the previous study (Priyadharshini, 2013) who reported that WBC count is less in case of ciprofloxacin induction and the rescue effect of vitamin C was effective gradually.

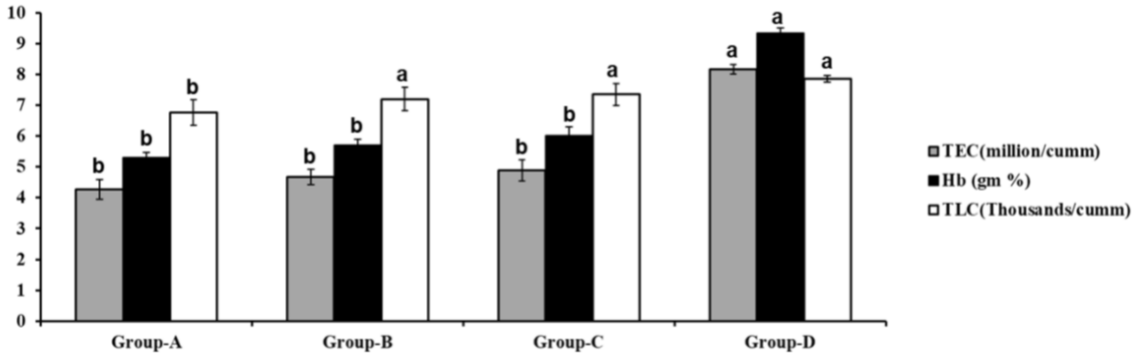
Table 2 shows a significant increase ($P<0.05$) in lymphocytes percentage in all treated group compared with control group but neutrophils values were significantly ($P<0.05$) decrease. No significant differences occurred between groups in the percentage of monocytes and eosinophils.

In contrast to the value of neutrophil in all treated group, the value of lymphocyte was increased significantly ($P<0.01$). Vitamin C is an immune enhancer that help white blood cell perform at their peak and quickens the immune system response. Previous study reported that vitamin C may increase the production of lymphocyte which help to defense against infection. (IBIS, 1998).

Table.1 Effect of ciprofloxacin in co-administration with vitamin C on TEC(million/cumm), Hb (gm %) and TLC(Thousands/cumm) in mice.

Hematological parameters	Mean ± SE				*P*- value
	Group-A	Group-B	Group-C	Group-D	
TEC(million/cumm)	4.27^{b}±0.32	4.67^{b}±0.25	4.89^{b}±0.34	8.17^{a}±0.16	0.01 (**)
Hb (gm %)	5.31^{b}±0.17	5.72^{b}±0.19	6.02^{b}±0.28	9.35^{a}±0.17	0.01 (**)
TLC(Thousands/cumm)	6.77^{b}±.41	7.20^{a}±.38	7.35^{a}±.36	7.86^{a}±.11	0.05 (*)

** = Significant at 1% level of significance.
* = Significant at 5 % level of significance.



a,b,c = Values with different superscripts in the same row differ significantly (P<0.01 &P<0.05).

Fig. 1. Hematological parameters (Mean ± SE) of mice after supplementation of ciprofloxacin in co-administration with vitamin C in different dosages.

Table 2. Effects of ciprofloxacin in co-administration with vitamin C on DLC; Neutrophil (%), Eosinophil (%), Lymphocyte (%) and Monocyte (%)in mice

Hematological parameters	Mean ± SE				*P* - value
	Group-A	Group-B	Group-C	Group-D	
Neutrophil(%)	21.0^{b}±2.24	22.60^{b}±3.44	23.00^{b}±1.58	29.00^{a}±1.58	0.01(**)
Eosinophil(%)	1.60±0.55	1.20±1.30	2.40±1.44	1.60±.55	NS
Lymphocyte(%)	72.00^{c}±1.58	73.80^{b}±3.35	75.00^{a}±1.87	68.00^{d}±1.58	0.01(**)
Monocyte(%)	1.40^{b}±0.55	1.80^{b}±1.10	2.60^{a}±0.89	1.40^{b}±0.89	0.01(**)

** = Significant at 1% level of significance.
NS=Not Significant
a,b,c = Values with different superscripts in the same row differ significantly (P<0.01).

Effect on Biochemical parameters

Concomitant administration of vitamin C with three different doses of ciprofloxacin in mice during the experimental period (14 days) produced a dose dependent significant decrease (P<0.05) in cholesterol and HDL values as compared with the control group (Table 1) and all the values within the treated groups were significantly different according to the doses and lowest values were in lower ciprofloxacin treated group. Treatment mice with vitamin C concomitantly with ciprofloxacin during

experimental period caused a significant increase ($P<0.05$) in TG only in highest ciprofloxacin treated group but other values did not affect significantly in compared with control. In case of SC values, the highest values were recorded in group A and B than that of group C&D.

The highest value of triglyceride level in the high dose treated group may be due to the excessive liver damage and other side effects of antibiotics (Basarann *et al.* 1993) but in lower doses of antibiotics, the side effects were suppressed by vitamin C (Afkhami & Shojaoddiny, 2007 and Yizhou *et al.* 2013)

A significant reduction of cholesterol level in all the treated group and this reduction is dose dependent. Previous studies show that effects of antibiotic were not so significant in change of cholesterol level but long term Vitamin C administration decrease the cholesterol level significantly. Our findings were also similar to those previous study (Akhilender, 2003; Yizhou *et al*. 2013 and Yousef *et al*. 2003).

HDL level is decreased in the treated group than in the control group. This may be due to the effects of antibiotic on liver, heart, blood vessels, lymphatic system and ultimately in serum quality. The Vitamin C normally increase the HDL, but in co-administration with Ciprofloxacin its effect was suppressed. This study was found in the similar finding of Afkhami & Shojaoddiny, 2007.

Gradually increasing creatinine level in mice may be due to the dose dependent effect of ciprofloxacin on kidney. Adikwu and Brambaifa (2012) investigated ciprofloxacin induced cardiotoxicity and hepatotoxicity. They found most prominent lesions induced by ciprofloxacin were in the kidney, liver, heart and elevated enzyme includes serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphate and gamma-glutamul transferase. The result obtained in present research is similar to that of Bergan *et al.* (1987) who investigated hepato-renal and hematological effects of ciprofloxacin in rats and found significant increase in aminotransferases, alkaline phosphatase, urea and creatinine levels. In our experimental also showed the significant effect of vitamin C as an antioxidant and did not reverse kidney function in higher ciprofloxacin treated groups than that of lowest doses group.

Table 3. Effects of ciprofloxacin in co-administration with vitamin C on TG (gm/dL), Cholesterol (gm/dL), HDL (gm/dL) and SC level (gm/dL) in mice.

Parameters	Mean ± SE				*P*- value
	Group A	Group B	Group C	Group D	
TG (gm/dL)	97.45^{a}± 6.53	84.08^{b}± 2.58	80.65^{b}±5.29	87.58^{b}± 2.12	0.01 (**)
Cholesterol (gm/dL)	156.17^{b}± 1.43	144.61^{c}± 6.88	138.53^{d}± 5.79	199.39^{a}± 10.9	0.01(**)
HDL (gm/dL)	44.14^{b}± 2.33	40.41^{c}± 0.77	33.59^{d}± 6.92	51.29^{a}± 2.44	0.01(**)
SC (gm/dL)	0.55^{a}±0.07	0.47^{a}±0.08	0.28^{b}±0.06	0.18^{b}±0.05	0.01(**)

** = Significant at 1% level of significance.

a,b,c = Values with different superscripts in the same row differ significantly ($P<0.01$)

Conclusion

In this study it is concluded that administration of vitamin C on ciprofloxacin induced toxicity is not more effective for restoring the values of TEC and Hb rather than the values of TLC and DLC. In addition, the restoring effects of vitamin C were more effective on lipid profiles that were altered due to ciprofloxacin induced toxicity. Further descriptive studies are needed to observe any toxic effect of ciprofloxacin like organ specific toxicity and antitoxic effect of vitamin C which will be beneficial for animal as well as human being.

Conflict of interest

The authors declare that they have no competing interests.

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