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Toxic outcomes of ciprofloxacin and gentamicin co-administration and possible ameliorating role for antioxidant vitamins C and E in Wistar Rats

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Abstract

The research aimed at evaluating the safety or toxicity outcomes of ciprofloxacin and gentamicin co-administration and the possible ameliorating role of vitamin C and E. Wistar albino rats were divided into five groups of five rats in each group. Animals were co-administered ciprofloxacin (7.14 mg/kg) and gentamicin (1.14 mg/kg) and treated with vitamin C (100 mg/kg), vitamin E (1000 iu) or a co-administration of vitamin C and E at the initial dose. After 11 days, various hematological, biochemical and histological studies were performed. The result showed that co-administration of ciprofloxacin and gentamicin significantly reduced hemoglobin concentration, packed cell volume, red blood cell count, alkaline phosphatase, total protein, albumin, cholesterol, superoxide dismutase and catalase. Increased blood level in total white blood cell count, serum urea, creatinine, total bilirubin, glutathione, malondialdehyde, C-reactive protein and creatine kinase were observed after ciprofloxacin and gentamicin co-administration. The co-administration of antioxidants vitamins (C and E) maintained plasma/serum hematological, biochemical and antioxidant status similar to control. Histological observation of the kidney and liver sections substantiated the biochemical findings.

Keywords: Ciprofloxacin, Gentamicin, Vitamins, Toxicity, Antioxidants, Biochemical

Introduction

Bacterial infections have been the major cause of diseases throughout the history of human population, with the introduction of antibiotics anticipated to counter this problem. However, bacteria have evolved to develop resistance to available antibiotics [1]. This is applicable to both gram positive and gram-negative bacteria. Multi drug resistant organisms like methicillin-resistent *staphylococcus aureus* (MRSA), vacomycin-resistent *enterococci* and certain gram-negative bacilli like *Pseudomonas aeruginosa* (Pa), *Acinetobacter baumanii* (Ab) are the causative agents for severe and lethal human infections. Many of the existing antibiotics have lost their potency in managing infections because the invading pathogens may acquire resistant genes enabling them produce enzymes like beta lactamase, carbepenemase, expresses efflux system or modify the drug target [2]. This alarming increase in drug resistance rate has necessitated the development and evaluation of alternative ways to curb the menace. The restoration of the interest of physicians in

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neglected antibiotic agents as well as combinatorial antibiotic therapy has been considered an important armament in the battle against antibiotics resistance [3]. Antibiotics combination has found wide application especially in improving clinical efficacy in patients where a given therapy is thought to have limitations. Combinational antibiotics therapies with different mechanisms of actions have been applied in the treatment of infections with the goal of producing a wider spectrum preventing the emergence of drug resistance and achieving a synergistic effect [4].

Gentamicin is an aminoglycoside antibiotic. It is effective against bacterial infections [5]. However, their efficacy is affected by toxicity especially nephrotoxicity, which leads to kidney damage. Ciprofloxacin is a commonly used antibiotic for the treatment of bacterial infections. It is a broad-spectrum fluoroquinolone active against *Pseudomonas aeruginosa* induced respiratory infections, acute or chronic osteomyelitis or osteochondritis, multi-drug resistant gram negative bacterial infections and so many others [6]. Although the co-administration of ciprofloxacin and genatmicin is not the first choice, it is clinically used mostly in resistant cases of bacterial infections caused by *Pseudomonas aeruginosa* (causative agent of urinary tract infections, skin infections, pneumonia), extended spectrum beta lactamase infections, Klebsiella pneumonia [7].

Vitamins have irreplaceable role in almost all biochemical reactions. They are ideal antioxidants capable of protecting tissues from oxidative stress [8]. Vitamin E (α-Tocopherol) is the primary membrane-bound, lipid-soluble, chain-breaking antioxidant that protects cell membranes against lipid peroxidation. Pre-treatment of vitamin E has been reportedly beneficial in preventing tissue damage in rats. Vitamin C (ascorbic acid) supplementation has a remedial benefit due to its ability to reduce oxidative stress by reacting with superoxide and hydroxide radicals as well as alkyl, peroxyl and alkoxyl radicals, thereby neutralizing these radicals, thus stopping the initiation and propagation of chain reaction [9].

The co-administration of two or more drugs is believed to be accompanied by a variety of therapeutic implications ranging from opposition, alteration, synergism, physical and chemical antagonism [10]. Clinically important interactions may occur in ciprofloxacin and gentamicin co-administration leading to drug resistance or systemic overexposure that may result in tissue toxicity. The safety or toxicity potentials of combinatorial administration of ciprofloxacin and gentamicin have not been evaluated fully. Thus, there is need to explore the safety/toxicity outcomes of this therapy, checking the possible biochemical roles of antioxidant vitamins (C and E) in such administration.

Materials and methods

Chemicals and animals

Pure and analytical grade chemicals procured from standard chemical dealers and Randox kits were used in the study. Female albino rats (wistar strain) weighing (85-140 g) were maintained under standard natural condition of light (12 h) and dark (12 h) cycle at (25$\pm$ 2) ˚C. The rats were fed on standard rat chow (Vital feeds) and water *ad libitum.* The usage of animals were in line with the rcommenations of Russow [11] regarding ethical standard for researches involving animal subjects and approved by the COLNAS committee on Research ethics, Michael Okpara University of Agriculture, Umudike.

Dose selection and preparation

Using doses for human as reference, doses were calculated and modified for rats using the method of Paget and Barnes [12]. The acceptable doses were: ciprofloxacin (7.14 mg/kg), Gentamicin (1.14 mg/kg), vitamin C (100 mg/kg) and vitamin E (14.29 IU/kg).
Ciprofloxacin solution was prepared by crushing and dissolving 25 mg ciprofloxacin in 25 ml of distilled water to give 1 mg/ml. It was administered twice a day at the dose of 7.14 mg/kg per oral route.
Vitamin C solution was equally prepared by crushing and dissolving 1000 mg of vitamin C in 10ml of distilled water to give 100 mg/ml. It was admistered twice a day at the dose of 100 mg/kg per oral route. One capsule of vitamin E (1000 IU) which is in the liquid form was administered orally at the dose 14.29 IU/kg body weight One ampoule of gentamicin (80 mg) was administered at a dose of 1.14 mg/kg body weight. It was administered once a day by intramuscular route.

Experimental design

Animals were divided into five groups of five animals each. **Group 1** served as control. **Group 2** was administered with ciprofloxacin (7.14 mg/kg) and gentamicin (1.14 mg/kg). **Group 3** received ciprofloxacin (7.14 mg/kg), gentamicin (1.14 mg/kg) and vitamin C (100 mg/kg). **Group 4** received ciprofloxacin (7.14 mg/kg), gentamicin (1.14 mg/kg) and vitamin E (14.29 iu/kg) while **Group 5** was treated with ciprofloxacin (7.14 mg/kg), gentamicin (1.14 mg/kg), vitamin C (100 mg/kg) and vitamin E (14.29 iu/kg). Ciprofloxacin, vitamin C and E were administered orally while gentamicin was administered through intramuscular route. All admintration lasted for 11 days. Blood samples were collected through ocular punture on the 12th day.

Hematological and biochemical assays

A portion of blood was collected into a well labelled anticoagulant specimen bottle for hematological analysis. Another portion was also collected into a well labelled specimen bottle without anticoagulant and were kept at room temperature for 1 h, centrifuged at 3000 rpm for 10 min to obtain serum. Serum was used for the analysis of total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, creatinine, urea, creatine kinase, C-reactive protein, total cholesterol, superoxide dismutase, catalase, glutathione and malondialdehyde (MDA) using Randox diagnostic kit (Randox kt, UK) according to the manufacturers instructions.

Assessment of hematological parameters

Hematological parameters such as hemoglobin, packed cell volume (PCV), red blood cell (RBC) count and white blood cell (WBC) count were analysed according to the method as described by Ochei and Kolhatkar [13]. Platelet count was done using the method described by Cheesbrough [14].

Estimation of biochemical parameters

Concentrations of total protein, albumin, total bilirubin, urea, creatinine, creatine kinase, C-reactive protein, glutathione, malondialdehyde, total cholesterol and the activities of AST, ALT, ALP, SOD, Catalase in blood serum was determined using standard biochemical methods [15, 16, 17, 18, 19, 20]

Statistical analysis

The data were analyzed with statistical products and service solution (SPSS) version 20.0. The statistically analyzed data were presented as mean ± standard deviation. Duncan multiple range test was used to compare means and statistical significance was set at $P<0.05$.

Results

The result in table 1 showed a significant ($p<0.05$) decrease in the red blood cell (RBC) count of group 2 rats (positive control) compared with the normal control. However, group 3 showed a non-significant ($p>0.05$) increase in RBC count compared to those of group 2 rats (positive control). Additionally, rats in groups 4 and 5 showed a non-significant ($p>0.05$) reduction in red blood cell count when compared with the normal control, but were significantly ($p<0.05$) increased when compared to the group 2 rats (positive control).

Hemoglobin (Hb) concentration significantly ($p<0.05$) reduced in group 2 rats compared with the normal control. However, groups 4 and 5 showed a significant ($p<0.05$) increase compared with both the normal control and positive control (group 2 rats). Additionally, Hb concentration group 3 were significantly ($p<0.05$) lower when compared with the normal control but significantly ($p<0.05$) higher when compared with the positive control.

Packed cell volume (PCV) significantly ($p<0.05$) reduced in group 2 rats when compared with the normal control. However, groups 3 and 5 showed a non-significant ($p>0.05$) reduction when compared with the normal control. Group 5 showed a significant ($p<0.05$) increase in PCV when compared with the positive control (group 2). Additionally, group 4 showed a non-significant ($p>0.05$) increase in PCV when compared with the normal control, but were significantly ($p<0.05$) higher when compared with the positive control (group 2).

The total white blood cell (TWBC) was significantly ($p<0.05$) increased in group 2 rats compared to the normal control. However, groups 3 and 4 showed a non-significant ($p>0.05$) increase when compared with the normal control, but were significantly ($p<0.05$) lower when compared with the positive control (group 2). Additionally, group 5 showed a significant ($p<0.05$) increase when compared with the normal control but were significantly ($p<0.05$) lower when compared with the positive control (group 2).

There was equally a non-significant ($p>0.05$) increase in platelet count of group 2 rats compared with the normal control. However, groups 4 and 5 showed a non-significant ($p>0.05$) reduction in platelet counts when compared with normal control but were significantly ($p<0.05$) lower when compared with the positive control (group 2). Additionally, rats in group 3 showed a non-significant increase when compared with both the normal and positive control rats.

The result in table 2 showed that the co-administration of ciprofloxacin and gentamicin had no significant effect on serum AST and ALT activity whereas ALT was significantly ($p<0.05$) reduced in group 5 rats when compared to those in group 2 (positive control).

Serum ALP activity significantly ($p<0.05$) reduced in group 2 rats (positive control) when compared to the normal control. Rats in group 3 and 5 showed a significant ($p>0.05$) reduction in ALP activity when compared to the normal control but were non-significantly ($p>0.05$) increased when compared to the rats in group 2 (positive control). Additionally, group 4 significantly

($p<0.05$) decreased when compared to the normal control but was non-significantly ($p>0.05$) lower when compared to group 2 (positive control).
A significant ($p<0.05$) reduction in serum protein and albumin concentration was observed in group 2 rats (positive control) when compared with the normal control. However, groups 4 and 5 showed a significant ($p<0.05$) increase when compared with both the normal control and positive control. Additionally, rats in group 3 showed a non-significant ($p>0.05$) increase in total protein and albumin when compared with the normal control but were significantly ($p<0.05$) higher when compared with the positive control (group 2).
Serum bilirubin showed a significant ($p<0.05$) increase in group 2 (positive control) when compared with the normal control (group 1). Rats in group 3 and 4 showed a significant ($p<0.05$) increase in total bilirubin concentration compared with the normal control but were non-significantly ($p>0.05$) lower compared with those of group 2 (positive control). Additionally, group 5 rats showed a non-significant ($p>0.05$) increase compared with the normal control but were significantly ($p<0.05$) lower compared with the group 2 rats (positive control).

Serum total cholesterol significantly ($p<0.05$) reduced in group 2 rats compared with the normal control (group 1). However, groups 3, 4 and 5 showed a non-significant ($p>0.05$) reduction when compared to the normal control but were significantly ($p<0.05$) increased compared with the group 2 rats (positive control).
Table 3 showed serum superoxide dismutase (SOD) and catalase activities were significant ($p<0.05$) reduced in group 2 rats when compared with the normal control (group 1). However, rats in groups 3, 4 and 5 showed a significantly higher SOD and catalase activity When compared with group 2 (positive control).
Serum GSH and MDA showed a significant ($p<0.05$) increase in concentration in group 2 rats when compared with the normal control (group 1). However, groups 3, 4 and 5 showed a non-significant ($p>0.05$) reduction when compared with the normal control, but were significantly ($p<0.05$) reduced compared with the group 2 rats (positive control).
Figure 1 showed that serum urea was significantly ($p<0.05$) increased in serum urea concentration of group 2 (positive control) rats when compared with the normal control (group 1). Rats in group 3, 4 and 5 showed a significant ($p<0.05$) increase when compared with the normal control, but were significantly ($p<0.05$) reduced when compared with rats in group 2 (positive control).
Table 2 showed serum Creatininine significantly ($P<0.05$) increased in group 2 rats (positive control) when compared with the normal control (group 1). Rats in group 3, 4 and 5 showed a significant ($p<0.05$) increase when compared with the normal control but were significantly ($p<0.05$) reduced when compared with the rats in group 2 (positive control).
Figure 3 showed serum C-reactive protein showed a significant ($p<0.05$) increase in group 2 rats when compared with the normal control (group 1). Rats in group 3 and 5 showed a significant ($p<0.05$) reduction when compared with the normal control and positive control (group 2) respectively. Additionally, a non-significant ($p>0.05$) increase was observed in group 4 rats when compared with the normal control but were significantly ($p<0.05$) reduced when compared with the positive control (group 2).
Figure 4 showed that serum creatine kinase activity showed a significant ($p<0.05$) increase in group 2 rats when compared with that of the normal control (group 1). Rats in groups 4 and 5

showed a significant ($p<0.05$) increase when compared with the normal control, but were significantly ($p<0.05$) reduced when compared with the positive control (group 2). However, rats in group 3 showed a non-significant ($p>0.05$) increased when compared with the normal control, but were significantly ($p<0.05$) reduced when compared with the positive control (group 2).

Table 1: Haematological indices of rats co-administered Ciprofloxacin and Gentamicin, treated with Vitamins C and E

Groups	PLATELET ($X10^9$/L)	RBC ($X10^{12}$/L)	HB (g/dL)	PCV (%)	TWBC (mm^{-3})
Group 1	161.25 ± 6.58^{ab}	4.40 ± 0.30^{b}	10.52 ± 0.18^{c}	47.50 ± 1.50^{a}	1500.00 ± 57.74^{c}
Group 2	170.00 ± 5.24^{a}	3.60 ± 0.34^{a}	7.72 ± 0.13^{e}	42.40 ± 0.81^{b}	2600.00 ± 109.54^{a}
Group 3	174.00 ± 6.96^{a}	3.64 ± 0.60^{a}	8.18 ± 0.15^{d}	45.00 ± 0.45^{ab}	1820.00 ± 66.33^{cb}
Group 4	152.00 ± 1.22^{b}	4.20 ± 1.00^{b}	12.34 ± 0.40^{b}	47.80 ± 0.37^{a}	1680.00 ± 101.98^{cb}
Group 5	153.00 ± 2.00^{b}	4.20 ± 0.45^{b}	13.00 ± 0.19^{a}	46.20 ± 1.28^{a}	1600.00 ± 63.25^{b}

Values are presented as mean ± standard deviation. *$p<0.05$ compared with the control group

Table 2: Liver function indices of rats co-administered Ciprofloxacin and Gentamicin, treated with Vitamins C and E

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Protein (mg/dl)	Albumin (mg/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)
Control	58.98 ± 1.99^{a}	52.18 ± 2.58^{ab}	31.75 ± 1.71^{b}	4.57 ± 0.20^{b}	1.35 ± 0.13^{b}	1.46±0.08a	4.86 ± 0.56^{c}
Group 2	58.24 ± 2.15^{a}	55.06 ± 2.82^{b}	25.20 ± 1.10^{a}	4.14 ± 0.20^{a}	1.01 ± 0.15^{a}	1.92±0.10c	3.74 ± 0.60^{a}
Group 3	58.62 ± 4.12^{a}	51.66 ± 3.03^{ab}	26.20 ± 2.95^{a}	4.69 ± 0.35^{b}	1.25 ± 0.21^{ab}	1.77±0.12b	4.24 ± 0.85^{ab}
Group 4	59.22 ± 1.92^{a}	54.58 ± 3.09^{b}	23.60 ± 3.13^{a}	6.44 ± 0.27^{c}	$2.02\pm$o.30^{c}	1.82±0.10bc	4.35 ± 0.90^{ab}
Group 5	60.60 ± 2.60^{a}	47.72 ± 4.94^{a}	27.00 ± 2.12^{a}	6.50 ± 0.40^{c}	1.71 ± 0.21^{c}	1.48±0.05a	4.79 ± 0.22^{c}

Values are presented as mean ± standard deviation. *$p<0.05$ compared with the control group

Table 3: Antioxidant status of rats co-administered Ciprofloxacin and Gentamicin, treated with Vitamins C and E

Groups	GSH	SOD (U/L)	Catalase (U/L)	MDA
Control	10.67 ± 0.30^{a}	10.57 ± 0.54^{b}	9.73 ± 0.46^{b}	0.73 ± 0.10^{a}
Group 2	12.56 ± 0.19^{b}	9.42 ± 0.41^{a}	8.58 ± 0.46^{a}	1.53 ± 0.12^{b}
Group 3	10.48 ± 0.26^{a}	10.94 ± 0.38^{b}	9.88 ± 0.52^{b}	0.72 ± 0.08^{a}
Group 4	10.63 ± 0.36^{a}	10.64 ± 0.55^{b}	9.43 ± 0.21^{b}	0.81 ± 0.05^{a}
Group 5	10.61 ± 0.18^{a}	10.89 ± 0.28^{b}	9.32 ± 0.30^{b}	0.89 ± 0.21^{a}

Values are presented as mean ± standard deviation. *$p<0.05$ compared with the control group

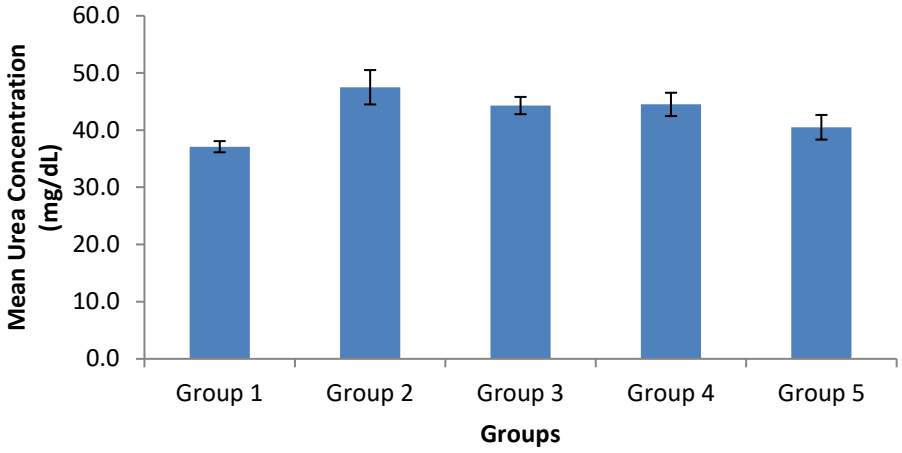

Figure 1: Serum urea concentration of rats co-administered Ciprofloxacin and Gentamicin, treated with Vitamin C and E

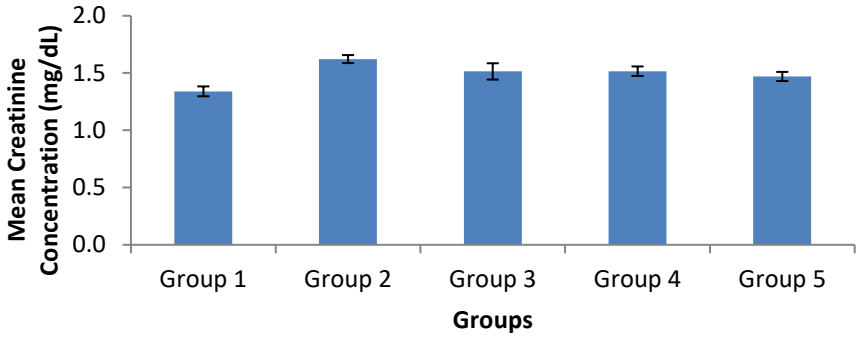

Figure 2: Serum creatinine concentration of rats co-administered Ciprofloxacin and Gentamicin, treated with Vitamin C and E

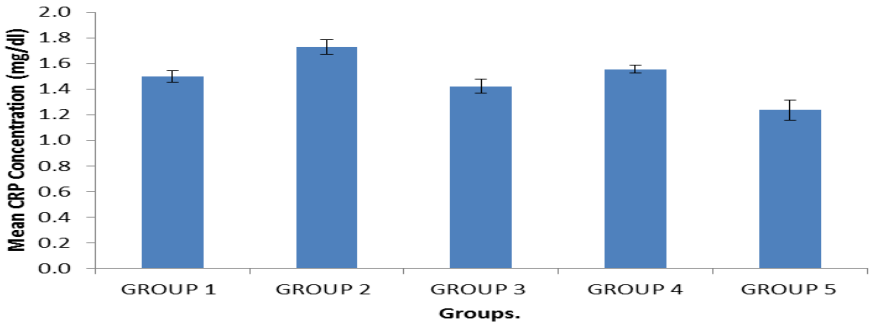

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Figure 3: Serum C - reactive protein (CRP) concentration of rats co-administered Ciprofloxacin and Gentamicin, treated with Vitamin C and E

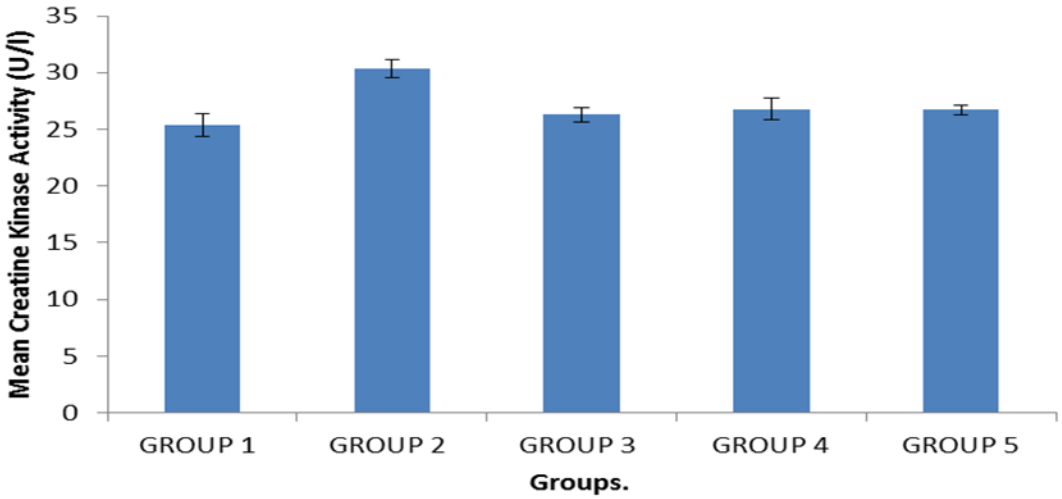

Figure 4: Serum creatinine kinase activity of rats co-administered ciprofloxacin and gentamicin, treated with Vitamin C and E

Discussion

Ciprofloxacin and gentamicin are antibiotics widely used to treat bacterial infections in humans and animals. As a result of antibiotic resistance, ciprofloxacin and gentamicin co-administration have found clinical application in the treatment of many bacterial infections [21]. The safety/toxicity potentials of combinatorial administration of ciprofloxacin and gentamicin have not been fully evaluated. Thus, this research therefore explored the safety/toxicity potentials of this administration and possible ameliorative roles of vitamins C and E in such administration.

The significant ($p<0.05$) reduction in levels of haematological parameters (Table 1) suggests a possible destruction of matured red blood cells and reduction in the rate of erythropoietin release from the kidney. Equally, the result is suggestive of a reduction in the oxygen carrying capacity of the blood and possibly a reduction in oxygen delivery to tissues. The reduction in the PCV could be due to blood loss or destruction of blood cells which might have occurred internally indicating a possible induction of anemia. Treatments with vitamin E alone and in combination with vitamin C significantly ($p<0.05$) increased the hitherto reduced red blood cell and PCV levels. This suggests a possible role of these antioxidants in hematopoiesis, enhancing oxygen delivery to tissues. All the antioxidant treatments (vitamin C, E and C+E) significantly increased the hemoglobin concentration of test rats suggesting their role in hemoglobin synthesis, possibly through either of enhanced iron absorption or maintaining the iron in its ferrous state. Jwad *et al.*, [22] equally reported that vitamin C and E treatment respectively restored the hematological indices (hemoglobin and packed cell volume) in lincomycin administered rats. Additionally there were no significant difference observed in the platelet count of ciprofloxacin and gentamicin co-

administered rats (group 2) compared to control. Total white blood cell (TWBC) count was significantly ($p<0.05$) elevated by ciprofloxacin and gentamicin co-administration in group 2 rats when compared with the normal control. This observation could be as a result of physiological inflammatory response which may have caused the immune system to increase the production of white blood cell [23]. All the antioxidant administrations significantly ($p<0.05$) reduced the total white blood cell.

Lipid peroxidation and its subsequent product MDA are typical examples of reactions that indicate oxidative stress. Membrane lipid peroxidation (in terms of MDA) reflects the damage to the cellular structure via the destruction of the double bonds in unsaturated fatty acids [24]. This study showed that co-administration of ciprofloxacin and gentamicin significantly ($p<0.05$) increased serum MDA concentration. This is indicative of lipoperoxidation and hence oxidative stress. The administration of the antioxidant (vitamin C, E and C+E) restored the serum MDA level, indicating their ameliorative roles. Several studies had reported that the administration of these antioxidants attenuates oxidative stress-induced increase in serum malondialdehyde concentration in rats [25, 26].

The extent of oxidative stress can further be assessed by assaying the serum levels of endogenous antioxidants (superoxide dismutase, glutathione and catalase). Studies have shown that gentamicin acts as an iron chelator and the iron-gentamicin complex formed is a potent catalyst for free radical generation, which also serves a factor for oxidative stress [27]. The result showed that the co-administration of ciprofloxacin and gentamicin significantly ($p<0.05$) decreased serum levels of superoxide dismutase (SOD) and catalase. The decrease observed could be as a result of free radical generation by co-administration of ciprofloxacin and gentamicin [28, 29]. Oxidative stress could lead to an alteration in the levels of these endogenous antioxidants. All the administrations of vitamins (C, E and C+E) significantly ($p<0.05$) increased the serum activities of superoxide dismutase (SOD) and catalase. This shows the ability of these antioxidant vitamins to quench the action of free radicals generated as a result of ciprofloxacin and gentamicin co-administration. The study also showed a significant ($p<0.05$) increase in plasma levels of Glutathione (GSH) in ciprofloxacin and gentamicin co-administered rats (group 2). This increase could have been as a result of the upregulation of de novo synthesis of glutathione in response to oxidative stress induced by the co-administration of ciprofloxacin and gentamicin. Treatment with the antioxidant vitamins (vitamin C, E and C+E) were seen to significantly ($p<0.05$) reduce the serum glutathione (GSH) level. This could also be attributed to the contribution of these antioxidant vitamins in mopping up the free radicals generated by the co-administration of ciprofloxacin and gentamicin.

Serum proteins have many functions including transport of other substances, immune defense, blood clothing. It is also a useful indicator of nutritional status, infection and various disorders. Albumin is the most abundant protein in blood plasma. It serves as an indicator to ascertain the state of the liver [30]. The present study revealed that the co-administration of ciprofloxacin and gentamicin caused a significant ($p<0.05$) reduction in serum protein and albumin concentrations. The decrease in serum protein is apparently due to a decreased blood albumin level. Decrease in albumin level may be due to increased urinary excretion arising from renal injury. Albumin as an antioxidant (carrier of thiol group) may have been used up in the process of combating oxidative

stress induced by these drugs. Earlier report had it that ciprofloxacin administration significantly decreased serum protein concentration of treated male rabbits [31] The treatment with both antioxidant vitamins mitigated the decrease in serum protein and albumin concentration observed in this study. This apparently could be due to the protection of the kidney from oxidative damage by the scavenging of the free radicals generated by ciprofloxacin and gentamicin co-administration thus reducing protein loss by the kidney. It could also be a resultant effect of albumin-sparing of these antioxidant vitamins.

Plasma alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) are diagnostic in nature. Their release above the normal physiological levels is employed in the assessment of liver damage and inflammatory hepatocellular disorders [32]. The result of the present study showed no significant effect of co-administration of ciprofloxacin and gentamicin on serum AST and ALT activities of test rats. Additionally, the administration of the antioxidant vitamins had no significant effect on these liver biomarkers.

Alkaline phosphatase (ALP) was found to be significantly ($p<0.05$) reduced by co-administration of ciprofloxacin and gentamicin. Metabolism of these drugs may have interfered with magnesium homeostasis. Magnesium is found to be one of the cofactors of ALP. Most studies have reported that there is a positive correlation between ALP activity and magnesium. Gentamicin had been found to decrease magnesium level [33]. This possibly could be by increased excretion of magnesium. Administration of the antioxidant vitamins had no significant effect on the already reduced serum alkaline phosphatase activity.
The result of the present study showed a significant ($p<0.05$) increase in the serum bilirubin level of ciprofloxacin and gentamicin co-administered rats. This elevation in bilirubin level observed in the present study could have arisen from a rapid breakdown of red blood cells which ultimately could lead to overproduction of bilirubin [34]. Only co-administration of vitamin C and E (group 5) restored the serum bilirubin concentration. This suggests a synergism between the two antioxidant vitamins in dousing the antibiotic induced breakdown of red blood cells.

Urea and creatinine are the most reliable indicators of renal efficiency [35] In the present study, co-administration of ciprofloxacin and gentamicin caused a significant ($p<0.05$) increase in serum urea levels of group 2 rats. This increase may be attributed to the generation of reactive oxygen radicals in renal cells [36]. This increased production of superoxide radical could lead to a decreased glomerular filtration rate [37]. The impairment of kidney function was indicated in the histopathological examination of sections of animal kidney of ciprofloxacin and gentamicin co-administered rats. All antioxidant treatments (vitamin C, E and C+E) significantly reduced the serum urea concentration. This result shows a protective effect of these antioxidants against the renal damage observed in the study. The possible mechanism could have been the quenching of reactive oxygen species (ROS) formed by ciprofloxacin and gentamicin co-administration thereby preventing the oxidant-induced cell damage.

The serum creatine kinase (MB) activity assay is an important sensitive diagnostic tool due to its high abundance in myocardiac tissues. The present study showed that co-administration of

ciprofloxacin and gentamicin caused a significant ($p<0.05$) elevation of serum creatine activity of group 2 rats. This suggests damage to the myocardiac tissues and thus a release of creatine kinase into circulation. Saraçoğlu *et al*. [6] had reported that the co-administration of ciprofloxacin and ofloxacin caused myocardiotoxicity in wistar rats. However, the antioxidant treatments (vitamin C, E and C+ E) significantly reduced the serum creatine kinase activity suggesting that these antioxidants could contribute to the maintenance of myocardiac membrane integrity.

C-reactive protein is an important marker of inflammation. The study showed that co-administration of ciprofloxacin and gentamicin significantly ($p<0.05$) increased the serum levels of C-reactive protein. The increase in C-reactive protein observed may be attributed to the ability of the drugs to activate nuclear factor KB [38]. This activation is enhanced by oxidative stress, thus leading to a cytokine-induced expression of cell adhesion molecules in the vascular endothelium, activating the production of tumor necrosis factor and interlukin-6-induced production of C-reactive protein [39-42]. It has been reported that increased level of C-reactive protein is an unspecific response to inflammation or tissue damage [39, 43-44]. Treatment with the antioxidant vitamins (vitamin C, E and C+E) were observed to significantly ($p<0.05$) reduce the serum C-reactive protein concentration. This possibly could be resulting from the ability of these vitamins to reduce the serum levels of inflammatory mediators (interlukin-6 and tumor necrosis factor) via the down regulation of hepatic mRNA expression [45-47].

The study equally showed that the co-administration of ciprofloxacin and gentamicin caused a significant ($p<0.05$) reduction in serum total cholesterol concentration of treated rats. Possibly the drugs may have inhibited the activity of 3-hydroxy, 3-methyl glutaryl CoA reductase (HMG-CoA reductase), the rate limiting enzyme in cholesterol biosynthesis or activated 7-alpha hydroxylase enzyme, stimulating the production of bile acids from cholesterol. The present study showed that the administration of the antioxidant vitamins restored the serum cholesterol level of the treated rats.

Conclusion

The present study has shown that the co-administration of ciprofloxacin and gentamicin for 11 days caused adverse changes in renal, hematological and other biochemical parameters. Some alterations in vital organs (kidney, heart and brain) were equally indicated by the histopathological examination. These adverse effects were possibly induced by oxidative stress resulting from free radical generation. However, the treatment with antioxidant vitamins (C and E) significantly attenuated these harmful effects.

Among all the antioxidant treatments, vitamin C and E co-administration presented the greatest ameliorative effect. Thus, the two antioxidant vitamins work in synergy to ameliorate adverse effects arising from ciprofloxacin and gentamicin co-administration.

Conflict of interest

There is no conflict of interest to declare.

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