Ciprofloxacin loaded castor oil based emulsion: Its antimicrobial, hematotoxicity and soft tissue pathophysiological study in Wister rats

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Abstract

The emergence of nanocarrier for drug delivery has been flanked with great achievements in the field of nanomedicine. It is however unsafe to assume their toxicological safety. This necessitated examining the toxico-dynamics of ciprofloxacin loaded castor oil based emulsion (COAB+Cp). The emulsions were prepared by mechano-chemical process. The effects of 10 mL per kg of body-weight (BW) of COAB+Cp was estimated in Adult Wistar rats (n=10) for 21 days vis-à-vis a control set up, treated with equal volume of distilled water. The biochemical and haematological parameters were conducted with histopathology of the heart, kidney, spleen and liver. Also, the antimicrobial study on *Bacillus. Subtilis* (BS) was carried out using the agar well diffusion method. No observable abnormal change in BW gain of both test and control animals. Treatment of animals with COAB+Cp did not cause any observable significant change at $P > 0.05$ in the blood-chemistry parameters and hematological indices. The photopathological examinations indicates that the histological architecture of vital organs (heart, liver, kidney and spleen) was not compromised in COAB+Cp treated animals. While the recorded value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of COAB+Cp corroborated its mean zone of inhibition (ZI) values, signifying a better bioactivity of COAB+Cp compared to Cp on BS. COAB+Cp has no cardiotoxic, hematotoxic and hepatotoxic effects on Wistar rats, with increased potency of the encapsulated Cp thus appears promising as a safe vehicle for oral delivery of ciprofloxacin.

Keywords: Emulsion; Nanocarrier; Photopathology; Cardiotoxic; Toxicokinetics

1.0 Introduction

Human being are classified as multicellular organisms [1]. These cells are suspended in a matrix; blood, and as such, the analysis of the blood reflects the state of the cells which are biomarker to the state of health of man or an organism [2, 3]. Over the years, the quest to solve the problems associated with drug delivery necessitated the development of various means of its delivery. This will foster patient compliance as well as improve the therapeutic potentials as well as the bioavailability of the encapsulated drugs without necessarily increasing the concentration [4, 5]. On this note, colloids have played a vital role in which series of emulsion have been formulated. With various application in nanomedicine and drug delivery either as a carrier or for imaging purposes through which bioactive materials can be transported into the body system [6, 7] This is due to its excellent surface properties or characteristics, thereby acting as excipient in the

manufacturing of drugs [8 -11]. In order to be suitable for this purposes, combination of other synthetic components (surfactant and co-surfactant) which has been approved as safe by Food and Drug Agency (FDA) are required to make up the formulation [12, 13]. It is therefore expedient to consider the safety of the mixture, that is, the formulations that makes up the drug carrier as well as the drug to be carried. Furthermore, Cp is a second generation fluoroquinolones that is used to treat gastrointestinal tract, respiratory tract and genitourinary tract infections. It functions by enzymatic-inhibition catalytic process to inhibit the growth of diseases-causing microorganisms through interfering with the replication process of DNA gyrase [14]. It is effective on *Bacillus subtilis*, a gram positive bacteria capable of growth within diverse environments including the gastrointestinal tracts. This microorganism is one of the main cause of pneumonia, pan-ophthalmitis, visceral abscess or musculoskeletal infections, and

tissue necrosis [15, 16, 17]. In this study, the action of COAB+Cp on *Bacillus subtilis* was to ensure that the drug retains its antimicrobial potential in the oil phase of the emulsion prior toxicity study of the drug-loaded emulsion on Wistar rats. On this note, this

2.0 Materials and Methods

2.1 Materials

Analytical grade ciprofloxacin from Sigma Aldrich, Germany. Polyethylene (20) sorbitanmonooleate (T 80), polyethylene (20) sorbitanmonolaurate (T 20); and polyethylene glycol (PEG 400) (NOF, Shanghai) were purchased from Evergreen Chemical industry, Idumota Lagos, Nigeria. Freeze-dried *Bacillus subtilis*. The clinical isolates of *Bacillus subtilis* which were collected from Federal Medical Centre (FMC), Idi Aba, Abeokuta, Nigeria were provided by the Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria.

2.2 Emulsification

The emulsification was carried out by weighing 0.075 g (0.3 % w/w) of Cp in a beaker containing 0.75 g (3 % w/w) castor oil. The mixture was homogenized until the drug was completely dissolved in the oil, then, 1.00 g (4 % w/w) of T 20, 4.00 g (16% w/w) of T 80, and 1.00 g (4% w/w) of PEG 400 and 18.25 g (73 % w/w) of distilled-deionized water were gently added. The mixture was homogenized using a magnetic stirrer (Faithful Huanghua SH-4C) at 800 rpm for 60 min [18, 19].

2.3 Management of animals and experimental design

Ten adult Wistar rats, weighing between 100 and 120 g were purchased from the animal breeding unit of the Department of Physiology and Pharmacology, Lead City University, Ibadan, Nigeria. All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health [20] and approved by Lead City University Ethical Review Board with protocol identification number; LCU/ERB/AN0017. The animals were randomly assigned to two groups, I and II containing five (5) animals each (n=5). The animals were acclimatized for two weeks before the commencement of the study. Group I animals served as control and group II animals were orally administered COAB+Cp (10

study aimed at evaluating the cytotoxicity of COAB+Cp on the suspended cells in blood of Wistar rats as well as its pathological effects on the structures of the heart, kidney, spleen and liver.

mL/kg bw) daily for a period of 21 days. After which they were sacrificed and blood samples and organs including the heart, liver, kidney and spleen were collected for analyses

2.3.1 Biochemical assays

The serum activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) was estimated spectrophotometrically (Axiom, England) by Reitman and Frankel method [21], serum total protein and albumin levels were analysed using Randox kits (Randox Laboratories Limited, England) as described by Tietz, [22]. The level of globulin was obtained by difference. Whole blood haemoglobin concentration was spectrophotometrically (Axiom, England) determined by Franco method [23] as described in cypress diagnostic Kit (Cypress Diagonistic, Belgium).

2.3.2 Histopathological Analysis

The tissues collected for histopathology retrieved from each rat were preserved in buffer 10% formalin solution for histopathological processing and examination. The fixed tissues were labeled accordingly, dehydrated by passing it through ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) solutions and cleared in xylene solutions baths to remove the alcohol, followed by infiltrating it in molten paraffin wax. Paraffin sections of the tissues were prepared and cut at 5 or 6 μ m thick with a rotary microtome and stained with haematoxylin and eosin according to Avwioro's method [24] the tissue sections were examined with light microscope.

2.4 Antimicrobial Study

2.4.1 Inoculums preparation

The bacteria strain was prepared overnight at 37 °C. The presence of turbidity in broth culture was adjusted equivalent to 0.5 McFarland standards to obtain standard suspension by adding sterile normal saline in Mueller-Hilton agar slants. The McFarland 0.5 standard provides turbidity comparable to bacterial suspension containing 1.5×10^8 CFU/mL and used at different dilutions in the proposed evaluation tests.

2.4.2 Determination of Antibacterial activities

Antibacterial activities of the drug samples were evaluated by the well plate agar diffusion method using the earlier method with modifications [25]. determined by measuring the zone of inhibition around each well [26].

2.4.3 Determination of minimum inhibitory concentration (MIC)

The MIC of both the drug and drug loaded emulsions were determined by the tube dilution technique, using a modified method Junaid *et al.*, [27]. The smallest concentration that inhibits the growth was taken as the MIC [28].

3.0 Results and Discussion

There was an observable significant difference at $P < 0.05$ in the mean ZI values of COAB+Cp compared to Cp at concentration 500, 125 and 62.15 mg/mL shown in Table 1. Also, the MIC and MBC of COAB+Cp were found to be less than Cp which supports the ZI result as regards the effectiveness and improved bio-potency of Cp in the emulsion phase. This could be due to the surface characteristic and better permeation rate of drug loaded emulsion through the cell membranes' of the microorganism.[30, 31]. It was observed that the antimicrobial potential of both the drug and the drug loaded emulsion reduces as concentration of the treatment reduces.

Table 1: The antimicrobial activities showing the zone of inhibition (ZI) of drugs and drug loaded emulsion on *Bacillus spp.*

Conc.(mg/mL)	COAB (mm)	Cp (mm)	COAB+Cp (mm)
500	0	17.00 ± 0.58**	23.33 ± 0.33**
250	0	16.67 ± 0.33*	17.00 ± 0.57 ^a *
125	0	12.33 ± 0.30**	15.00 ± 0.50**
62.50	0	9.00 ± 0.58*	11.00 ± 0.40*
31.25	0	6.00 ± 0.57**	8.000 ± 1.00**

Data are means of three replicates (n = 3): mean ± SD; ** Shows significant difference at $P < 0.05$ while * Shows no significant difference between Cp and COAB+Cp at $P > 0.05$

2.4.4 Determination of minimum bactericidal concentration (MBC)

The MBC is the lowest concentration of antibiotic agent that kills at least 99.9% of the organism. MBC of Cp and COAB+Cp on the clinical isolate of *Bacillus subtilis* were carried out according to Doughari method [29].

2.5 Statistical Analysis

All statistical analysis were performed using SPSS software 16.0 (SPSS Inc., Chicago, IL) and Prism Graphpad 6.0. The level of significance was set at $P < 0.05$. The results are presented as the mean ± SD.

Figure 1: The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of Cp and Cp-loaded nanoemulsion on *Bacillus spp.*

Some of the signs and symptoms of unhealthy side effects of drugs or drug vehicles include excessive weight gain/loss. In this study, treatment of the rats with COAB+Cp compared with the control group as shown in Table 2 revealed a steady increase in weight gain of the animal. This however suggested no weight loss in the test animals which shows that COAB+Cp does not interfere with food metabolism, nutrient absorption and utilization.

Table 2: Effects of COAB+Cp on Average Body Weight of the Experimental Animals

Days	Control (Kg)	COAB+Cp (Kg)
1	121.10±10.00	125.00±3.53
3	125.90±9.61	127.00±7.07
5	127.00±10.00	128.40±9.90
7	133.05±12.53	131.60±9.19
9	141.75±10.41	134.20±7.78

11	145.70±12.10	134.40±10.61
13	151.35±8.16	136.20±9.90
15	151.75±10.41	138.80±12.02
17	154.15±11.93	140.20±8.49
19	157.00±13.23	141.00±7.07
21	161.10±13.00	145.00±7.10

n=5, Data are represented as the mean±SD

Furthermore, damage to soft tissues particularly liver is often characterized by abnormality in serum activity of enzyme markers such as ALT, AST and ALP. High levels of bilirubin and urea, as well as marked decrease in white blood cells and hemoglobin content may be due to lyses of red blood cells [32]. Liver is one of the vital organs in the body that is prone to toxicity due to its function as the primary site for the metabolism of most ingested substances in the body [33]. During the process of hepatic metabolism, there is a tendency for the build-up of harmful toxic substances that are capable of altering

the morphology of the liver thereby resulting to the leakage of enzymes (ALT, AST and ALP) from the cytosol of the hepatocytes into the circulation [34, 35]. These enzymes, particularly ALT and AST are clinical markers that determines the functionality of liver. Also, total plasma protein which is a sum of albumin and globulin are essential blood parameter in the effective functioning of the body's system. Hence, it is expedient that its plasma concentration exists within certain clinically defined range. Marked deviations from this physiological concentration range could trigger unhealthy metabolic state of the organism in question. The results obtained in this study as shown in Figure 2 proposed that there was no observable significant difference in all the serum parameters of the treated animal when compared to the control at $P > 0.05$. Except in urea which a significant difference was observed between the control and treated rats. This was suggested to be due to the protein content of the treatment.

Figure 2: The Serum parameters of control and Cp-loaded emulsion of Wistar rats

Blood is regarded as life fluid because it is made up of series of suspended cells and these cells are essential life supporting organs which helps in maintaining homeostatic process. The state of the body and its functionality could be reflected by the deviation from the norm in the following blood-related indices such as total protein level, enzyme activity, hematological profile (leucocytes, erythrocytes, neutrophils, and hemoglobin and lymphocyte counts). These are therefore the primary pathological reflectors of the health status of humans and animals [36, 37]. The response of the body defense mechanism against a foreign invasive

pathogenic substance is reflected by the increase in the secretion of these immunogenic parameters; Leucocytes, neutrophils, lymphocytes and monocytes in which their protective response is apt. Conversely, the protective mechanism provided by these body defense mechanism could be suppressed due to the persistence in the toxicity of the invasive substance, thereby resulting in the reduction of these parameter through a process described as immunological suppression. This could however result to severe compromise in the normal physiological functions of the body system which can expose the animal to further toxin and pathogenic attacks resulting to

death. The result of the hematological parameter in this study presented in Table 2 indicates that there was no observable significant difference in the hematological parameters as shown in the test of

significance noted at $P < 0.05$. This however connotes that COAB+Cp does not pose any threat to the functionality of the treated animals compared to the control animals.

Table 3: Hematological parameters of control and COAB+Cp

Parameters	Control	Treatment	P value
PCV (%)	40.00 $\pm$ 5.2*	45.00 $\pm$ 1.47*	0.3928
Hb (g/dl)	15.95 $\pm$ 0.065*	14.10 $\pm$ 0.87*	0.0777
RBC $\times 10^{12/L}$	8.175 $\pm$ 0.28*	8.075 $\pm$ 0.46*	0.8587
WBC $\times 10^9/L$	6.825 $\pm$ 0.28*	5.650 $\pm$ 0.55*	0.1070
Neutrophils (%)	29.25 $\pm$ 1.65*	29.50 $\pm$ 4.63*	0.9611
Lymphocytes (%)	67.25 $\pm$ 1.38*	67.50 $\pm$ 4.63*	0.9604
Eosinophils (%)	1.000 $\pm$ 0.00*	0.5000 $\pm$ 0.29*	0.1340
Monocytes (%)	1.000 $\pm$ 0.00*	1.500 $\pm$ 0.30*	0.1350
Basophils (%)	1.500 $\pm$ 0.28*	1.000 $\pm$ 0.00*	0.1340
MCV (fL)	48.45 $\pm$ 4.79*	56.06 $\pm$ 2.20*	0.1988
MCH (pg)	19.58 $\pm$ 0.70*	17.58 $\pm$ 1.16*	0.1902
MCHC(g/dl)	42.06 $\pm$ 5.57*	31.28 $\pm$ 1.22*	0.1077

Data are means of four replicates ($n = 4$) $\pm$ SEM; * Shows a significant difference at $P < 0.05$ while * Shows no significant difference between Control and COAB+Cp at $P > 0.05$. Hb, hemoglobin; PCV, packed cell volume; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell

Furthermore, the pathological study of the soft tissues is an important parameter that is very essential and cannot be underestimated. Its assessment helps to reveal the impacts of the xenobiotic substance on the soft tissue's proper functionality. The observation in the photomicrographs of the section of heart in Plates 3A and B showed a normal appearance of the myofibers. Likewise the liver and spleen. More so, the kidney is an important organ that houses the nephron, which is responsible for ultrafiltration and reabsorption of fluid in the body. Although, earlier studies reported that acute kidney injury is as a result of tubular necrosis but in recent time, studies have shown that necrosis is not the main form of cell death [38]. Persistent use of drug on daily bases could also result to the alteration of the physiological structure of kidney due to its function as a filter. Proper hydration is one of the precautionary measures to reduce the effect of drugs on the kidney [39]. In this study, mild congestion of interstitial blood vessels, degeneration and necrosis of tubular epithelia tissue was observed in the photomicrograph of a section of kidney treated with COAB+Cp (Plate 2B). This could be due to the persistent administration of treatment as well as poor intake of water for proper hydration of the treated rats. Physiological alteration in the morphology of the spleen and liver was not observed.

This study indicates that administration of COAB+Cp to animals did not jeopardize the morphology of the liver as substantiated by the photomicrograph of the treated animals (Plate 4G) compared to the control counterparts (Plate 4H). It was noted that the pathological studies of the soft tissues examined in this study was in line with the hematological and serum chemistry's results. This shows that the functionality and the integrity of the blood suspended cells were not jeopardized. This findings further attests to the apparent safety of COAB+Cp as a potential vehicle for drug delivery and targeting.

Plate 1: Sections of the heart from control (A) and treated (B) rats (x400; H & E).

Plate 2: Sections of the kidney from control (C) and treated (D) rats (x400; H & E)

Plate 4: Sections of the Liver from control (E) and treated (F) rats (x400; H & E)

Plate 3: Sections of the spleen from control (C) and treated (D) rats (x400; H & E)

4.0 Conclusion

The findings of this study shows that COAB+Cp has the potential to encapsulate the drug due to its effects on *Bacillus subtilis*. And at the investigated

dosage of 10 mL/kg BW, the emulsion was observed to have no cardiotoxic, hematotoxic and hepatotoxic effects on Wistar rats, without any obvious alterations in the physiological structure of the soft tissues and as such appears promising as a safe vehicle for oral drug delivery and targeti

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