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SERUM PROHEPCIDIN AND INTERLEUKIN-6 IN CHLORAMPHENICOL CHALLENGED LIVER OF WISTAR RAT MODELS

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ABSTRACT

The role of the disturbances of hepcidin metabolism, the master regulator of iron homeostasis synthesized in the liver, has been raised in cases of impaired liver function. The aims of this study were to evaluate the serum levels of prohepcidin, serum iron, interleukin-6, liver enzymes: aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) and total bilirubin, on chloramphenicol-administered Wistar rat models, and also investigate the ameliorative property, if any, of *Telfairia occidentalis* on chloramphenicol-induced hepatocyte damage. The hepatocytes of 120 male Wistar rats (130-150g) were challenged in clinical conditions of chloramphenicol administration. Prohepcidin and interleukin-6 were measured by an enzyme linked immunosorbent assay (ELISA) technique, and AST, ALT, ALP and total bilirubin were measured spectrophotometrically. 60 rats served as controls, *while 60 rats were test animals.* The control sub groups were given *Telfairia occidentalis* leaf extract (50 mg/ml) and ferric hydroxide (50 mg/5ml) respectively with rat chow, for 21 days, while the test groups were administered Chloramphenicol (86mg/kg body weight) for 21 days, with normal feeding and treated as control sub-groups.

A significant decrease ($p<0.05$) in serum prohepcidin level, and IL-6, was observed in chloramphenicol administered rat models, while serum AST, ALT, and ALP activities were elevated ($p<0.05$) in the rat models. Administration of *Telfairia occidentalis* to the rat models greatly improved liver function and enhanced humoral immunity of the test animals. In conclusion, serum prohepcidin level was decreased in chloramphenicol-induced liver impairment and *Telfairia occidentalis* had restorative and immune boosting properties.

INTRODUCTION

The liver plays an important biochemical role in the digestion, metabolism, detoxication and elimination of substances from the body[1]. It is the primary site of metabolism of both endogenous substances and exogenous compounds such as drugs and toxins, a process of biotransformation. The liver is a large organ and has ability to carry out its functions with extensive reserve capacity.

Hepcidin is a peptide hormone synthesized by the liver that regulates iron homeostasis in humans and other mammals[2]. It is produced by the hepatocytes in response to iron overload and inflammatory stimuli[3-5].

Iron, an essential element for all living organisms, is required for metabolic processes which include oxygen transport, DNA synthesis and energy production. Excess iron is harmful to the body through the generation of oxygen radicals[6].

KEYWORDS: *Prohepcidin, Chloramphenicol, liver enzymes, interleukin-6, Iron, Telfairia occidentalis,*

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Hepcidin acts by down-regulating both iron absorption and iron release by enterocytes and macrophages' in response to high iron levels and inflammatory cytokines such as interleukin-6[5]. Studies have indicated that hepcidin deficiency is implicated in many iron metabolism disorders[3,9]. In addition to iron deficiency or iron overload, some of the diseases that can occur in dysregulation of iron homeostasis include enzyme destruction, or degenerative organ diseases of the liver, splsen and kidney[10].

The drug chloramphenicol, a bacteriostatic antimicrobial, has a broad spectrum of activity. It is active against a wide variety of Gram-positive and Gram-negative bacteria, and most anaerobic organisms[11]. Chloramphenicol is metabolized by the liver to the inactive form, chloramphenicol glucuronate. It is widely used, available and cheap and thus, prone to abuse. Some serious adverse effects have been ascribed to chloramphenicol administration: reversible bone marrow suppression[11], aplastic anaemia[12], increased risk of childhood leukaemia[13], and gray baby syndrome[14]. However, there is paucity of information on the effect of chloramphenicol on the synthesis of hepcidin by the liver and its consequences on iron homeostasis.

Telfairia occidentalis (Ugu), a member of the family Cucurbitaceae commonly called fluted gourd or pumpkin, is a vegetable food crop indigenous to Nigeria and other West African countries. Some studies have attributed haematological indices (increased haemoglobin and haematocrit levels) to this vegetable[15-16].

The present study, while determining the serum levels of prohepcidin, iron and interleukin-6, would also investigate the effects of *Telfairia occidentalis* on the hepatocytes of rat models under clinically challenged chloramphenicol administration over a period of time. Ameliorative property of *Telfairia occidentalis* if any would be compared against a reference salt, ferric hydroxide (Orofer®)[18].

Therefore, the aims of the present study were to determine the effect of prolonged chloramphenicol abuse on the extent of liver damage and on the liver's role in iron homeostasis by determining the levels of serum prohepcidin, serum iron, interleukin-6, liver enzyme activities: Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosephatase (ALP), and serum total bilirubin, and to investigate the ameliorative effect, if any, of Telfairia occidentalis on chloramphenicol-induced hepatocyte damage.

MATERIALS AND METHODS

Materials:

The experimental animals for the study were male Wistar rats procured and housed in the animal house of the Anatomy Department, School of Basic Medical Sciences, University of Benin, Benin City. Animals were allowed to acclimatize for two weeks before experimentation.

A total number of one-hundred and twenty (120) rats, 12-14 weeks old, weighing between 130-150g were used. These animals were divided into two (2) main groups: the control group and the test animals. Each group comprised sixty (60) male Wistar rats. These were subdivided into 3 groups of twenty (20) rats per sub group. In the control group, first sub group (n=20) was given normal rat chow and water freely and no other treatment. The second sub-group (n=20) was given *Telfairia occidentalis* leaf extract (Ugu) (50 mg/ml) for 21 days in addition to food and water. The third sub-group (n=20) was given ferric hydroxide polymaltose (trade name Orofer®) (50 mg/5ml) for 21 days, as

well as rat chow with food and water. Ferric hydroxide polymaltose (Orofer®)) was bought at a reputable Pharmacy in Benin City. Treatments were administered through orogastric tube, ensuring maximum utilization of what was given.

The test group was administered Chloramphenicol (Clofencol®) given through orogastric feeding for 21 days, at a dose of 86 mg/kg body weight as previously described by Ebaid[17]. Chloramphenicol, a prescription drug, was bought at a reputable Pharmacy in Benin City. The three (3) sub-groups in addition to their diets of rat chow and water were treated as follows: sub-group 1(n=20): chloramphenicol only, sub-group 2 (n=20): chloramphenicol and *Telfairia occidentalis* (50mg/ml), and sub-group 3 (n=20): chloramphenicol and Ferric hydroxide polymaltose 50mg/5ml. The treatments lasted for 21 days.

After 21 days, the rats were sacrificed by anaesthetizing with chloroform. Blood was removed by cardiac puncture and collected into non-anticoagulant specimen containers for serum sample preparation. Serum specimens were obtained by centrifuging coagulated blood at 3000g for five (5) minutes and stored at -65°C for analyses that could not be done immediately.

The liver was quickly removed and stored into universal bottles containing fixative 10% formol-saline for histological examinations. Heamatoxylin-eosin stain was used for cell morphology.

Plant Extract: Aqueous *Telfairia occidentalis* (Ugu) leaf extracts (800 g) were prepared in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin-City. Fresh Telfairia occidentalis leaves were bought from the local market (New Benin Market). The leaves were weighed (800g) and ground (about 800ml of distilled water added) to increase surface area for extraction. It was then filtered to separate the fibre and filtrate. The filtrate was freeze-dried to yield a fine powdery concentrate. This was done at the Energy Centre, University of Benin, Benin City.

Measurement of liver enzymes

The serum transaminases (aspartate transaminase and alanine transaminase) were measured spectrophotometrically using reagent kits from Randoz Laboratories Ltd, Ardmore, Diamond Road, Crumlin, Co. Antrim, UK.

Diagnostic reagent kit from Teco Diagnostics, 1268N. Lakeview Ave. Anaheim, CA92807, was used to analyse alkaline phosphatase.

Measurement of serum prohepcidin and rat interleukin-6

Prior to analysis, serum samples stored at -65°C were allowed to thaw at room temperature before analysis. Serum Prohepcidin and rat Interleukin-6 were assayed according to the manufacturer's instructions, using DRG Diagnostics ELISA products from DRG Instruments; Germany. Results of ELISA assays were read using Plate Reader: Stat Fax Model 303/PLUS, Serial No: 303-9024 by Awareness Technology Inc. Palm City, USA.

Serum iron assay

Serum iron analysis was performed in Martlet Environmental Research Laboratory Limited, Benin City, using atomic absorption spectrophotometric method. The instrument was Atomic Absorption Spectrophotometer (AAS) model SOLAAR 969 Unicam Series and the flame used was Air acetylene flame. All reagents were Analar Grade.

Statistical analyses were conducted using standard error of mean (SEM) and analysis of variance (ANOVA) with Student-Newman-Keul Post-test to compare control animals with the treated groups, and also compare variations within the treated groups. A P value < 0.05 was considered to be significant. Statistical analysis was performed using GraphPad Instat3 software.

RESULTS

In figure1, control rats were compared with chloramphenicol-treated rats for serum prohepcidin level. There was a significant decrease for serum prohepcidin level in chloramphenicol-treated rats (P<0.05). Serum prohepcidin level was significantly increased in rats fed ferric hydroxide polymaltose (P<0.05). Treating chloramphenicol-fed rats with Telfairia occidentalis greatly increased the serum level of prohepcidin.

Figure 2 showed control rats compared against chloramphenicol-treated rats for serum interleukin-6 (IL-6). The differences in their mean values were statistically significant (P<0.05) in some of the sub-groups. Decreased levels: rats fed *Telfairia occidentalis* (P<0.05), chloramphenicol-treated rats (P<0.05), and increased levels: rat fed *Telfairia occidentalis (P<0.05),* chloramphenicol+ T. occidentalis-treated rats (P<0.05), and chloramphenicol+ Ferric hydroxide polymaltose -treated rats (P>0.05).

In figure 3, serum total ALP activity for control rats were compared against chloramphenicol-treated sub-groups. In chloramphenicol-treated rats, ALP parameter was elevated (P<0.05, and also for chloramphenicol+ T. occidentalis-treated and chloramphenicol+ Ferric hydroxide polymaltose -treated sub-groups (P<0.05).

Figure 4 showed serum AST activity for chloramphenicol-treated rats compared against control animals. Serum AST activity was increased in Chloramphenicol-treated sub-group (P<0.05), and chloramphenicol+ Ferric hydroxide polymaltose-treated rats (P<0.05), but decreased in Chloramphenicol+ *T. occidentalis*- treated rats (P<0.05) and in control rats given T. occidentalis (P<0.05) and Ferric hydroxide polymaltose (P>0.05).

In figure 5, serum ALT activity for chloramphenicol-treated rats was compared against control animals. Serum ALT activity was increased in Chloramphenicol-treated sub-group (P<0.05), and chloramphenicol+ Ferric hydroxide polymaltose-treated rats (P<0.05), decreased in Chloramphenicol+ *T. occidentalis* treated rats (P>0.05) and in control rats fed *T. occidentalis* (P>0.05).

In figure 6, serum total bilirubin level for chloramphenicol-treated rats was compared against control animals. In Chloramphenicol-treated rats serum total bilirubin level was not significantly increased (P>0.05). For control rats fed *T. occidentalis,* and Chloramphenicol+ ugu-treated rats, serum total bilirubin level was decreased (P<0.05). Changes in the other sub-groups were not significant (P>0.05).

Serum Prohepcidin

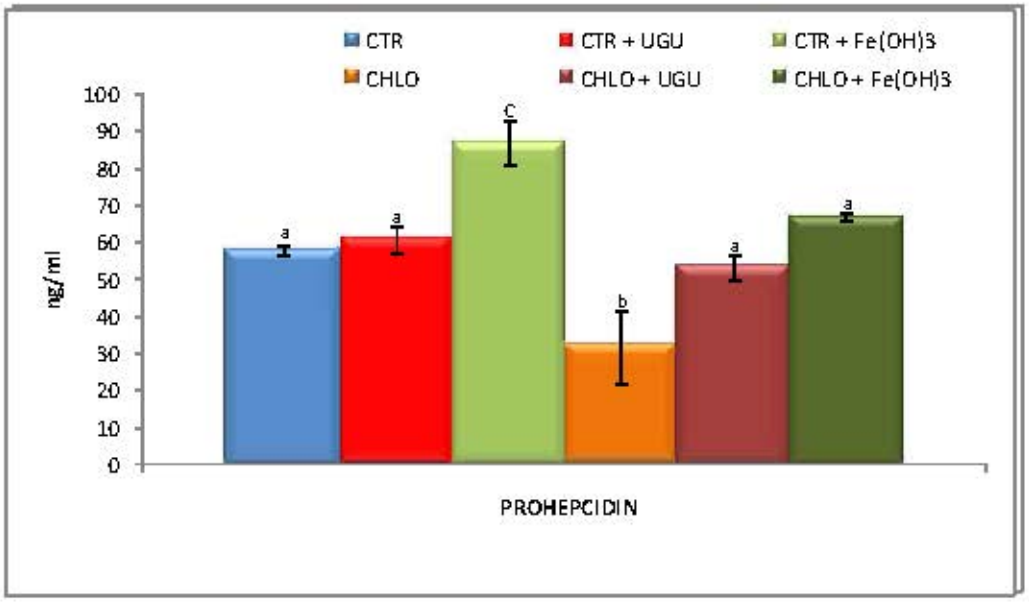

Fig.1, shows from left to right serum level of Prohepcidin for Control rats, Controls+Ugu, Controls+ferric hydroxide, Chloramphenicol-treated rats, Chloramphenicol+Ugu, and chloramphenicol+ ferric hydroxide -treated rats. Different superscript letters differ significantly from each other at P<0.05.

Serum Interleukin-6 (IL-6)

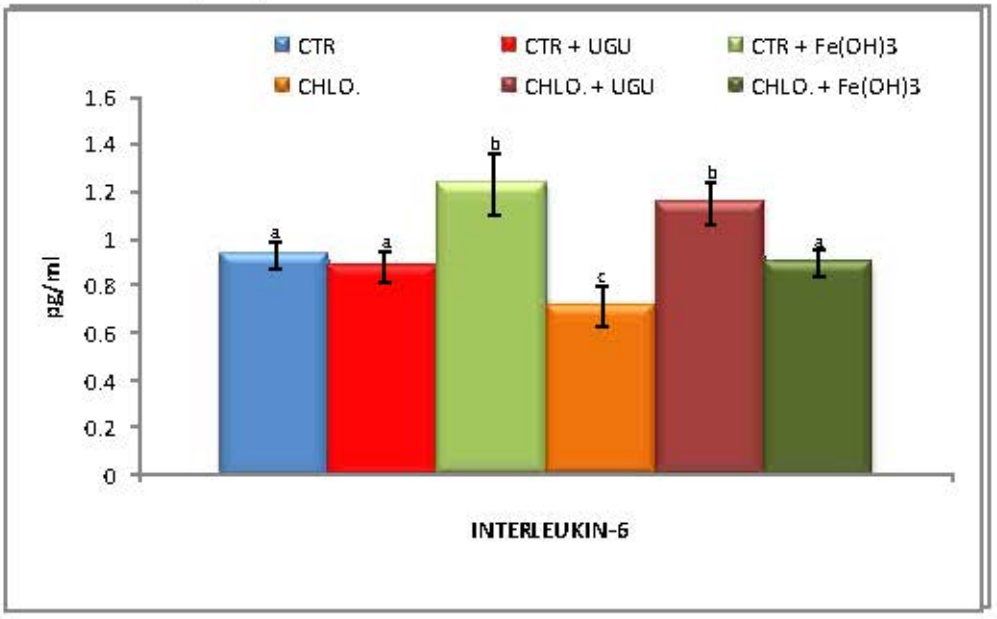

Fig 2 shows from left to right serum level of Interleukin-6 (IL-6) for Control rats, Controls+Ugu, Controls+ferric hydroxide, Chloramphenicol-treated rats, Chloramphenicol+Ugu, and chloramphenicol+ ferric hydroxide-treated rats. Different superscript letters differ significantly from each other at P<0.05

Serum total ALP

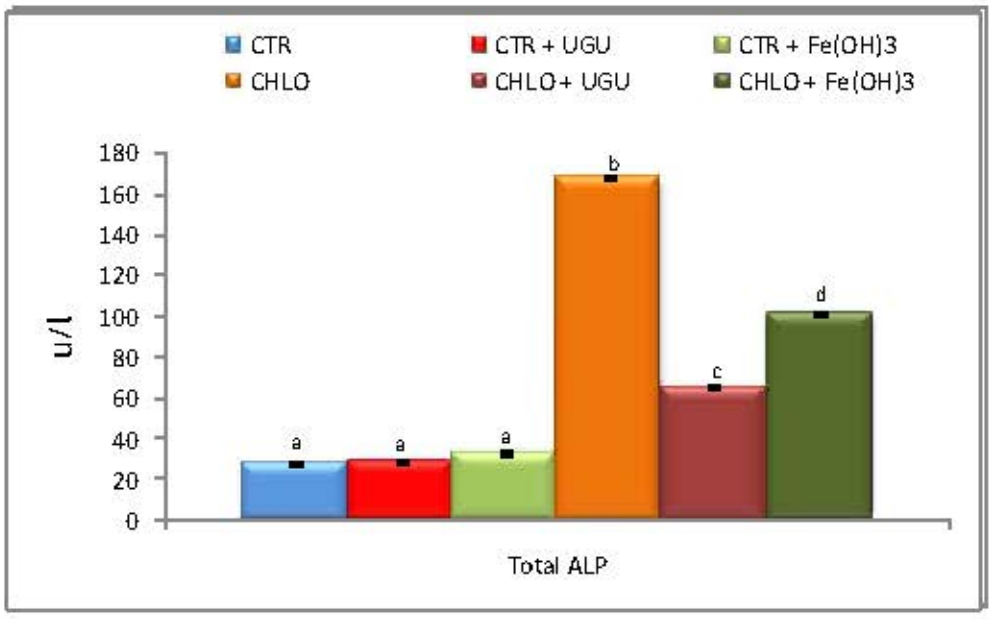

Fig. 3 shows from left to right serum total ALP activity for control rats Control rats, Controls+Ugu, Controls+ferric hydroxide, Chloramphenicol-treated rats, Chloramphenicol+Ugu, and Chloramphenicol+ ferric hydroxide-treated rats. Different superscript letters differ significantly from each other at P<0.05.

Serum AST

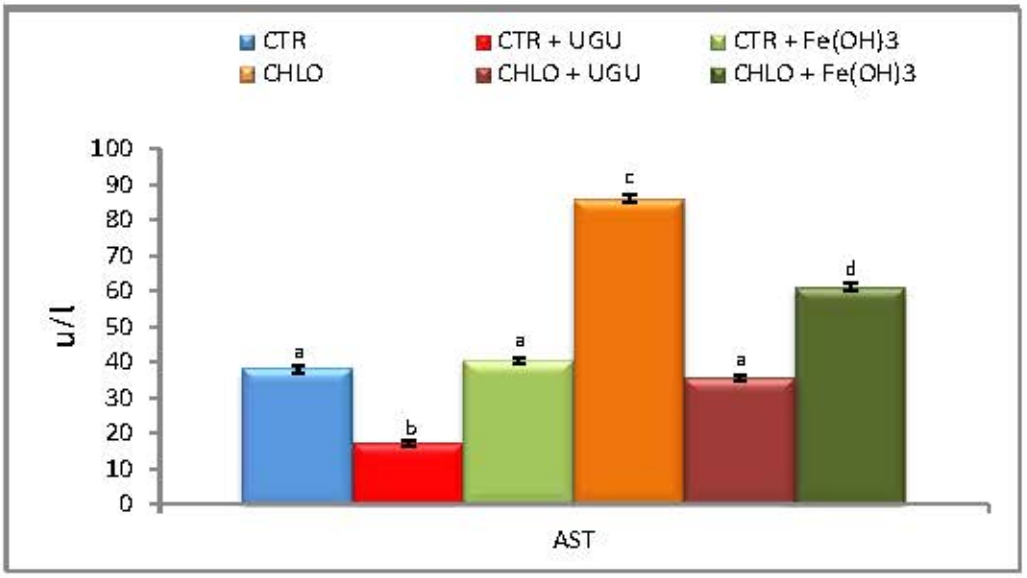

Fig.4: shows from left to right Serum AST activity for Control rats, Controls+Ugu, Controls+ ferric hydroxide, Chloramphenicol-treated rats, Chloramphenicol+Ugu, and chloramphenicol+ ferric hydroxide-treated rats. Different superscript letters differ significantly from each other at P<0.05.

Serum ALT

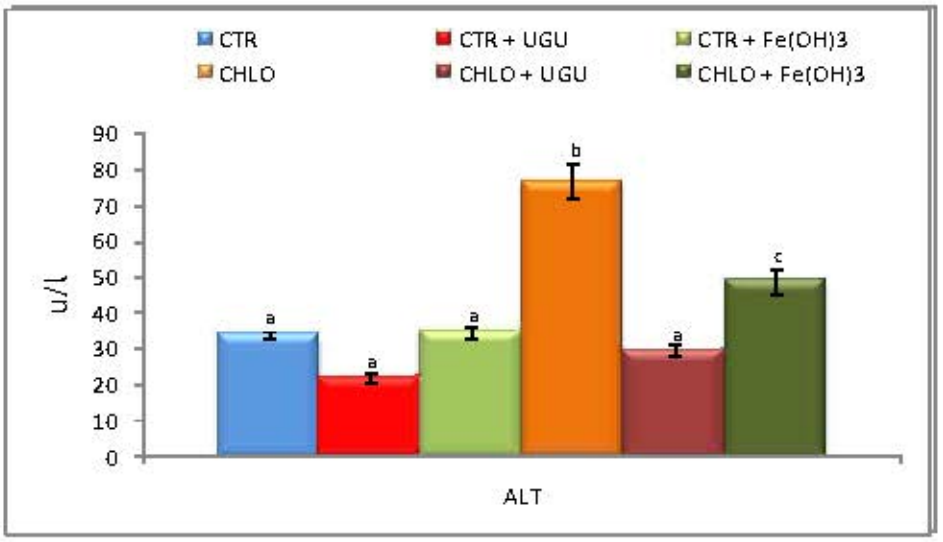

Fig. 5 shows from left to right serum ALT activity for Control rats, Controls+Ugu, Controls+ ferric hydroxide, Chloramphenicol-treated rats, Chloramphenicol+Ugu, and chloramphenicol+ ferric hydroxide-treated rats. Different superscript letters differ significantly from each other at P<0.05.

Serum Total Bilirubin

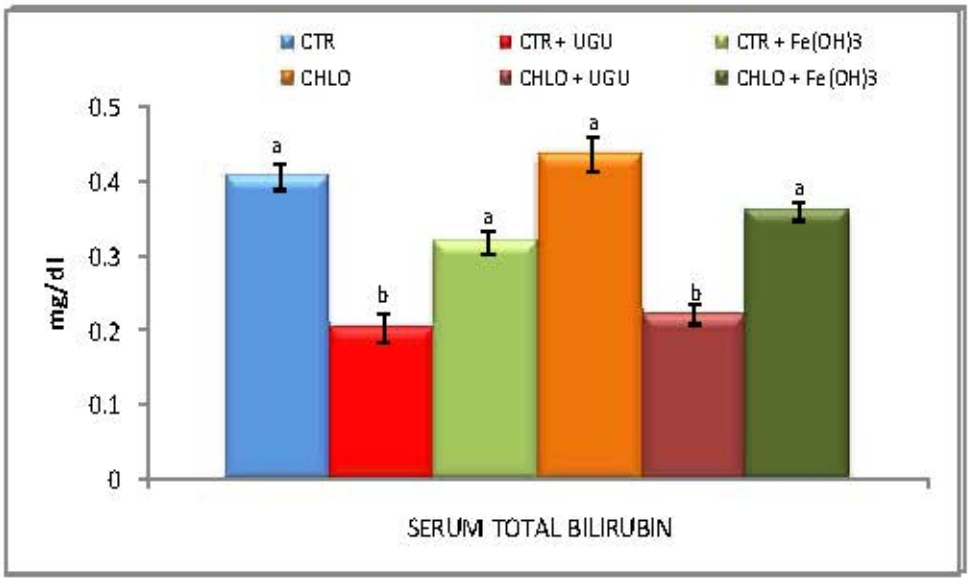

Fig. 6: shows from left to right Serum total bilirubin for Control rats, Controls+Ugu, Controls+ ferric hydroxide, Chloramphenicol-treated rats, Chloramphenicol+Ugu, and chloramphenicol+ ferric hydroxide -treated rats. Different superscript letters differ significantly from each other at P<0.05.

DISCUSSION

Investigating the liver status of chloramphenicol administered rat models in the present study revealed decreased levels of serum prohepcidin. Liver function parameters, AST and ALT were also found to be altered by chloramphenicol administration. Changes in the serum iron levels of the experimental animals correlated with the serum prohepcidin level.

The study has demonstrated that prolonged use of chloramphenicol might have deleterious effect on the hepatocytes as indicated by the elevated serum AST, ALT and ALP activities. Serum AST and ALT are sensitive makers in the diagnosis of hepatic damage as they are cytoplasmic enzymes released into circulation upon hepatic cellular damage. Previous study by Nwanjo[18], also reported increased activities in halofantrine toxicity in Wistar rats. Their work corroborates with the present study which revealed that chloramphenicol was hepatotoxic as indicated by the raised transaminases. Drug that affects the biliary tree raises ALP activity. Chloramphenicol administration might have affected the biliary tree and thus, raised the serum ALP activity in the rat models.

Serum interleukin-6 level was found decreased in chloramphenicol administered rat models. Interleukin-6, a cytokine which plays a role in cell mediated immune response is known to positively influence hepcidin expression[19]. The low level of interleukin-6 reported in this study suggested that the adverse effect of chloramphenicol on the hepatocyte did not activate interleukin-6 production, and which in turn, did not activate hepcidin production.

Serum bilirubin levels were increased although not significantly. The effect of chloramphenicol administration on the biliary tree was not too adverse as to have caused extra hepatic obstruction.

Administration of *Telfairia occidentalis* greatly enhanced the hepatic status of the test animals. The serum levels of prohepcidin and interleukin-6 were all improved, while serum transaminase activities were decreased. Probably, the high iron content of *Telfairia occidentalis* had helped to obliterate the effect of anaemia induced by chloramphenicol in the study animals. The work of Osuntoki and Sanusi[16] ascribed haematological property to *Telfairia occidentalis*. Thus, *Telfairia occidentalis* effectively ameliorated the adverse effect of chloramphenicol on the rat models, and the hepatocytes conditions were greatly improved upon its administration.

Hypothesizing therefore, the data from the present study suggest that the effects of drugs such as chloramphenicol on hepatocytes could modulate iron metabolism and thereby influence serum hepcidin level. This hypothesis is reinforced within the *Telfairia occidentalis* sub group which exhibited improved hepatocyte function and improved serum prohepcidin and iron levels. Conversely, rat models treated with chloramphenicol only, exhibited hepatotoxicity. Further study is necessary to investigate the effect of hepcidin expression in prolonged chloramphenicol administration.

In conclusion, chloramphenicol administration caused hepatotoxicity, did not increase serum prohepcidin level while Telfairia occidentalis was an effective ameliorating agent. Chloramphenicol which is metabolized by the liver to its inactive form could be toxic with prolonged usage. Therefore, it would be necessary to monitor liver function status of patients on prolonged chloramphenicol therapy because of the adverse effect of this drug on the hepatocytes.

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Conflict of interest
The authors state that there is no conflict of interest.

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