Effect of Long-Term Oral Administration of Chloramphenicol on Hepato-Renal and Oxidative Stress Biomarkers in *Sprague dawley* Rats

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ABSTRACT

Chloramphenicol was discovered from Streptomyces venezuelae and synthetically produced in 1948. This investigation aimed on the effect of long-term oral administration of chloramphenicol on hepato-renal and oxidative stress biomarkers in Sprague dawley rats. After each of the ten rats in the experimental group consumed 250 mg of chloramphenicol dissolved in 15 milliliters of distilled water on a daily basis for 30 days, five milliliters of blood specimen were taken from each of them and dispensed into lithium heparin anticoagulated bottle respectively. The remaining ten rats, which served as controls, were not administered with chloramphenicol or any other antibiotics. The rats in the experimental group demonstrated mean values of alanine aminotransferase (21.16 ± 2.81) U/L, aspartate aminotransferase (20.27 ± 2.60) U/L,, alkaline phosphatase (37.10 ± 2.92) U/L, creatinine (32.17 ± 0.88) µmol/L, urea (6.74 ± 0.24) mmol/L, glutathione peroxidase (4.78 ± 0.61) µmol/L and malondialdehyde (4.71 ± 0.57) mmol/L as being statistically significant when compared to that of the control group alanine aminotransferase (4.02 ± 0.83) U/L, aspartate aminotransferase (3.97 ± 0.64) U/L, alkaline phosphatase (12.80 ± 1.75) U/L, creatinine (32.12 ± 0.87) µmol/L, urea (6.71 ± 0.22) mmol/L, glutathione peroxidase (2.15 ± 0.10) µmol/L and malondialdehyde (2.02 ± 0.06) mmol/L respectively. In conclusion, taking 250 mg of chloramphenicol diluted in 15 milliliters of distilled water every day for 30 days may trigger hepato-oxidative stress disorders in Sprague dawley rats

KEYWORDS: *Hepato-renal biomarkers, Oxidative stress biomarkers, Chloramphenicol consumption, Long term effect, Sprague dawley rats*

1. INTRODUCTION

Chloramphenicol was initially identified in 1947 as a naturally transpiring chemical compound synthesized by the soil and compost-dwelling microorganism *Streptomyces venezuelae*. In 1948, two epidemics of typhoid fever in Bolivia and Malaysia served as compelling evidence of the effectiveness of this antibiotic. As a result, the United States Food and Drug Administration granted approval for its usage as the initial broad-spectrum antibiotic (Donald, 2012).

Due to its wide-ranging antibacterial capabilities and ability to easily enter tissue and fluid, it is widely acknowledged and accepted. In addition to effectively treating acute bronchitis, this medication was successfully employed to address many viral ailments during the 1950s (Donald, 2012). After gaining significant popularity, the utilization of this medication began to decrease in the 1960s due to the discovery of two separate impacts on the bone marrow, resulting in aplastic anaemia and dose-dependent toxicity. This toxicity can lead to anaemia, which can be restored by discontinuing the medicine. The grey syndrome is an additional indication of chloramphenicol poisoning in babies (Flegg *et al.,* 1992).

The medicine is called D-(-)-threo-1-p-nitrophenyl-2-dichloroacetamido 1, 3-propandiol at the molecular level. The substance possesses a crystalline structure, which appears white in colour, and has a neutral nature. It readily dissolves in alcohol but does not dissolve in water. The substance has four stereoisomers, two of which are D-erythro and L-erythro chemical isomers. Lerythro's bacteriostatic efficiency is significantly lower, at only 2%, compared to D-erythro's 98%. In addition, succinate and palmitate are two esterified versions of this medication that have been found to be ineffective against microbes (Pooja *et al.,* 2011).

This medication is a broad-spectrum antibiotic available in liquid or pill form. It has been proven to be specifically effective against a variety of organisms that are the primary causes of numerous diseases in both domestic animals and humans (Arriola-Dechert, 2006). This medication can be administered intravenously or via parenteral routes such as infusion, eye drops, or ear drops. Alternatively, it can be ingested in the form of oral capsules. Considering the significant potential for toxicity and negative impacts, it is advised to limit the dosage of this medication to therapeutic levels not exceeding 50 mg/kg/day. The recommended approach is to take the medication in divided doses at intervals of 6 hours (Statpearls, 2021). Infections produced by organisms exhibiting a moderate level of resistance may necessitate an escalation in dosage to 100 mg per kilogramme per day. If an escalation in dosage is necessary, it is imperative to closely monitor the patient, and the dosage should be promptly reduced to 50 mg/kg/day. In addition, the dosage is reduced to 25 mg/kg/day for neonates and patients with compromised renal or hepatic function (Statpearls, 2021).

Multiple studies have demonstrated the detrimental effects of prolonged chloramphenicol usage on human health (Saba *et al.,* 2000). Notwithstanding these firmly established findings, the majority of individuals continue to manage typhoid without obtaining a prescription from a medical doctor or other duly certified healthcare professional. Hence, it is imperative to carry out

this study in order to ascertain the impact of prolonged usage of this medication on the biochemical markers of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (liver biomarkers), creatinine, urea (kidney biomarkers), glutathione peroxidase, and malondialdehyde (oxidative stress biomarkers) in *Sprague dawley* rats.

2. MATERIALS AND METHODS

2.1. Study Area

The study area was the Department of Medical Laboratory Science, located in the Faculty of Basic Medical Sciences within the College of Health Sciences at Niger Delta University, situated at Wilberforce Island, Bayelsa State, Nigeria.

2.2. Ethical Approval

Approval was obtained from the College of Health Ethical Committee, and the research was conducted in strict compliance with the National Guidelines for Animal Research.

2.3. Experimental Design

The study exclusively utilized an animal model.

2.4. Inclusion and Exclusion Criteria

The *Sprague dawley* rats utilized in this work were between 3 and 4 months old. The rats included in this group are all healthy males with a weight range of 131.0 ± 0.5 g. Rats exhibiting symptoms of disease were not included.

2.5. Animal Study

The study included 21 male *Sprague dawley* rats, aged 3-4 months, with an average weight of around 131.0 ± 0.5 g.

The rats were procured from the Pharmacology Department of Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, and subsequently transferred to the animal house of the Department of Medical Laboratory Science at Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. They were housed there for a period of 2 weeks to allow for acclimatization and to monitor for any physical deformities or illnesses that would render them unsuitable for use in this study. Throughout this procedure, the rats were housed in well-ventilated iron steel cages and provided with pre-mixed rat feed and water.

2.6. Pilot Study

The LD_{50} of chloramphenicol, which is the minimal dose that causes fifty percent mortality in experimental rats, was determined. This study utilized 16 rats, aged 3-4 months and weighing 131.0 ± 0.5 g. The rats were divided into four groups, each consisting of four rats, labelled as A,

B, C, and D. The rats in groups A, B, C, and D were administered chloramphenicol doses of 175 mg, 250 mg, 500 mg, and 750 mg, respectively, via oral administration.

Following the trial, all rats underwent a 24-hour period of surveillance to detect any indications, symptoms, or mortality. The presence of these observations in group C suggests the LD_{50}, which was determined using arithmetic calculations as outlined by Nwachukwu *et al.* (2015).

2.7. Sub-Chronic Toxicity Study

The study used 20 rats aged between 3 and 4 months, with an average weight of 131.0 ± 0.5 g. The rats were randomly divided into two groups.

2.8. Experimental Group

The group comprised 10 male *Sprague dawley* rats, with an age range of 3-4 months and a weight of 131.0 ± 0.5 g. Every rat received a daily dose of 250 mg of chloramphenicol, which was dissolved in 15 ml of distilled water, for a duration of 30 days.

2.9. Control Group

The rats in this group were free from chloramphenicol administration

Anaesthetization of the rats by the chloroform technique, took place after the completion of this experiment which was followed by the cardiac withdrawal of 5 ml blood specimen from each of them into lithium heparin anti-coagulated bottles respectively which was used alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine, urea, C-reactive protein, interleukin 6, glutathione peroxidase and malondialdehyde investigations.

2.10. Sample Size Determination

This was determined using the Resource Equation approach as outlined by Wan and Wan (2017).

2.11. Laboratory Procedures

2.11.1 Measurement of Alanine Aminotransferase

The colorimetric approach described by Emmanuel *et al.* (2020) was employed in this study, using the kit of Randox Laboratories Limited located at 55 Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom.

2.11.2 Measurement of Aspartate Aminotransferase

The colorimetric method developed by Egoro *et al.* (2021), previously described by Randox Laboratories Limited located at 55 Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom, was employed.

2.11.3 Measurement of Alkaline Phosphatase

The colorimetric method developed by Randox Laboratories Limited, located at 55 Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom, and later updated by Egoro *et al.* (2021), was employed.

2.11.4 Measurement of Creatinine

The Jaffe reaction method, as defined by Randox Laboratories Limited at 55 Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom, with modifications by Emmanuel *et al.* (2021), was employed.

2.11.5 Measurement of Urea

The described Urease Berthelot method by Randox Laboratories Limited 55, Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom as modified by Emmanuel *et al.* (2021) was used.

2.11.6 Measurement of Glutathione Peroxidase

The modified ultra violet method provided by Bio-diagnostic, located at 29 Tahreer Street, Dokki, Giza, Egypt, as outlined by Kshipra *et al.* (2018), was utilized.

2.11.7 Measurement of Malondialdehyde

The modified ultra violet procedure provided by Bio-diagnostic, located at 29 Tahreer Street, Dokki, Giza, Egypt, as outlined by Wali *et al.* (2020), was utilized.

2.12. Statistical Analysis

The data collected from both the experimental and control groups were analyzed using SPSS version 23.0. The mean and standard deviation were used to express the results, while the differences between the two groups were assessed using the student's "t" test. A p-value less than 0.05 was deemed to be statistically significant.

3. RESULTS AND DISCUSSION

Table 1 Mean ± SD of Long-Term Consumption of Chloramphenicol on Hepatic Biomarkers in *Sprague dawley* Rats (Experimental Group) Compared with the Control Group

Parameters	Control group (n=10)	Experimental group (n=10)	p-value	Remark
ALT (U/L)	4.02 ± 0.83	21.16 ± 2.81	0.01	S
AST(U/L)	3.97 ± 0.64	20.27 ± 2.60	0.01	S
ALP(U/L)	12.80 ± 1.75	37.10 ± 2.92	0.01	S

KEYS: ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase, S = Statistically significant, n = Number of rats

Table 1 demonstrates that the average values of the hepatic enzyme biomarkers, namely alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, were considerably higher ($p = 0.01$) in the experimental rats compared to the control group.

Table 2. Mean ± SD of Long-Term Consumption of Chloramphenicol on Renal Biomarkers in *Sprague dawley* Rats (Experimental Group) Compared with the Control Group

Parameters	Control group (n=10)	Experimental group (n=10)	p-value	Remark
Creatinine (µmol/L)	32.12 ± 0.87	32.17 ± 0.88	0.87	NS
Urea (mmol/L)	6.71 ± 0.22	6.74 ± 0.24	0.76	NS

KEYS: NS = Not statistically significant, n = Number of rats

Table 2 demonstrates that the average levels of the renal biomarkers, creatinine ($p = 0.87$) and urea ($p = 0.76$), did not undergo significant changes in the experimental animals when compared to the control group.

Table 3. Mean ± SD of Long-Term Consumption of Chloramphenicol on Oxidative-Stress Biomarkers in *Sprague dawley* Rats (Experimental Group) Compared with the Control Group

Parameters	Control group (n=10)	Experimental group (n=10)	p-value	Remark
GPx (µmol/L)	2.15 ± 0.10	4.78 ± 0.61	0.02	S
MDA (mmol/L)	2.02 ± 0.06	4,71 ± 0.57	0.02	S

KEYS: GPx = Glutathione peroxidase, MDA = Malondialdehyde, S = Statistically significant, n = Number of rats

Table 3 demonstrates that the average levels of the oxidative-stress indicators, glutathione peroxidase ($p = 0.02$) and malondialdehyde ($p = 0.02$), were considerably higher in the experimental rats compared to the control group.

This study involved comparing the average values of the measured biomarkers in the experimental *Sprague dawley* rats with those of the control group. Table 1 displays the hepatic enzyme biomarkers that exhibited notable increases in the experimental group of *Sprague dawley* rats compared to the control group. Specifically, alanine aminotransferase ($p = 0.01$), aspartate aminotransferase ($p = 0.01$), and alkaline phosphatase ($p = 0.01$) showed significant elevations. The observed elevations, consistent with the findings of Saba *et al.*, 2000, may be linked to the prolonged use of this antibiotic. This prolonged use may have caused strain on the liver as it tried to break down the antibiotic, resulting in the generation of harmful free radicals that can damage the liver. This condition likely caused the release of these enzymes into the bloodstream.

Table 2 displayed the average values of renal biomarkers in the experimental group of *Sprague dawley* rats, in comparison to the control group. The results indicated that there were no statistically

significant differences (p = 0.87) in the levels of creatinine and urea (p = 0.76) compared to those of the control group, respectively. The discovery, which suggests that the kidney is not negatively affected by long-term ingestion of chloramphenicol, contradicts the prior research conducted by Saba *et al.*, 2000. However, this observation suggests that the kidneys may possess the capacity to endure and remove the byproducts of this antibiotic in rats that have received it regularly for an extended duration of 30 days.

The average levels of oxidative-stress indicators in the rats of the experimental group showed a statistically significant increase in glutathione peroxidase (p = 0.02) and malondialdehyde (p = 0.02) compared to the control group, as shown in Table 3. The notable increases in these indicators of oxidative stress may be linked to their involvement in defending against the heightened production of free radicals. These rises may indicate oxidative stress resulting from the buildup of antibiotic metabolites due to prolonged daily intake over a 30-day period. However, this is the result obtained in the current study.

CONCLUSION

Prolonged administration of chloramphenicol over a period of 30 days can lead to an increase in hepatic enzyme biomarkers, specifically alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, in addition to inducing oxidative stress in *Spague dawley* rats.

Recommendation

This antibiotic, which requires careful administration, should be used strictly according to the prescription of physician or trained healthcare personnel.

Disclosure of conflict of interest

No conflict of interest

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