Influence of Azithromycin Treatment on Hepatic Lipid Peroxidation and Antioxidant Defence Systems of Rats

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ABSTRACT

Aim: Azithromycin is a semisynthetic macrolide antibiotic commonly indicated for use in middle ear, upper and lower respiratory tract infections, bronchitis, and community-acquired pneumonia with a well-established safety and efficacy profile. The antibiotic is usually well tolerated; however, it has been associated with rare cases of progressive cholestatic hepatitis and fulminant hepatic failure. This study was therefore designed to examine the effect of two preparations of azithromycin; Zithromax 250® (5.6 mg/kg body weight, b.w.) and Zithromax 500® (11.2 mg/kg b.w.) on some hepatic and renal function indices, oxidative stress and antioxidant defense system in rats.

Study Design: Toxicological, Histological and Biochemical study.

Place and Duration of study: Biochemistry Unit, Department of Chemical Sciences, Faculty of Natural Sciences, Ajayi Crowther University, Oyo, Nigeria between March 2012 and April 2012.

Methodology: Thirty rats (Wistar strain) weighing between 180 – 200 g were randomly assigned into three treatment groups of ten animals each; Group A (Control) did not receive any drug, Group B received Zithromax 250® (5.6 mg/kg b.w) and Group C received Zithromax 500® (11.2mg/kg b.w) twice daily for seven days.

Results: Urea, Creatinine and Bilirubin levels were significantly (p=0.05) elevated in the plasma of the rats that received Zithromax 250® and Zithromax 500® by 20.0% and 35.0%, 78.0% and 130.0%, and 14.0% and 47.4% respectively when compared to the control. Activities of some marker enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Gamma-glutamyl transferase (GGT) were also significantly increased (p=0.05) in the plasma of treated animals by 19.0% and 36.6%, 27.0% and 36.4%, 16.0% and 23.5%, and 43.9% and 80.5% respectively. Also, there was a significant increase (p=0.05) in plasma Triglycerides, Total cholesterol, HDL- and LDL-cholesterol in the treated animals by 20% and 31.2%, 19% and 35.4%, 24% and 74.3%, and 33% and 35.2% respectively. Furthermore, Zithromax 250® and Zithromax 500® significantly (p=0.05) reduced the levels of hepatic Ascorbic acid, Glutathione (GSH) and activities of Glutathione-S-transferase (GST) by 35%, 66.3% and 34% and 56.1%, 45% and 50%, respectively. In addition, there was a significant decrease in the activities of hepatic Catalase (CAT), and Superoxide dismutase (SOD) by 67.4% and 43.0%, and 43% and 50% respectively in the two treated group. These were accompanied by a significant increase (p=0.05) in hepatic lipid peroxidation (MDA) by 81% and 91.6% respectively in the treated groups. The histology of the liver revealed sinusoidal and portal congestion and a mild periportal cellular infiltration by mononuclear cells, by Zithromax 500®. Likewise, kidney histopathology revealed severe cortical congestion and hemorrhage and few tubules containing protein casts in the treated group.

Conclusion: In conclusion, the administration of Zithromax 250® and Zithromax 500® induced marked renal and liver damage, oxidative stress and altered the antioxidant status in rats.

Keywords: Azithromycin, liver and renal damage, oxidative stress, antioxidants

1. INTRODUCTION

Azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin, Fig. 1), an erythromycin semisynthetic derivative is formed by inserting a methyl-substituted nitrogen in place of the carbonyl...
group at the 9a position of the aglycone ring. The resulting dibasic 15-membered ring macrolide derivative is more appropriately referred to as an “azalide.” This structural modification gives azithromycin several distinct advantages over erythromycin: makes the compound more stable in acid, significantly increases the serum half-life, improved pharmacokinetic profiles, gastrointestinal tolerability, oral bioavailability and tissue penetration, and results in increased activity against gram-negative organisms when compared with erythromycin [1]. Its extensive coverage against all the major respiratory pathogens, excellent safety profiles and desirable tissue distribution into the infection sites make azithromycin the drug of choice for the treatment of respiratory tract infections [2]. Azithromycin is frequently prescribed for the treatment of middle ear and upper respiratory tract infections, bronchitis, and community-acquired pneumonia [3].

Macrolide antibacterial agents including azithromycin are lipophilic and are extensively distributed in body fluids and tissues. They exert their antibacterial effects by reversibly binding to the 50s subunit of the bacterial ribosome. This interaction inhibits RNA-dependent protein synthesis by preventing transpeptidation and translocation reactions [4]. The advanced macrolide – azithromycin is a structural analogue of erythromycin that has similar mechanism of action.

![Fig. 1: Azithromycin](image)

Certain macrolide antibiotics have been documented to be hepatotoxic in animals [5] and human subjects in clinical studies [6, 7, 8]. Macrolide antibiotics are also known to alter the physiological redox homeostasis leading to oxidative stress [9, 10], and lipid peroxidation. Cells require oxygen for certain metabolic processes including the metabolism of xenobiotic and in the process certain destructive species referred to as reactive oxygen species (ROS) are generated [11]. The cell protects itself from these ROSs, by the actions of certain low molecular weight substances such as glutathione, vitamin A, E, and C (the non enzymic antioxidants) and enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT) etc.

The effects of azithromycin on liver damage has been well documented in clinical studies [6, 7, 12, 8], however, information regarding the hepatotoxic and renal effects of azithromycin as well as its effect on the antioxidants status in nonclinical studies is still lacking. Therefore, the objective of this study was to investigate the effect of azithromycin on the markers of renal and hepatic toxicity, hepatic redox status and lipid peroxidation in rats.

### 2. MATERIAL AND METHODS

#### 2.1 Chemicals and Reagent

Azithromycin (Zithromax® 250 mg and 500 mg tablets) was a product of Pfizer, New York, Glutathione, 1-Chloro, 2, 4-dinitrobenzene (CDNB), 5, 5-dithio bis-2-nitrobenzoic acid (DTNB), epinephrine, and hydrogen peroxide (H₂O₂) were all purchased from Sigma Chemical Company (London, UK). Assay kits for alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), Urea, Creatinine, total Bilirubin, Total cholesterol, triglycerides (TG), high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol were obtained from Randox® laboratories ltd (Antrim, UK). All other reagents used were of analytical grade.
2.2 Animals and Treatments

2.2.1 Animals

In bred 5-6 week-old male rats (Wistar strain) weighing 200±20g were used in this study. The rats were bred and housed in the animal house of the Department of Chemical sciences, Ajayi Crowther University, Oyo, Nigeria. They were kept in wire meshed cages at room temperature and under controlled light cycle (12-hr light: dark). They were fed with commercial rat chow (Ladokun feeds, Ibadan, Nigeria) and supplied water ad libitum. All experiments were carried out in the Biochemistry laboratory of Ajayi Crowther University, Oyo, Nigeria and conducted without anaesthesia and protocol conforms to the guidelines of the National Institute of Health [13] for laboratory animal care and use.

2.2.2 Treatments

Thirty (30) healthy male albino rats (Wistar strain) were randomly divided into three groups of 10 rats each. Group I (Control) received physiological saline, while groups II and III were administered Zithromax 250® (5.6 kg/ b.w) and Zithromax 500® (11.2 kg/ b.w) respectively as presented in Table 1. The treatments were administered in divided doses, twice daily for seven days. The rats were sacrificed 24hrs after the last treatment.

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (n=10)</td>
<td>Control (Normal saline)</td>
</tr>
<tr>
<td>Group II (n=10)</td>
<td>Zithromax 250® (5.6mg/kg.b.w)</td>
</tr>
<tr>
<td>Group III (n=10)</td>
<td>Zithromax 500® (11.2mg/kg.b.w)</td>
</tr>
</tbody>
</table>

2.3 Collection of blood samples for plasma preparation

Blood was collected from the inferior vena cava of heart of the animals into heparinized tubes, and the rats were sacrificed by cervical dislocation. Plasma was prepared by centrifuging blood samples for ten minutes at 3000 x g in an Eppendorf (UK) bench centrifuge. The clear supernatant was used for the estimation of plasma lipid profiles and enzymes.

2.4 Preparation of cytosolic fractions

The liver, excised from rat, blotted of blood stains, rinsed in 1.15% KCl was homogenized in 4 volumes of ice-cold 0.01 M potassium phosphate buffer, (pH 7.4). The homogenates were centrifuged at 12,500 g for 15 min at 4°C and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquoted and used for enzyme assays.

2.5 Renal and liver functions test

Plasma Creatinine, urea and bilirubin determination was done using Randox® diagnostic kits. Methods for Creatinine assays are based on colorimetric alkaline picate methods Jaffe [14] with Creatinine-picrate complex measured at 492nm. The urea determination method was based on the Fenton reaction Tietz et al. [15] with the Diazine chromogen formed absorbing strongly at 540nm. The dimethy sulphoxide method by Tietz, et al. [16] was used for bilirubin determination. The dimethyl sulphoxide form a coloured compound with maximum absorption at 550nm.
2.6 Determination of plasma AST, ALT, ALP and GGT activities

Plasma AST, ALT, ALP and GGT activities were determined using Randox® diagnostic kits. Determination of AST and ALT activities were based on the principle described by Reltman and Frankel [17]. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine at 546nm and ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm. ALP was determined in accordance with the principles of Tietz [15]. The p-nitrophenol formed by the hydrolysis of p-Nitrophenyl phosphate confers yellowish colour on the reaction mixture and its intensity can be monitored at 405nm to give a measure of enzyme activity. GGT activity was measured based on a modification of the method described by Theodorsen et al. [18] using Randox diagnostic kits.

2.7 Determination of plasma lipid profiles

The plasma total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were determined using Randox® diagnostic kits and the determination were based on CHOD-PAD enzymatic colorimetric method of Trinder [19]. Cholesterol in the presence of Cholesterol Oxidase and Peroxidase enzymes produced 4-(p)-benzoquinone-monoiminophenazone whose absorbance is read at 546 nm. Low-density lipoprotein (LDL and VLDL) and Chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (High density lipoprotein) fraction which remains in the supernatant is determined enzymatically. The Triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence.

2.8 Non-enzymatic antioxidants and lipid peroxidation Assay

Hepatic vitamin C was determined chemically according to the method of Erel et al. [20] using dinitrophenyl hydrazine (DNPH), while hepatic glutathione was determined according to the method of Jollow et al. [21]. The chromophoric product resulting from the reaction of Ellman’s reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm which was read in a spectrophotometer. Reduced GSH is proportional to the absorbance at 412 nm. The extent of lipid peroxidation (LPO) was estimated by the method of Vashney and Kale [22], the method involved the reaction between malondialdehyde (MDA; product of LPO) and thiobarbituric acid to yield a stable pink chromophore with maximum absorption at 532 nm.

2.10 Antioxidant enzymes assay

The procedure of Misra and Fridovich [23] as described by Magwere et al. [24] was used for the determination of hepatic superoxide dismutase (SOD) activity by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 and 30°C. Hepatic catalase activity was determined according to the method of Asru [25] by measuring the reduction of dichromate in acetic acid to chromic acetate at 570 nm. Hepatic Glutathione S-transferase (GST) activity was determined by the method described by Habig et al. [26] using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

2.11 Protein determination

Protein determination of plasma and all fractions was estimated by the method of Lowry et al. [27] using bovine serum albumin as standard.

2.12 Histopathological studies

The method of Baker and Silverton [28] was employed for the processing of liver for histopathological studies.
2.13 Statistical analysis

Results were expressed as mean of 10 replicates ± SD. Data obtained were subjected to one-way Analysis of Variance (ANOVA) and complemented with Student’s t-test using Stat Pac Statistical Software. Statement of statistical significance was based on p<0.05.

3. RESULTS AND DISCUSSION

3.1 Effect of Azithromycin on the levels of plasma Creatinine, Urea and Bilirubin in rats

Table 2 shows the effects of Azithromycin treatment on plasma Creatinine, Urea and Bilirubin level. Azithromycin (as Zithromax 250mg® and Zithromax 500mg®) treatment significantly(p=0.05) increased the plasma Creatinine, Urea and Bilirubin levels in the rats by 78% and 130%, 21% and 35%, and 14% and 47.4%, respectively when compared with control.

3.2 Effect of Azithromycin on the levels of plasma enzymes in rats

Effects of Azithromycin (Zithromax 250mg® and Zithromax 500mg®) treatment on Plasma Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and γ-Glutamyl transpeptidase (GGT) activities in rats were shown in Table 3. Zithromax 250mg® and Zithromax 500mg® treatments significantly(p=0.05) increased the plasma ALP, ALT, AST and GGT activities in the rats by 16% and 127.4%, 20% and 36.4%, 20% and 36.6% and 43% and 80.5% respectively when compared with control.

3.3 Effect of Azithromycin on the levels of plasma lipid profiles in rats

The effects of Azithromycin (Zithromax 250mg® and Zithromax 500mg®) treatment on Plasma Lipid Profiles in rats are presented in Table 4. Zithromax 250mg® and Zithromax 500mg® treatment significantly increased the plasma Total Cholesterol, HDL- and LDL- Cholesterol concentrations in the rats by 19% and 35.44%; 24% and 74.32%; and 33% and 35.19% respectively when compared with control (p=0.05). Similarly, plasma Triglyceride level was also significantly increased in the treated group by 20% and 31.2% respectively when compared with the control (p=0.05).

3.4 Effect of Azithromycin on the levels of hepatic antioxidant enzymes in rats

Table 5 shows the effects of Azithromycin (Zithromax 250mg® and Zithromax 500mg®) treatment on Hepatic Superoxide dismutase (SOD) and Catalase (CAT) activities in rats. Zithromax 250mg® and Zithromax 500mg® treatment significantly decrease the SOD and CAT activities in the liver of rats by 27% and 50%; and 30% and 50% respectively when compared with control (p=0.05). Hepatic glutathione-S-transferase (GST) activity was also significantly reduced following treatment with Zithromax 250mg® and Zithromax 500mg® by 45% and 56.1% respectively (Fig. 4).

3.5 Effect of Azithromycin on the levels of hepatic non-enzymatic antioxidant and lipid peroxidation in rats

The hepatic Vitamin C concentration is shown in Fig 2 following treatment with Azithromycin (Zithromax 250mg® and Zithromax 500mg®). The Vitamin C level was significantly decreased by 35% and 66.3% respectively in the treated groups when compared with the control (p=0.05). Also, the GSH level was significantly decreased by 34% and 67.4% respectively in the treated groups when compared with the control (Fig.3). Furthermore, hepatic lipid peroxidation (MDA) level was significantly increased by 81% and 91.7% in the treated groups respectively when compared with the control (Fig.5).

3.6 Effect of Azithromycin on the histopathology of kidney and liver of rats

Plates 1 and 2 are photomicrographs showing the effect of azithromycin on the liver and kidney respectively. The Zithromax 250 mg resulted in mild periportal cellular infiltration by mononuclear cells
A sinusoidal and portal congestion with mild periportal cellular infiltration by mononuclear cells was observed in the Zithromax 500mg group (Plate 1C). In the kidney, Zithromax 250 mg, produces protein casts in the tubular lumen (Plate 2B), while, severe cortical congestion and haemorrhage, and few tubules containing protein casts was observed in the Zithromax 500mg group (Plate 2C).

**Table 2: Effect of Azithromycin on Plasma Creatinine, Urea and Bilirubin levels in rats.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CREATININE (mg/dl)</th>
<th>UREA (mg/dl)</th>
<th>TOTAL BILIRUBIN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.14±0.01</td>
<td>46.8±0.83</td>
<td>0.57±0.09</td>
</tr>
<tr>
<td>Zithromax 250mg®</td>
<td>0.25±0.01 (78.5%)*</td>
<td>56.4±1.5 (20.5%)*</td>
<td>0.65±0.01 (13.4%)*</td>
</tr>
<tr>
<td>Zithromax 500mg®</td>
<td>0.32±0.01 (130%)*</td>
<td>63.2±1.9 (35%)*</td>
<td>0.85±0.03 (47.4%)*</td>
</tr>
</tbody>
</table>

*The values are the Means + SD (range) for ten rats in each group; *significantly different from the control p=0.05; Values in parenthesis represent percentage (%) increase.

**Table 3: Effects of Azithromycin on Plasma Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and γ-Glutamyl transpeptidase (GGT) activities in rats.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>ALP (U/l)</th>
<th>ALT (U/l)</th>
<th>AST (U/l)</th>
<th>GGT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>103.2±1.9</td>
<td>52.2±3.1</td>
<td>61.2±3.6</td>
<td>8.2±0.8</td>
</tr>
<tr>
<td>Zithromax 250mg®</td>
<td>120+2.9 (16.2%)*</td>
<td>66.2±2.2 (26.8%)*</td>
<td>73.4±1.6 (19.9%)*</td>
<td>11.8±0.8 (43.9%)*</td>
</tr>
<tr>
<td>Zithromax 500mg®</td>
<td>127.4±1.5 (23.5%)*</td>
<td>71.2±1.9 (36.4%)*</td>
<td>83.6±3.1 (36.6%)*</td>
<td>14.8±0.8 (80.5%)*</td>
</tr>
</tbody>
</table>

*The values are the Means + SD (range) for ten rats in each group; *significantly different from the control p=0.05; Values in parenthesis represent percentage (%) increase.

**Table 4: Effects of Azithromycin on Plasma Lipid Profiles in rats.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TOTAL-cholesterol (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
<th>LDL-cholesterol (mg/dl)</th>
<th>TRIGLYCERIDE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.2±1.9</td>
<td>14.8±0.8</td>
<td>21.6±1.5</td>
<td>43.6±2.6</td>
</tr>
<tr>
<td>Zithromax 250mg®</td>
<td>49.2±1.4 (19.4%)*</td>
<td>18.4±0.5 (24.3%)*</td>
<td>28.8±1.3 (33.3%)*</td>
<td>52.6±2.0 (20.6%)*</td>
</tr>
<tr>
<td>Zithromax 500mg®</td>
<td>55.8±1.8 (35.4%)*</td>
<td>25.8±2.1 (74.3%)*</td>
<td>29.2±1.6 (35.2%)*</td>
<td>57.2±4.1 (31.2%)*</td>
</tr>
</tbody>
</table>

*The values are the Means + SD (range) for ten rats in each group; *significantly different from the control p=0.05; Values in parenthesis represent percentage (%) increase.

**Table 5: Effects of Azithromycin on Hepatic Superoxide dismutase (SOD) and Catalase activities in rats.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>SOD Activity (units)</th>
<th>CATALASE Activity (μmole H₂O₂ consumed/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8±0.7</td>
<td>0.36±0.02</td>
</tr>
<tr>
<td>Zithromax 250mg®</td>
<td>7.4±1.1 (7.5%)*</td>
<td>0.26±0.01 (28%)*</td>
</tr>
<tr>
<td>Zithromax 500mg®</td>
<td>4±0.7 (50%)*</td>
<td>0.18±0.02 (50%)*</td>
</tr>
</tbody>
</table>

*The values are the Means + SD (range) for ten rats in each group; *significantly different from the control p=0.05; Values in parenthesis represent percentage (%) decrease. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.
Figure 2: Influence of Azithromycin on hepatic Vitamin C concentration in Rats.  
*The values are the Means + SD (range) for ten rats in each group *significantly different from the control, p=0.05.

Figure 3: Influence of Azithromycin on Hepatic reduced Glutathione (GSH) level in Rats.  
*The values are the Means + SD (range) for ten rats in each group *significantly different from the control, p=0.05.
Figure 4: Influence of Azithromycin on Hepatic Glutathione-S-transferase (GST) activity in Rats. The values are the Means + SD (range) for ten rats in each group *significantly different from the control, p=0.05.

Figure 5: Influence of Azithromycin on Hepatic Lipid peroxidation (MDA) level in Rats. The values are the Means + SD (range) for ten rats in each group *significantly different from the control, p=0.05.
Plate 1: Effect of Azithromycin on histology of the Liver cell. A - Control: No visible lesions seen; B - 250 mg Azithromycin: There is mild periportal cellular infiltration by mononuclear cells. C - 500mg Azithromycin: There is sinusoidal and portal congestion and there is also mild periportal cellular infiltration by mononuclear cells.

Plate 2: Effect of Azithromycin on histology of the Kidney cell A - Control: No visible lesions seen; B - 250 mg Azithromycin: There are protein casts in the tubular lumen; C - 500mg Azithromycin: There is severe cortical congestion and haemorrhage. There are also few tubules containing protein casts.
3.7 Discussion

Azithromycin (Zithromax®) is an azalide, a subclass of macrolide antibiotics, structurally modified from erythromycin. Its efficacy and potential to inhibit microorganisms had led to its increased use. It is well-known that Azithromycin is widely used to treat or prevent certain bacterial infections, most often those causing middle ear infections, strep throat, pneumonia, typhoid, and sinusitis. However, an increasing number of evidence indicates that it has risk of hepatotoxicity and cardio-toxicity as adverse effect [8, 29].

Oxidative stress mediated by reactive oxygen species (ROS) has been implicated as a common link between chronic liver damage and hepatic fibrosis [30]. The human body contains an array of antioxidant defense mechanisms (non-enzymatic and enzymatic antioxidants) to remove harmful ROS as soon as they are formed and to prevent their deleterious effects [31]. The non-enzymatic antioxidants include vitamins E and C, and reduced glutathione (GSH), while the enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx).

In the present study, the effect of two preparations of azithromycin; Zithromax 250® (5.6mg/kg.b.w) and Zithromax 500® (11.2 mg/kg.b.w) on markers of renal and hepatic toxicity, lipid profile and antioxidant status was investigated in rats. We observed that the two preparations of Azithromycin induce renal and liver failure in the rats. This was obvious from the renal and liver function test as plasma concentration of creatinine, urea and bilirubin increased significantly in the treated groups, suggesting impairment of renal and liver function. Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys [32] and their serum/plasma concentrations are commonly used to screen for renal or cardiovascular diseases [33, 34]. Elevation of the plasma levels of creatinine and urea is an indication of abnormal renal function [35]. Increases in the plasma bilirubin concentration are generally the consequence of bile accumulation as a result of impairment to intrahepatic or extrahepatic bile flow, increased production associated with accelerated erythrocyte destruction, or altered bilirubin metabolism [36, 37, 38].

An integrated evaluation of data from the plasma levels of ALT, AST, ALP and GGT has been shown to be crucial in the identification of hepatobiliary injury [39]. Hepatotoxic drugs has been shown to cause damage to the liver cell membranes which makes marker enzymes like AST, ALT and ALP leak into serum and show increased activities [40]. Hepatic causes of increased serum ALT and AST activities are known to include hepatocellular necrosis, injury, or regenerative or reparative activity [41, 42, 38]. Plasma/serum ALP elevation has been attributed to increased osteoblastic activity and hepatobiliary diseases [43]. The elevated level of aminotransferases and phosphatases observed in the 5.6 and 11.2 mg/kg dose of Zithromax® treated animals is highly indicative of hepatic toxicity [44, 45].

The hepatotoxic effects of azithromycin appear to progress through generation of free radicals and ROSs as evident in reduction in the levels of enzymic and non-enzymic antioxidants. In the present investigation, we observed a significant reduction in the activities of SOD, CAT and GST in the liver of azithromycin- treated animals. The significantly decreased activity of the ROS scavenging enzyme, SOD and CAT, by exposure to azithromycin, conforms to previous report on macrolide antibiotics [46]. This may be due to the damaging effects of free radicals possibly generated by the action of the drug.

The antioxidants enzymes CAT and SOD represent the primary intracellular antioxidants defence mechanism against oxidative stress [20]. Catalase is a tetrameric hemoprotein present in the liver cells and erythrocytes at high concentration [47]. It is generally accepted that H₂O₂ can be detoxified by catalase which removes it when present at high concentration. Catalase is known to be inhibited by ROS such as superoxides anion which converts it to ferroxy and ferryl states that are inactive forms of enzymes [48]. SOD catalyzes the dismutation of superoxide radicals (O₂⁻) to hydrogen peroxide (H₂O₂) and molecular oxygen (O₂). SOD therefore protects catalase against inhibition by...
superoxide anion. Thus the balance of this enzyme system may be essential to get rid of superoxide
anion and peroxides generated in subcellular compartments of the liver. The antioxidant enzymes,
Superoxide dismutase and Catalase constitute a mutually supportive team of defense against reactive
oxygen species.

Glutathione-S-Transferase (GST) is a family of multifunctional isozymes found in all eukaryotes,
catalyzing both glutathione dependent conjugation and reduction reactions [49]. One main function of
GST is to catalyze the biotransformation of xenobiotics, including drugs detoxification in the
mercapturic acid pathway, leading to the elimination of toxic compounds [50], and also acting as an
antioxidant enzyme [51]. In this study, GST activities were inhibited in animals treated with the two
preparations of Azithromycin. This observation, therefore, suggests that these drugs may alter the
expression and activities of antioxidant enzymes as a result of toxic metabolites generated during
their biotransformation.

The level of reduced GSH is a measure of non-enzymic antioxidant and cellular redox status of cells
in higher animals [52]. In addition to vitamin A and β-carotene, Ascorbic acid (Vitamin C) is known to
represent the first line of antioxidant defence [53, 54] and this vitamin is likely to be most susceptible
to free radical oxidation. Ascorbate is a good free radical scavenger due to its chemical properties
[55]. Studies have shown that the redox state of intracellular vitamin C is controlled by the intracellular
level of GSH [56]. Our results showed that Azithromycin treatment decreased the overall redox
status in the liver as indicated by a significant decrease in the level of hepatic GSH and Vitamin C.
The observed decrease in GSH and Vitamin C levels further confirmed the formation of reactive
oxygen species or toxic metabolites from the two preparation of Azithromycin.

The thiobarbituric acid assay is a satisfactory means of assessing lipid peroxidation (LPO) through
assay of MDA in biological samples. The elevated level of malondialdehyde (MDA) in the liver of
Azithromycin treated rat is a clear manifestation of excessive formation of free radicals and activation
of lipid peroxidation systems. The level of MDA is a good indicator of the degree of lipid peroxidation
and tissue damage. The accumulation of LPO products observed in this study agrees with previous
studies on certain macrolide antibiotics [57, 58]. The significant increase in the level of lipid
peroxidation may be related to the reduction in the enzymic and non enzymic antioxidant systems,
leading to accelerated oxidation of lipids. Lipid peroxidation has been shown to cause a significant
alteration in the structural integrity and functions of cell membranes [59].

Lipid profile analysis revealed a significant increase in the plasma level of total cholesterol, LDL-
cholesterol, HDL-cholesterol and triglycerides by the two preparations of Azithromycin. It has been
suggested that cholesterol is a general indicator of the level of lipid in the circulation [60] and together
with polyunsaturated fatty acids (PUFA) are the main components of LDL. PUFA is the substrate
required for MDA formation and the amount of peroxidised lipid formed may be related both to the
amount of substrate and to the level of lipid peroxidation (LPO). Oxidation of LDL-cholesterol is known
to result in the depletion of lipoprotein antioxidants and subsequent accumulation of cholesterol esters
[59].

In the present investigation, we observed that azithromycin induced renal and hepatic toxicity in rats
as explained by the photomicrograph of rat kidney and liver sections. The histological alterations
observed in the liver of azithromycin - treated rats characterized by sinusoidal and portal congestion,
and mild periportal cellular infiltration by mononuclear cells coupled with the presence of protein casts
in the kidney tubular lumen as well as severe cortical congestion and haemorrhage is an indication of
disruption of cellular architecture. All these abnormalities might have resulted due to the formation of
highly reactive radicals because of oxidative threat caused by the drug which disrupted normal
cellular functioning of the liver and the kidney.
4. CONCLUSION

In summary, azithromycin induced renal and hepatic damage in rats and its mechanism of toxicity appears to proceed through the generation of free radicals or depletion of the antioxidant systems.

COMPETING INTERESTS

We declared that there are no competing interests.

AUTHORS’ CONTRIBUTIONS

This research work was carried out in collaboration between the two authors – the experimental designed, the study, the statistical analysis, Interpretation of biochemical findings and preparation of journal manuscript.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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