Alteration in Antioxidant Status and Apoptotic Genes in Rat Liver Following Treatment with Gatifloxacin

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ABSTRACT

Aim: Gatifloxacin (GTX) - an 8-methoxy fluoroquinolone antibacterial agent is known to be very effective in the treatment of respiratory and urinary tract infections. This study investigates the toxic potentials of GTX in Wistar rat liver.

Methodology: Adult rats were exposed to oral doses (10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg) of gatifloxacin for five days. Thereafter, biomarkers of oxidative stress were assessed spectrophotometrically while the levels of expression of *Bcl2l1*, caspases 3, 8 and 9 were assessed using reverse transcription polymerase chain reaction technique.

Results: The results showed that GTX induces hepatic oxidative stress as well as increased expression of proapoptotic genes.

Conclusion-These alterations observed at the lowest dose of 10 mg/kg showed that gatifloxacin exposure could induce hepatic apoptosis.

Keywords: Alteration, Antioxidant Status, Apoptotic Genes, Rat Liver, Treatment and Gatifloxacin.

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1. INTRODUCTION

GTX is a fourth-generation fluoroquinolone, a family of antibiotics that inhibits the bacteria enzyme DNA gyrase and topoisomerase IV (Emmanuelle et al., 2009) in gram positive and gram negative organisms as well as anaerobes such as mycoplasma, chlamydia, legionella and mycobacteria (Kumar, Dhivya, & Vijayakumar, 2011). GTX penetrates well into leukocytes, delivers active drug to sites of infection and plays important role in the treatment of intracellular pathogens (Kumar et al., 2011).However, gatifloxacin like other fluoroquinolones has been reported to produce several side effects including hepatotoxicity, joint defects and phototoxicity with complications like liver damage, purpura and dysglycemia (Park-Wyllie, Juurlink, & Kopp, *2006*).

Liver damage in rat characterized by hepatic portal congestion and cellular infiltration by mononuclear cells as well as elevation in the activities of plasma biomarkers of liver damage like alkaline phosphatase, alanine transaminase, aspartate aminotransferase and gamma-glutamyl transferase as result of exposure to graded doses of gatifloxacin has been reported (Olayinka, Ore, & Adeyemo, 2015). Also it has been reported that fluoroquinolone antibiotics generate ROS and cause oxidative stress (Afolabi and Oyewo, 2014).The present study therefore evaluated the influence of four different doses of Gatifloxacin on some proapoptotic genes and antioxidant status in Rat liver.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

Gatifloxacin was obtained from Sigma-Aldrich, St. Louis, MO. EASYspin Plus® was obtained from Aidlab Biotechnologies Co., Ltd, Beijing, China while RNAhold® and *EasyScript*® one-step RT-PCR kit was obtained from TransBionovo Co., Ltd. Beijing, China. Other chemicals and reagents were of analytical standard and purchased from Sigma-Aldrich.

2.2 Experimental Animals and Procedure

Twenty-five (25) inbred male Wistar rats (130±30 g) were used for this research. The animals were maintained on standard 12-h light and dark cycles and granted access to water and feed, *ad libitum*. The animals were allowed to acclimatize for three weeks before commencement of the experiment. The experiment was approved by the Covenant University Ethical Committee (CU/BIOSCRECU/BIO/2016/004) and carried out according to the guidelines of the committee. The animals were randomly allotted into five (5) experimental groups after the initial 2 weeks of acclimatization. Group 1 served as control, while the remaining groups received varying doses of gatifloxacin thus: group 2 (10 mg/kg bw), group 3 (20 mg/kg bw), group 4 (40 mg/kg bw) and group 5 (80 mg/kg bw) orally for 5 days. The rats were anaesthesized under light ether and sacrificed Twenty-four (24) hours after the last dosage. The liver was excised immediately and its portion for oxidative stress assays were processed appropriately (Graham, 2002), while other portions were cryopreserved in RNAhold® for RNA analysis.

2.3 Biochemical Analysis

The level of lipid peroxidation was quantified by assessing the concentration of thiobarbituric acid reactive substances (TBARS) as described by Buege and Aust, 1978. Superoxide dismutase's activity was determined as described by Marklund and Marklund, 1974 by measuring the rate of autooxidation of pyrogallol at 420 nm. The level of reduced glutathione (GSH) concentration was quantified according to the method of Ellman, 1959. The Lowry method was used for the determination of protein concentration as described by Gallagher and Desjardins, 2011.

2.4 Gene Expression Analysis

The expression level of certain apoptotic genes (Table 1) were quantified using relative reverse transcriptase polymerase chain reaction (RT-PCR) techniques as described by Chaudhry, 2016 with appropriate modifications.

Table 1- List of genes studied and the sequences of Gene Specific Primers

Bcl2l1	Bcl-2-like 1	Forward: TTTTGCTGAGTTACCGGCGA	NM_001033672.1
		Reverse: GCCACAAGGGTAGCCAGAAT	
Casp3	Caspase 3	Forward: GAGCTTGGAACGCGAAGAAA	NM_012922.2
		Reverse: TAACCGGGTGCGGTAGAGTA	
Casp8	Caspase 8	Forward: AGAGAAGCAGCCTATGCCAC	NM_022277.1
		Reverse: CCCCGAGGTTTGCTCTTCAT	
Casp9	Caspase 9	Forward: GCGCGACATGATCGAGGATA	NM_031632.1
		Reverse: TCTCCATCAAAGCCGTGACC	
β-ACTIN	Actin, Beta	Forward: GTCAGGTCATCACTATCGGCAAT	NM_031144.3
		Reverse: AGAGGTCTTTACGGATGTCAACGT	

The level of transcription of the genes relative to β-actin was quantified using Image J® software Rotimi, Bankole, Adelani, & Rotimi, 2016.

2.5 Statistical Analysis

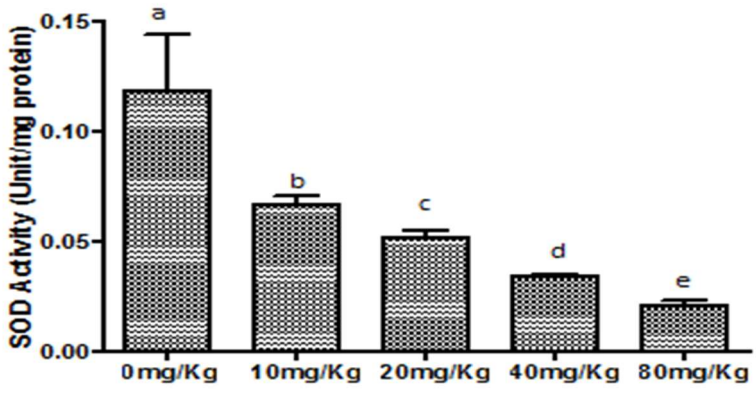
Data were expressed as mean ± SEM and analysis of variance was carried out to test for the level of homogeneity at $p < 0.05$ among the groups. Heterogeneous groups were subjected to Duncan's multiple range post hoc test.

3. RESULT

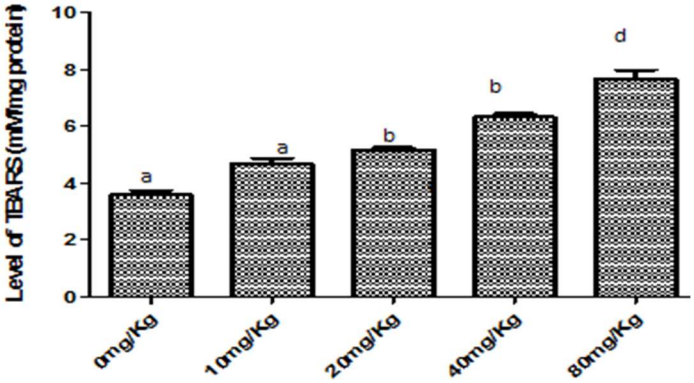
3.1 Gatifloxacin Induced Oxidative Stress in Rat Liver

The levels of GSH and TBARS as well as the activity of SOD were assessed in the liver of the rats (Figure 1, a-c). Gatifloxacin resulted in a dose-dependent significant ($p<0.05$) reduction in the level of hepatic GSH and SOD activity with a concomitant significant ($p<0.05$) dose-dependent increase in the levels of TBARS.

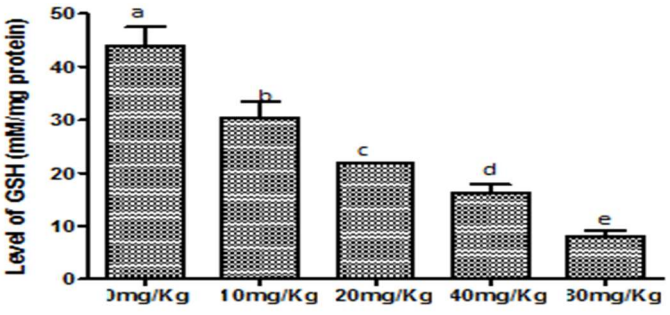
a)



(a) the activity of superoxide dismutase



(b) level of liver thiobaribituric acid reactive substances,

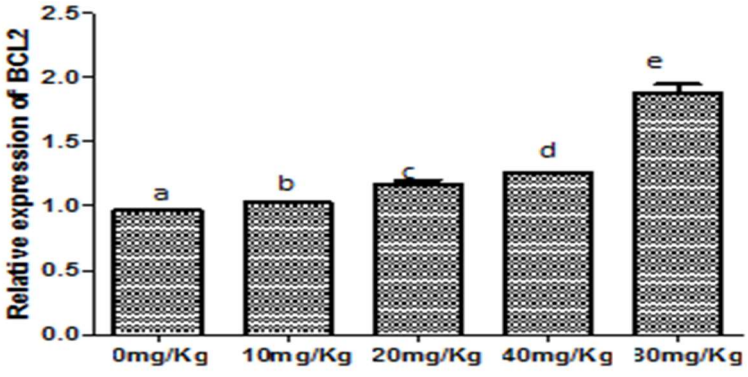


c)

(c) the level of liver liver reduced glutathione

3.2 Gatifloxacin Modulated the Expression of Genes Involved in Apoptosis in Rat Liver

The expression of *Bcl2l1*, *Casp3* and *Casp8* are illustrated in figure 2 (a-c). There was a significant ($p < 0.05$) increase in the expression of *Bcl2l1 and Casp8* in the liver of rats treated with gatifloxacin with a further increase in group treated with 80 mg/kg. A significant ($p < 0.05$) dose-dependent increase was also observed in the levels of expression of *Casp3*, however dosage of gatifloxacin beyond 10 mg/kg had no significant ($p > 0.05$) effect on the expression of *Casp3*.

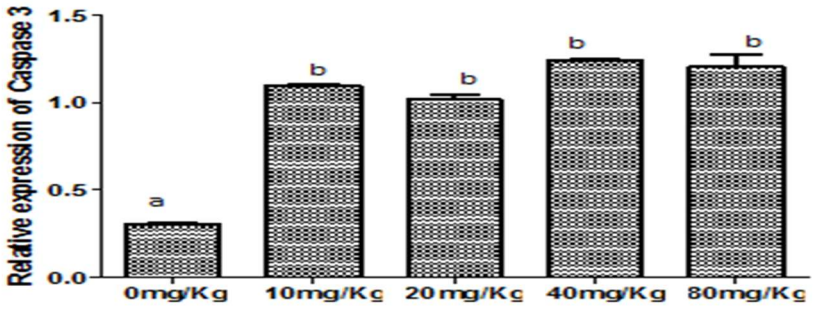


a)

(a) The levels of expression of *Bcl2l1* in the liver

(b) the levels of expression of caspase 8 in the liver, (c) the levels of expression of caspase 3 in the liver

c)



b) the levels of expression of caspase 8 in the liver

4. DISCUSSION

Fluoroquinolones have been shown to generate reactive oxygen species (ROS), inducing oxidative stress in human and experimental animals (Talla and Veerareddy, 2011). The potential toxicity of four doses of GTX was investigated in rats Liver. Our findings showed that gatifloxacin induced oxidative stress. Naturally, lipid peroxidation is prevented by the body's enzymic antioxidant defense system. SOD is an important scanvengers of superoxide ion and hydrogen peroxide. Reduction of SOD activity in the liver following exposure of rats to the doses of GTX is indicative of increased production of ROS. This is similar to the findings of Talla and Veerareddy, 2011. One of the products of lipid peroxidation is thiobaribituric acid reactive substances (TBars). Lipid peroxidation has been described as a biomarkers of tissue damage (Gutteridge, 1995).

The apparent increase in TBars formation indicates that GTX is capable of inducing oxidative stress. Reduction of GSH concentration in the liver by GTX is a symptom of decrease in the overall redox status of the liver, resulting from the formation of reactive oxygen species or toxic metabolites by the four doses of GTX. This observation is in agreement with findings of Olayinka et al., 2015. Apoptosis (programmed cell death) which is a naturally occurring cell death process is needed for the normal development and homeostasis of all multicellular organisms(Schwartzman, & Cidlowski,1993).This process is required also for removing damaged, infected, or potentially neoplastic cells. However, both too little and too much apoptotic cell death can lead to adverse biological consequences (Nagata, 1996). It has been reported that certain fluoroquinolone antibiotics increases expression level of proteins involved in apoptosis (Liang et al., 2016).Our result showed that GTX administration resulted in the upregulation of proapoptotic genes.

5. CONCLUSION

Our study shows that gatifloxacin altered antioxidant status and triggered the expression of apoptotic genes in rat liver in a dose dependent manner. Data from the present study coupled with increased frequency of Kidney damage might pose a reason for the withdrawal of gatifloxacin from market in some countries.

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