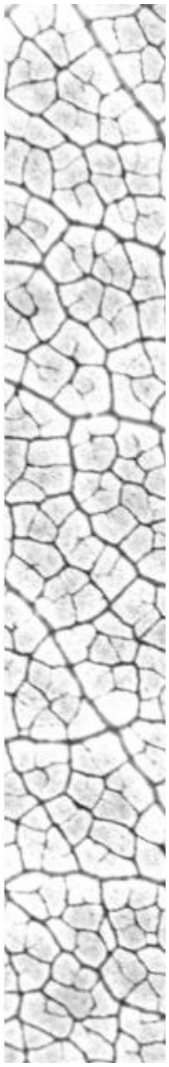

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In vivo effects of different doses of ciprofloxacin on glutathione reductase activity in some rat tissues

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Abstract
This study evaluated the effect of a fluoroquinolone antibiotic, ciprofloxacin on the enzymatic activity of glutathione reductase (GR) in the brain, cardiac, small intestine, and testes tissues of rats. GR enzymes contribute to maintaining appropriate concentrations of intracellular glutathione (GSH). Seventy-two adult male albino rats were divided into four groups (I–IV). Rats in group I (control) were injected with sterile distilled water, whereas animals in experimental groups (II-IV), were injected intraperitoneally with ciprofloxacin in single doses of 100, 150, and 200 mg/kg, respectively. Dosages effects of antibiotic were investigated at three specific periods (in the first, the third, and the seventh hour). The obtained results showed that brain GR activity in ciprofloxacin groups was inhibited compared to the control group all periods ($p < 0.05$). After seven hours, ciprofloxacin found to significantly inhibited the enzyme activity in cardiac tissues especially in group IV ($p < 0.05$). In the II and III groups, however, the small intestine reductase enzyme activity was not affected by ciprofloxacin doses, excluding group IV, where the enzyme activity decreased after seven hours of treatment ($P < 0.05$). As for the GR enzyme activity in rat testis, it was a decrease compared to the control in the treated groups from the start of the third hour to the end of the period ($P < 0.05$).

Keywords: Glutathione Reductase (GR), Glutathione, Ciprofloxacin, oxidative stress, enzyme

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INTRODUCTION

Fluoroquinolones are a class of broad-spectrum antibiotics, which are effective against both Gram-negative and Gram-negative bacteria. They are agents that can exterminate bacteria via inhibiting the activity of the two enzymes; DNA gyrase and topoisomerase IV, and this in turn will prevent cell division and leading to bacteria death (Idowu and Schweizer 2017). Ciprofloxacin is a fluoroquinolone antibiotic, which is widely used to treat several infections such as urinary, respiratory, biliary tract, and sexual-transmitted diseases (Kim and Hooper 2014). Although fluoroquinolones are well sustained by patients, they also cause some health problems, including intestinal disturbance, joint toxicity, and the harmful effects on the central nervous system and other symptoms (Golomb et al. 2015, Michalak et al. 2017; Ozung, et al, 2017).

In the normal healthy case, reactive oxygen species (ROS) are generated as a natural product of the cellular metabolism process and play a major role in signal pathways. On the other hand, oxidative stress indicates high cellular levels of reactive oxygen species that are associated with the development of cardiovascular, neurodegenerative, cancer, and other diseases (Snezhkina et al. 2020). The living cell protects itself from the harmful effects of ROS via activating relating antioxidant defense systems, such as glutathione reductase (Patlevič et al. 2016).

Glutathione reductase (GR) has been detected in plant and animal cells and even bacteria, where it is associated with the cytoplasm, mitochondria, and nuclei (McQueen 2010). GSH reductase is a flavoprotein that utilizes NADPH as the reducing co-factor to convert glutathione from the oxidized form (GSSG) to (GSH) which is the reduced form (Prast-Nielsen et al. 2011). So, these enzymes are responsible for maintaining GSH levels in the cells. For this reason, it plays a vital role in protecting the cell by detoxification from xenobiotics, including drugs, carcinogenic and toxic compounds (Main et al. 2012, Hasanuzzaman et al. 2019). Accordingly, inhibition of the GR enzyme leads to a decrease in the GSH concentration versus high concentrations of xenobiotics in the tissues, which causes serious diseases (Ballatori et al. 2009).

Based on the literature review, few studies have examined the effect of drugs such as ciprofloxacin on GR enzyme activity in vivo conditions. In the present

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Time (hour)	Control	Experimental groups			Inter group comparison (p- value)
	Group I	Group II	Group III	Group IV	
Brain GR Activity (Units per gram of protein)					
1	3.648 ± 0.062	3.473 ± 0.103aA	3.212 ± 0.069aB	2.968 ± 0.080aC	0.001
3	3.630 ± 0.150	2.895 ± 0.174bA	2.190 ± 0.094bB	1.437 ± 0.086bC	0.001
7	3.573 ± 0.104	2.208 ± 0.073cA	1.145 ± 0.097cB	0.742 ± 0.091cC	0.001
p-values (depending on time)	N.S.	0.001	0.001	0.001	
Heart GR Activity (Units per gram of protein)					
1	1.512 ± 0.069	1.903 ± 0.086aA	1.797 ± 0.044aB	1.540 ± 0.039aC	0.001
3	1.515 ± 0.067	1.625 ± 0.024bA	1.347 ± 0.052bB	1.222 ± 0.050bC	0.001
7	1.500 ± 0.041	1.310 ± 0.068cA	1.157 ± 0.090cB	0.720 ± 0.046cC	0.001
p-values (depending on time)	N.S.	0.001	0.001	0.001	
Small intestine GR Activity (Units per gram of protein)					
1	2.178 ± 0.055	2.167 ± 0.085aC	2.228 ± 0.110bB	2.342 ± 0.053aA	0.001
3	2.198 ± 0.071	2.257 ± 0.087aB	2.360 ± 0.041aA	2.425 ± 0.056aA	0.001
7	2.177 ± 0.066	2.062 ± 0.044bA	1.923 ± 0.059cB	1.677 ± 0.068bC	0.001
p-values (depending on time)	N.S.	0.001	0.001	0.001	
Testis GR Activity (Units per gram of protein)					
1	1.975 ± 0.047	2.390 ± 0.075aA	2.107 ± 0.060aB	1.827 ± 0.103aC	0.001
3	2.013 ± 0.052	1.843 ± 0.109bA	1.647 ± 0.052bB	1.277 ± 0.059bC	0.001
7	2.007 ± 0.066	1.548 ± 0.088cA	1.192 ± 0.047cB	0.663 ± 0.086cC	0.001
p-values (depending on time)	N.S.	0.001	0.001	0.001	

Values are presented as mean ± standard deviation with 6 rats in each group.The different lowercase letters following values (in the same column) showed significant time-dependent changes, regardless of doses of ciprofloxacin. The different capital letters following values (in the same row) showed significant changes between different ciprofloxacin doses groups, regardless of time-dependently

study, we investigated the effects of ciprofloxacin with different doses on glutathione reductase activity in the brain, heart, small intestine, and testis of rats.

MATERIAL AND METHODS

Chemicals

Ciprofloxacin (hydrochloride) was purchased from Cayman Chemical Co. (Ann Arbor, MI). All other chemicals and reagents used in the current study were commercially obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A)**.**

Experimental design

Seventy-two adult male Albino rats (290±19 g) were randomized into four groups (I–IV), each group consists of 18 animals. The first of these groups was the control group (group I), in which the rats were injected with sterile distilled water using IP injection. Animals in the three treated groups (II-IV) received ciprofloxacin as a single dose (100, 150, and 200 mg/kg, respectively) by intraperitoneal injection. After administration, six rats of each group were anesthetized and killed at 1st, 3th, and 7th hours. This study received approval from the ethics committee at the University of Anbar**.**

Preparation of supernatant from rat tissues

Brain, heart, small intestine, and testes of rats were rinsed in ice-cold saline solution (1%). Subsequently, the tissues were homogenized in cold Tris-HCl buffer solution (pH 7.4), using a Polytron Homogenizer. Then, the homogenates of rat tissues were centrifuged at 11000xg for 15 min (MPW-350R, Korea), and the supernatant was obtained and stored at -80°C for next biochemical assays**.**

Enzyme activity and protein determination

GR activity was determined spectrophotometrically according to the method of Carlberg and Mannervik (1985). The rate of NADPH oxidation was monitored at (340 nm, 25°C). Protein content was measured at 595 nm according to the procedure of Bradford (1976), using bovine serum albumin as a standard**.**

Statistical Analysis

All statistical analysis and the graphs were performed by GraphPad Prism software (version 8). The statistical differences between the different means were determined following two-way ANOVA and Tukey's multiple comparison tests. The differences were regarded as statistically significant when $P < 0.05$.

RESULTS

Details of the effects of different doses of the ciprofloxacin antibiotic on GR activities in the brain, cardiac, and testes of rats are shown in **Table 1**. As presented in **Fig. 1**, in all groups of animals injected with ciprofloxacin (II-IV), the glutathione reductase activities were lower than the control group (I) during the same period ($p < 0.05$). Remarkably, a significant decreased considering brain GR activity were observed over time. Under this, the GR activity was in its minimum level at the end of the seventh hour compared to the 1st hour in which the enzymatic activity was slightly affected ($p < 0.05$) and declined gradually after three hours up to seven hours as the investigated enzyme recorded the

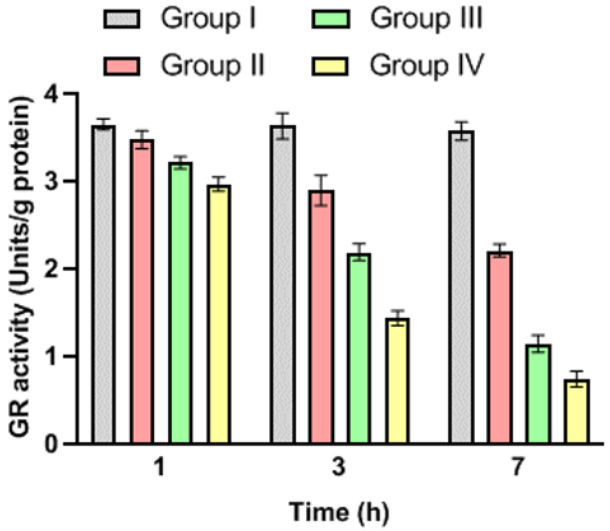

Fig. 1. Influences of different doses of ciprofloxacin on GR enzyme activities of rat testis

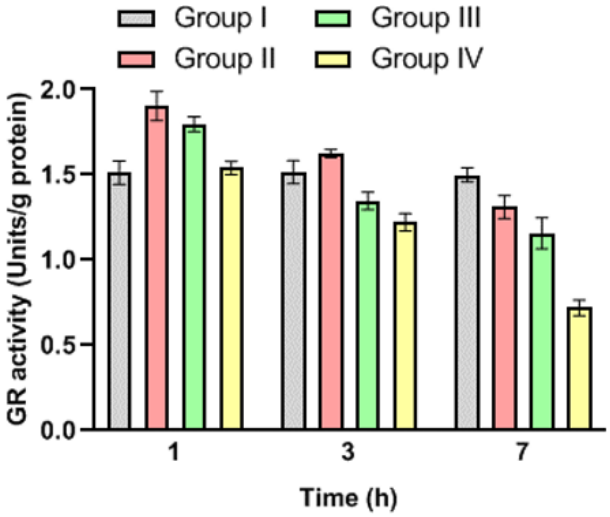

Fig. 2. Influences of different doses of ciprofloxacin on GR enzyme activities of rat heart

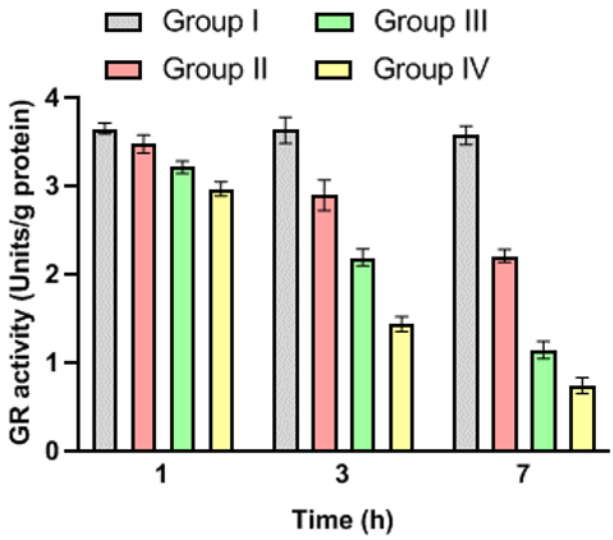

Fig. 3. Influences of different doses of ciprofloxacin on GR enzyme activities of rat small intestine

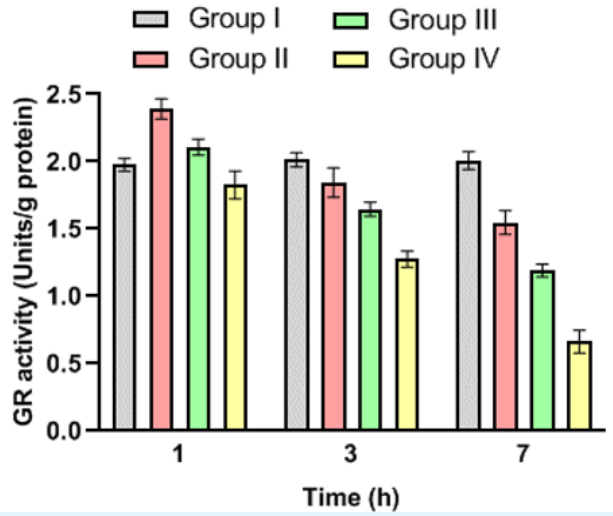

Fig. 4. Influences of different doses of ciprofloxacin on GR enzyme activities of rat testis

lowest activity ($p < 0.05$). In a group of rats that received 100 mg of ciprofloxacin (group II), the cardiac GR enzyme activity was augmented on the 1st hour as well as the third hour ($p < 0.05$), a contrast to the control group (**Fig .2**).

However, a reduction in antioxidant enzyme activity was observed after seven hours of ciprofloxacin administration ($p < 0.05$). For group III, the heart enzyme activity was raised in the 1st hour compared to group (I), but it soon decreased from the third hour to the end of the seventh- hour ($p < 0.05$). In heart of rats that were injected with 200 mg of ciprofloxacin (group IV), GR activity was not affected at the first hour ($p < 0.05$), while it was dropped on the third hour, the inactivation was increased at the end of the next period compared to the control group ($p < 0.05$).

For small intestine tissue, the GR enzyme activities in groups (II-IV) were almost higher than the control in the first hour (**Fig. 3**), then it was heightened after three hours ($p < 0.05$). But, a significant decline in the activities of the small intestine GR enzyme in experimental groups was observed after seven hours ($p < 0.05$). The testicular GR enzyme activities of both, II and III groups were higher than group I (control) by the 1st hour ($p < 0.05$), whereas the enzyme activity of group II was lower than group I for the same time ($p < 0.05$). However, the testis GR enzyme activities of three treated groups (II-IV) were inactivated from the third hour and even in the next period ($p < 0.05$, **Fig. 4**).

DISCUSSION

Under normal physiological conditions, there is a cellular balance between ROS formation and development of defense mechanisms such as antioxidant enzymes (Pisoschi and Pop 2015). Oxidative

stress occurs when ROS levels overwhelm antioxidant levels, which may lead to severe damage in cellular components of proteins, lipids, carbohydrates, and nucleic acids (Katerji et al. 2019).

Some studies have confirmed that a certain type of antibiotics including fluoroquinolones may induce intracellular ROS formation as part of its mechanisms to kill bacteria (Van Acker et al. 2016). As an endogenous antioxidant, the GR enzyme plays a fundamental role in the equilibrium of the intracellular redox status, in addition to the protection of macromolecules from oxidative damage (Zhu et al. 2018). Recently, some studies have been achieved to explain the importance of the GR enzyme and determine its functions. So far, some inhibition studies have been performed on GSH reductase in some animal tissues, including the effects of some drugs on enzyme tasks (Kocaoğlu et al. 2019). The study of the drug effects on the activity of enzymes in living conditions helps in understanding the physiological activity of these enzymes and the potential unwanted drug reactions (Bibi 2008).

In vivo tests, we assessed the effect of ciprofloxacin on GR enzyme activity at different doses. The activities of the reductase enzyme in the brain tissue suffered inhibition due to the three doses of the antibiotic, in comparison with the control group. Weak inhibition was detected in the first hour in almost all groups. Notably, the inhibitory effects against GR enzyme activities were increased after three hours in the experimental groups. Furthermore, GSH reductase was continued to lose its activity over time reaching the seventh hour. This data confirms the harmful effects of specific ciprofloxacin doses (150 and 200 mg) on the activity of brain GR enzyme.

In III and IV groups, a considerable inhibition of cardiac GR enzyme activities occurred after three hours. Thereafter, the enzyme activities were inhibited in all antibiotic groups at the following time. The ciprofloxacin doses had a stimulating effect considering the GR enzyme of the small intestine tissues, particularly in the first and third hours compared to group I, nevertheless a significant inhibitory effect developed after the longest duration of seven hours in all rat groups subjected to ciprofloxacin.

Our results revealed that the inhibitory effects of ciprofloxacin doses on the testis GR enzyme were comparable to that in the rat brain. Meanwhile, the investigated enzyme was less affected by ciprofloxacin doses in the testicular tissues. It has been demonstrated that levofloxacin, as one of the fluoroquinolones, depletes the antioxidant status in the liver and pulmonary rat cells (Olayinka et al. 2015). Besides, Dawood and colleagues reported in a recent study that ciprofloxacin doses have adverse effects on GST; another cellular antioxidant (Dawood et al. 2020). In the current study, the impacts of different ciprofloxacin doses on GR enzyme activity was determined at different periods in some rat tissues. The obtained results from in vivo experiments are pivotal to explain the physiological traits of this enzyme and to highlight the toxicity of ciprofloxacin.

CONCLUSION

The selected doses of ciprofloxacin had an inhibitory action against GSH reductase in the tested rat organs. Under this, in brain tissues, the three ciprofloxacin doses inhibited the GR activities throughout the entire periods of the test. As for cardiac tissues revealed inhibited enzymatic activity in III and IV groups after three hours followed by stronger inhibition after seven hours of administration. On the other hand, the small intestine expressed no adverse effect of ciprofloxacin relating to the GR enzyme activity except for group IV after the seventh hour. Also, the three ciprofloxacin doses in the rat testicle manifested their most potent inhibitory effect on the investigated enzyme activity after seven hours. These findings confirm the harmful effects of ciprofloxacin on the health of patients, despite its efficacy against bacteria, hence the doctors must prescribe safe alternative antibiotic.

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