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In vivo biochemical evaluations of some β-lactam group antibiotics on glutathione reductase and glutathione S- transferase enzyme activities

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ABSTRACT

Objectives: The aim of this study was to investigate whether some of the cephalosporin group antibiotics have inhibition effects on GR and GST enzymes with important functions in the metabolic pathway.
Methods: In this study, some selected cephalosporin group antibiotics on GST and GR enzyme was carried out using 96 rats. 16 groups (16 × 6) were created from these rats, divided to another 4 groups (4 × 24). The resulting groups were named as sham groups, cefazolin groups, cefuroxime groups and cefoperazone groups, respectively. The antibiotics used were injected to cefazolin, cefuroxime and cefoperazone groups. The inhibition effects of the antibiotics were measured in the different time intervals (1st, 3th, 5th, 7th). The statistical investigation of the results was performed using the SPSS software program.
Results: Results revealed the complex effects of the tested substances on GR and GST activity at different time intervals and in different tissues ($p < 0.05$). This indicated that the tested substances could be exposed to different interactions *in vivo*.
Conclusion: The tested antibiotics showed some significant inhibition effects on the GST and GR enzyme activity in some tissues of brain, eye and muscle. The interaction of enzyme - the drug is a key factor to highlight the toxicological mechanism. For this reason, the results obtained from *in vivo* experiments are crucial to explane the physiological properties of the enzymes.

1. Introduction

The functions of antioxidants in living creatures are very important. Glutathione S- transferase (GST) and glutathione reductase (GR) are antioxidant enzymes and present various tissues. Glutathione S- transferase (GST) isoenzymes are simultaneously located in various tissues, hence they have a multifunctional effects. GST constitutes 10% of the cytosolic proteins that provide an oxidative function and catalyzes conjugation reactions of xenobiotic and toxic compounds. In this way, GST provides a protection against oxidants, and that facilitates the formation of metabolism of these compounds [1,2]. The proteins exhibiting GST activity are separated from three families, and these are microsomal, cytosolic and mitochondrial [3]. Microsomal GST consists of a protein structure plays a key role in glutathione (MAPEG) and eicosanoid metabolism, therefore these family has taken microsomal GST family name [4]. Cytosolic is one of the detoxification enzymes and has hetero and homo dimers [5]. Although mitochondrial and cytosolic GST are different in many properties, both of them contain water-soluble enzymes [6]. Mitochondrial and cytosolic GST families are separated into eight classes depend on their substrate structure and amino-acid sequence. These eight classes are alpha (α), omega (ξ), theta (θ), sigma (δ), mu (μ), kappa (κ), pi (π), zeta [7]. GST enzymes are highly effective in genetic variations and drug biotransformation with catalytic supplements. Additionally, GST also affects the cellular defense of toxicities from environmental agents. In particular, alpha (α), pi (π) and mu (μ) GST are effective in removing toxins such as 4-hydroxynonenal forming as a result of peroxidation of lipids and acrolein originating from cigarette smoke [8]. Glutathione reductase (GR) is another important enzyme and triggers the redox reaction of glutathione. The amount of GR in the temozolomide cells providing resistance is higher than sensitive cells. Some studies have reported that the decreasing amount of GR in medicated therapies and increasing the sensitivity of cisplatin and temozolomide drugs. However, it has been observed that the amount of GR enzyme in sensitive cells increases the resistance against

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chemotherapy [9,10]. Additionally, GR catalysis the electron transfer in some molecules such as reduced pyrimidine nucleotide and disulfide. As mentioned above, the reactions that occur in the cellular environment and GR catalyzed ensure the protection of GSH-GSSG (reduced and oxidized glutathione) [11].

Antibiotics play an important role in intracellular activities such as prevention of bacterial infection, facilitating digestion and delivery of food to target cells [12]. Cefuroxime antibiotic is very effective in protecting against *Staphylococcus aureus* with gram-positive bacterial species that causes bone and soft tissue infections [13,14]. Additionally, so many treatments on infections such as meningitis, pneumonia, septicemia, urinary infection, and peritonitis are performed using cefuroxime. The usage of cefuroxime is easy, and some papers have reported that cefuroxime can be used in the treatment in serious infections with sequential administration [15]. This antibiotic is known as a third- β-lactam generation piperazine and semi-synthetic antibiotic exhibit a fairly large spectrum of activity on grams positive, and on negative bacteria [16]. Cefoperazone has been widely tested for the treatment of calf diseases (pneumonia and diarrhea) [17], joint and bone diseases of horses [18], and effective penetration of the pancreas [19]. Cefazolin has a potention prevent to the effect of gram negative bacteria [20]. The antibiotics (Cefuroxime, cefoperazone and cefazolin) used in this study are cephalosporin group antibiotics. Cephalosporins are semi-synthetic beta-lactam antibiotics and are widely used in the treatment of human and animal diseases [21]. Beta-lactam antibiotics inhibit the cell wall biosynthesis by binding the proteins in the microbial membrane to the penicillin and cause cell death [22]. Generally, cephalosporins are divided into three groups as first, second and third generation according to antibiotic characteristics and discovery rankings [23]. Although the first group of cephalosporins shows effective activity against gram-positive bacteria, they are inefficient against enterococci and staphylococci. Additionally, the second group is highly active against salmonella and *Escherichia coli* bacteria [24].

The effects of cefuroxime, cefoperazone and cefazolin antibiotics on GSTs isoenzymes in some tissues such as heart, liver, and kidney have been investigated in our previous study [1]. In this study, for the first time, the effects of these antibiotics (cefazoline, cefuroxime and cefoperazone) on GR and GSTs enzyme activities present in some tissues such as eye, brain, and muscle were investigated *in vivo* time-dependently (1st, 3th, 5th, 7th hours).

2. Materials and methods

All chemicals, GSTs, GR, antibiotics and solvents used were of analytical grade with the highest degree of purity and were purchased from Sigma-Aldrich, Co.

2.1. The process of experiments

The approval of ethics committee was taken from Van Yuzuncu Yıl University to conduct the study. Herein, 96 rats, each 3 mounth old, were used in the study. 16 groups (16 × 6) were created from these rats, divided to another 4 class groups (4 × 24). The resulting groups were named as sham groups (1st, 3th, 5th, 7th hours), cefazolin groups (1st, 3th, 5th, 7th hours), cefuroxime groups (1st, 3th, 5th, 7th hours) and cefoperazone groups (1st, 3th, 5th, 7th hours), respectively. The drug was not given to rats of the sham groups, and injector into their intraperitoneal regions were dipped and then removed. Cefazolin groups were injected with 50 mg/kg cefazolin as intraperitoneal and these groups were operated under the anesthesia at 1st, 3th, 5th and 7th hours, respectively. Then, the target tissue was taken and stocked at −80 °C. Cefuroxime groups were injected 25 mg/kg cefuroxime and the procedure in the cefazoline groups was repeated. In the same way, cefoperazone groups were injected 50 mg/kg cefoperazone and the process in the others group was treated. The steps of the operation are summarized in Fig. 1.

2.2. The preparation procedure of eye, brain and muscle tissues

A buffer solution containing 1.8 mL Tris-HCl (20 mM, pH 7.4) was prepared and added to the tissue (1/10, gram / volume) which to be used in the experiments. The resulting slurry was stirred by homogenizer (Ultra Turrax-T25, Made in Japan) as given in elsewhere [25] . This homogenized mixture was centrifuged at 30 °C at 15.000 rpm for 30 min. The top layer on the mixture was placed in another tube. In the last case, all samples were stocked at −80 °C for using in further experiments [26].

2.3. Measurements of GR and GST enzyme activities

Enzymatic activity was measured spectrophotometrically at 25 °C according to the method of Carlberg and Mannervik [27]. The assay system contained 0.75 mM Tris-HCl buffer, pH 7.0, including 1 mM EDTA; 1 mM GSSG; and 0.1 mM NADPHin a total volume of 1 mL. The decrease in absorbance at 340 nm was followed with a Agilent techonology Spectrophotometer Carry 60 UV Vis. The reaction was initiated by adding the enzyme solution. One enzyme unit is defined as the oxidation of 1 μmol of NADPH/min under the assay conditions.

GSTs activity was measured at 25 °C using 1-chlro-2,4-dinitrobenzene as a model substrate. The assay system included phosphate buffer (pH 6.5), GSH (20 mM) and 1-chlro-2,4-dinitrobenzene (25 mM). A spectrophotometer was employed to estimate the changes in absorbance at 340 nm for 3 min [28,29].

2.4. Determining protein amount in enzymes

The amount of protein in the enzymes was determined using Bradford method [30]. In determination of the amount of protein measurements, bovine serum albumin was taken as a standard enzyme model as described elsewhere [31].

2.5. Statistical analysis of the study

In order to determine descriptive statistical of the data of activities of enzymes (GR and GSTs), descriptive statistics were expressed as the mean and standard deviations. All the statistical calculations were performed by using SPSS 15 computer package programe. The separately and together effect of groups and time intervals on parameter were examined using two-way ANOVA method. The difference of among groups and the difference of time intervals were tested by using Turkey posthoc test. The importance of statistical data was determined as 5% ($p < 0.05$), and values of p which is smaller than 0.5 were taken as meaningful values.

3. Results

The effects of the antibiotics tested on GR and GSTs activities in eye, brain and muscle tissues are detailed Tables 1 and 2, respectively. Brain GR enzyme activities of cefazolin, cefuroxime and cefoperazone groups were decreased compared to sham groups in the same time intervals ($p < 0.05$). Brain GR activity in the same drug groups' are inhibited during in the first 3 h, activated after other time intervals ($p < 0.05$). However, the brain GR activities for cefazolin, cefuroxime, and cefoperazone were lower than the activities obtained from sham groups in the same time even at the end of 7th hour ($p < 0.05$, Table and Fig. 2).

The eye GR activitiy of cefazolin, cefuroxime, and cefoperazone groups was low almost in the whole compared to sham groups in the same time intervals ($p < 0.05$). When change in the same drug groups time-dependently was examined, eye GR activities of cefazolin group was inhibited during the first 3rd h, in cefuroxime group during 7th h and in cefoperazone group during ^{5th} h ($p < 0.05$). However, eye GR activities of cefoperazone groups were started to increase after from at 5th h ($p < 0.05$, Table 1 and Fig. 4).

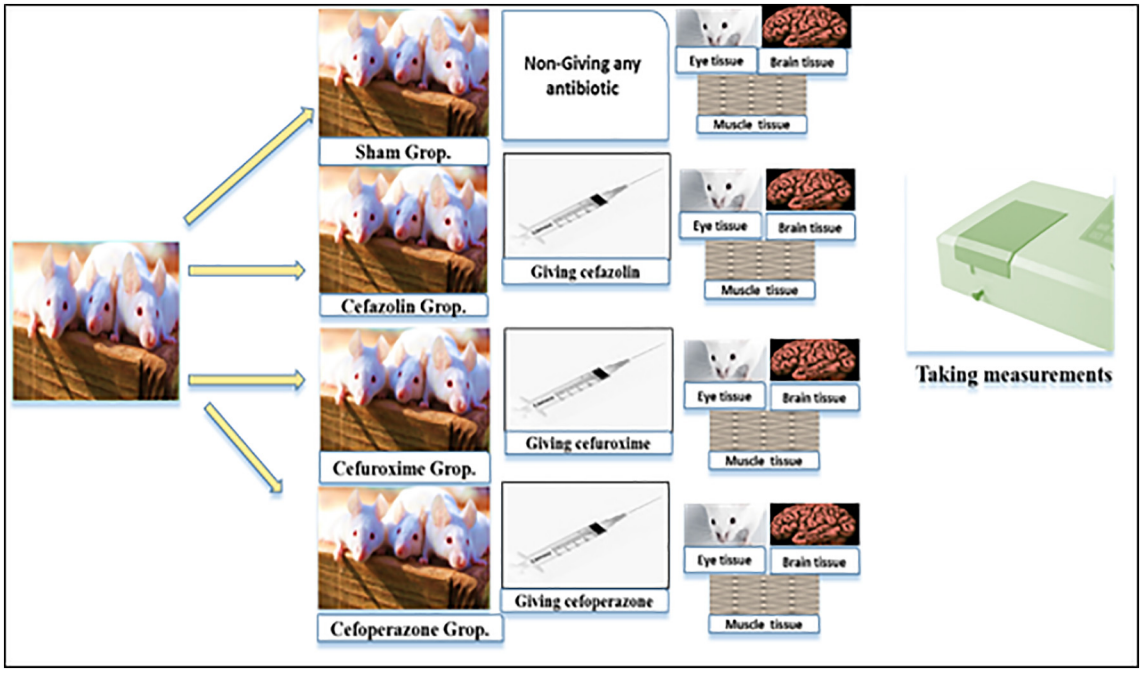

Fig. 1. The schematic representation of the experimental process.

The muscle GR activities of the cefazolin group at 1st hour was lower than the sham group in the same time ($p < 0.05$). However, the muscle GR activities of the other drugs groups were higher than the sham group almost in the whole time intervals ($p < 0.05$). Additionally, muscle GR activity both cefazolin and cefuroxime groups was enhanced during the first 5 h and started to decrease in the next time intervals ($p < 0.05$). But, a decline for muscle GR enzyme activity in cefoperazone group was observed from the 1^{sth} h to the end of 7^{sth} h ($p < 0.05$) (Table 1 and Fig. 6).

As seen Table 2 and Fig. 3 The brain GSTs activities of cefazolin, cefuroxime, and cefoperazone groups were increased compared to the sham groups almost all the same time periods ($p < 0.05$). The brain GSTs activity of cefuroxime and cefoperazone groups was inhibited during the first 3 h, and it was activated in next time periods ($p < 0.05$) (Table 2 and Fig. 3).

When eye GSTs activities were examined, almost all eye GSTs enzyme activities of cefazolin, cefuroxime, and cefoperazone groups were lower than the sham group in the same time period ($p < 0.05$). Additionally, eye GSTs enzyme activity in between of cefazolin groups was inhibited in the first 3 h, then it was began to be activated in the other time intervals ($p < 0.05$). The eye GSTs activities in tissue of cefuroxime groups was inhibited during the first 5 h time dependently, and then it began to be activated in the next time intervals ($p < 0.05$). The other results are detailed in Table 2 and Fig. 5.

The muscle GSTs activity of all the whole drugs groups was lower than the sham group at 5th and 7th hours ($p < 0.05$). When change in

Table 1
Effects of tested antibiotics on GR activities in different tissue homogenates time-dependently.

Time (hour)	Sham groups	Cefazolin groups	Cefuroxime groups	Cefaperazon groups	*P* Values (intergroup)
Brain GR Level (U/g protein)					
1	3.44 ± 0.18^{aA}	$2.57 \pm 0.0.26^{aB}$	$2.49 \pm 0.0.13^{bB}$	1.74 ± 0.14^{aB}	0.003
3	1.85 ± 0.14^{cA}	$0.93 \pm 0.0.12^{bB}$	0.95 ± 0.07^{cB}	0.39 ± 0.04^{cC}	0.001
5	3.19 ± 0.21^{bA}	$0.42 \pm 0.0.06^{cD}$	0.61 ± 0.03^{cC}	1.24 ± 0.06^{bB}	0.001
7	3.23 ± 0.15^{bA}	$1.95 \pm 0.0.11^{bD}$	2.63 ± 0.26^{aC}	2.73 ± 0.02^{aB}	0.001
P values (time-dependently)	0.001	0.001	0.001	0.001	
Eye GR Level (U/g protein)					
1	6.69 ± 0.19^{aA}	$4.09 \pm 0.0.24^{bC}$	3.46 ± 0.19^{aC}	5.06 ± 0.35^{aB}	0.004
3	$4.97 \pm 0.0.25^{cA}$	2.42 ± 0.22^{cC}	2.86 ± 0.25^{bB}	2.95 ± 0.27^{cB}	0.001
5	$4.56 \pm 0.0.36^{dB}$	5.37 ± 0.28^{aA}	1.92 ± 0.19^{dC}	1.29 ± 0.07^{dC}	0.001
7	$5.03 \pm 0.0.19^{bA}$	1.18 ± 0.04^{dD}	2.05 ± 0.15^{cC}	4.62 ± 0.58^{bB}	0.001
P values (time-dependently)	0.001	0.002	0.001	0.001	
Muscle GR Level (U/g protein)					
1	1.00 ± 0.12^{bB}	0.80 ± 0.08^{cB}	1.55 ± 0.05^{bA}	1.54 ± 0.05^{aA}	0.004
3	0.94 ± 0.04^{cC}	1.01 ± 0.03^{cC}	1.64 ± 0.22^{bA}	1.35 ± 0.06^{aB}	0.001
5	1.28 ± 0.11^{aB}	2.26 ± 0.19^{aA}	2.01 ± 0.14^{aB}	1.35 ± 0.04^{aB}	0.001
7	1.23 ± 0.12^{aB}	1.70 ± 0.22^{bA}	1.16 ± 0.02^{cC}	1.18 ± 0.01^{bC}	0.001
P Values (time-dependently)	0.001	0.001	0.001	0.012	

$^{a,b,c,d}p < 0.05$: The a, b, c and d in the same drug groups (in the same column) exhibited significant changes time-dependently.
$^{A,B,C,D}p < 0.05$: The A, B, C and D in the same time intervals (in the same line) exhibited significant changes between different drug groups.

Table 2
Effect of tested antibiotics on GST activities in different tissue homogenates time dependently.

Time (hour)	Sham groups	Cefazolin groups	Cefuroxime groups	Cefaperazon groups	P values (intergroup)
Brain GST Level (U/g protein)					
1	5.94 ± 0.20^{aC}	5.74 ± 0.39^{aC}	6.48 ± 0.44^{bB}	8.32 ± 0.24^{aA}	0.003
3	4.84 ± 0.40^{bB}	5.53 ± 0.41^{bB}	5.72 ± 0.12^{cB}	6.65 ± 0.30^{cA}	0.829
5	4.32 ± 0.11^{cC}	5.71 ± 0.31^{aB}	7.25 ± 0.33^{aA}	7.38 ± 0.34^{bA}	0.001
7	4.70 ± 0.34^{bD}	5.69 ± 0.21^{bC}	8.96 ± 0.66^{aA}	6.60 ± 0.37^{cB}	0.002
P values (time-dependently)	0.002	0.001	0.001	0.001	
Eye GST level (U/g protein)					
1	2.85 ± 0.36^{aA}	2.02 ± 0.08^{aA}	0.87 ± 0.06^{cB}	2.59 ± 0.0.24^{cA}	0.025
3	2.99 ± 0.13^{aA}	0.88 ± 0.06^{dC}	1.36 ± 0.17^{bB}	2.67 ± 0.20b^{A}	0.001
5	2.32 ± 0.08^{bB}	1.78 ± 0.17^{bC}	0.63 ± 0.03^{dD}	3.08 ± 0.21a^{A}	0.001
7	299 ± 0.11^{aB}	1.60 ± 0.13^{cC}	2.51 ± 0.22^{aA}	3.22 ± 0.21^{aA}	0.006
P Values (time-dependently)	0.001	0.001	0.001	0.001	
Muscle GST Level (U/g protein)					
1	1.17 ± 0.13^{bA}	1.10 ± 0.07^{aA}	0.98 ± 0.08^{aB}	0.74 ± 0.05^{bC}	0.001
3	0.83 ± 0.04b^{cA}	0.87 ± 0.06^{bA}	0.84 ± 0.02^{bA}	0.63 ± 0.03^{bB}	0.001
5	1.33 ± 0.12^{bA}	0.50 ± 0.04^{bB}	1.38 ± 0.10^{aA}	1.14 ± 0.09^{aA}	0.001
7	1.73 ± 0.11^{aA}	1.41 ± 0.10^{aA}	0.46 ± 0.09^{cC}	1.07 ± 0.06^{aB}	0.001
P Values (time-dependently)	0.002	0.007	0.001	0.001	

$^{a,b,c,d}p < 0.05$: The a, b, c and d in the same drug groups (in the same column) exhibited significant changes time-dependently.
$^{A,B,C,D}p < 0.05$: The A, B, C and D in the same time intervals (in the same line) exhibited significant changes between different drug groups.

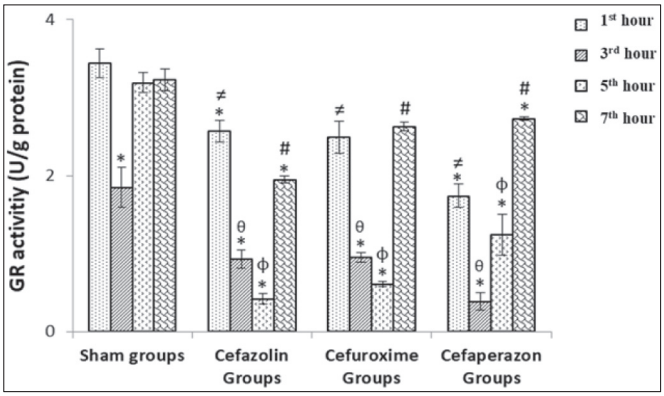

Fig. 2. Comparison of the effect of different drugs on the activity of GR enzyme in brain tissue *p: The same groups in different time intervals exhibit meaningful values ($p < 0.05$), ≠p: 1st h exhibited meaningful values compared to the sham group ($p < 0.05$), θp: 3th h exhibited meaningful values compared to the sham group ($p < 0.05$), ϕp: 5th h exhibited meaningful values compared to the sham group ($p < 0.05$), #p: 7th h exhibited meaningful values compared to the sham group ($p < 0.05$).

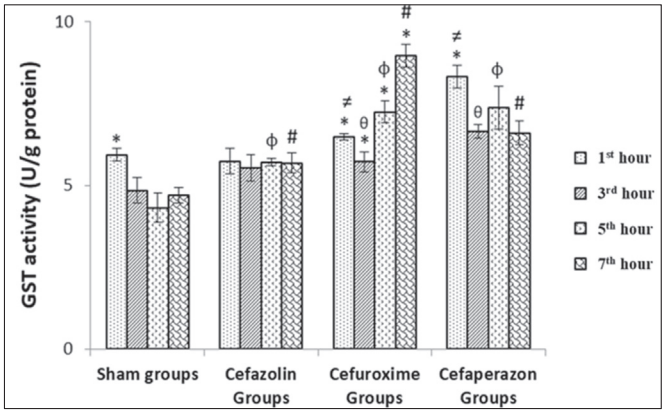

Fig. 3. Comparison of the effect of different drugs on the activity of GST enzyme in brain tissue *p: The same groups in different time intervals exhibit meaningful values ($p < 0.05$), ≠p: 1st h exhibited meaningful values compared to the sham group ($p < 0.05$), θp: 3th h exhibited meaningful values compared to the sham group ($p < 0.05$), ϕp: 5th h exhibited meaningful values compared to the sham group ($p < 0.05$), #p: 7th h exhibited meaningful values compared to the sham group ($p < 0.05$).

muscle GSTs activities of the same drug groups time-dependently were examined, an inhibition was observed in the GSTs activity of groups with cefazolin during in the first 5 h, and then an activation was seen in muscle GSTs activity. In the other drug groups, the muscle GSTs activities were inhibited during in the first 3 h while then an activation was observed in other time intervals ($p < 0.05$). The other results were detailed in Table 2 and Fig. 7.

4. Discussion

Antioxidant enzymes have crucial functions in living metabolism. Hence, nowadays various studies related to antioxidant enzyme activities have been conducted. Normally, in living creatures, the number of antioxidants and production of free radicals in the living tissues have an balanced level. However, if this balanced level changes because of the changing the number of free radicals in a living organism, oxidative stress and some pathogenic diseases associated with oxidative stress can be seen in [32]. Especially, because of increasing level of exogenous free radical due to environmental pollution, the usage of alcohol and drug, exposure to X-ray and UV can lead to damaging of protein, carbohydrate and lipids in living organisms. When the amount of free radicals is increased, the number of endogenous antioxidants cannot be adequate because of the increase of consumption of these antioxidants.

GSTs are available in many important tissues such as heart, liver, and kidney. Due to the availability of GSTs in many tissues, the analysis of GSTs enzyme activity can be easily conducted. Literature contains various studies related to the effect of various different molecules and compounds on GSTs enzyme activity [33,34]. The most tested compounds and drug groups on GSTs enzymes are glutathione analogues, bifunctional inhibitors, ethacrynic acid, haloenol lactones, prodrugs and tocopherols [35]. It has been reported in a study that the ihibition of GSTs is increased with the enhancing of Hypericin dosage. Also, it was determined the observed inhibition type was not a competitive inhibition [36]. Additionally, Türkan et al. conducted some studies of *in*

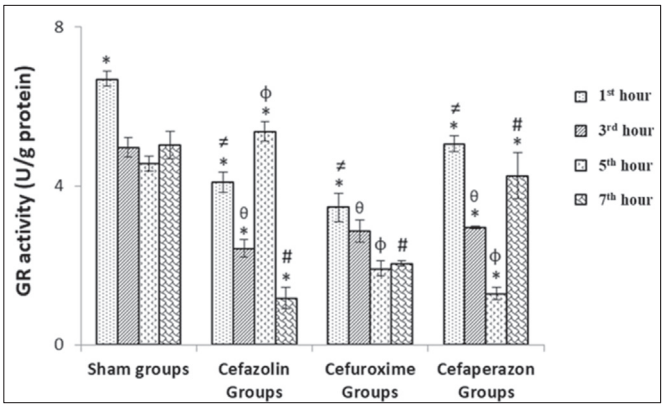

Fig. 4. Comparison of the effect of different drugs on the activity of GR enzyme in eye tissue *p: The same groups in different time intervals exhibit meaningful values ($p < 0.05$), ≠p: 1st h exhibited meaningful values compared to the sham group ($p < 0.05$), θp: 3th h exhibited meaningful values compared to the sham group ($p < 0.05$), Φp: 5th h exhibited meaningful values compared to the sham group ($p < 0.05$), #p: 7th h exhibited meaningful values compared to the sham group ($p < 0.05$).

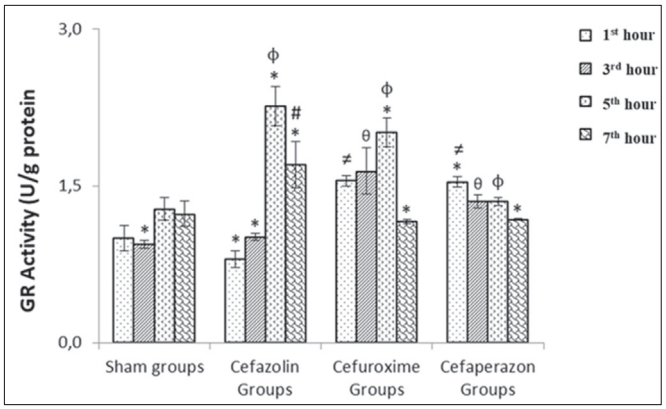

Fig. 6. Comparison of the effect of different drugs on the activity of GR enzyme in muscle tissue *p: The same groups in different time intervals exhibit meaningful values ($p < 0.05$), ≠p: 1st h exhibited meaningful values compared to the sham group ($p < 0.05$), θp: 3th h exhibited meaningful values compared to the sham group ($p < 0.05$), Φp: 5th h exhibited meaningful values compared to the sham group ($p < 0.05$), #p: 7th h exhibited meaningful values compared to the sham group ($p < 0.05$).

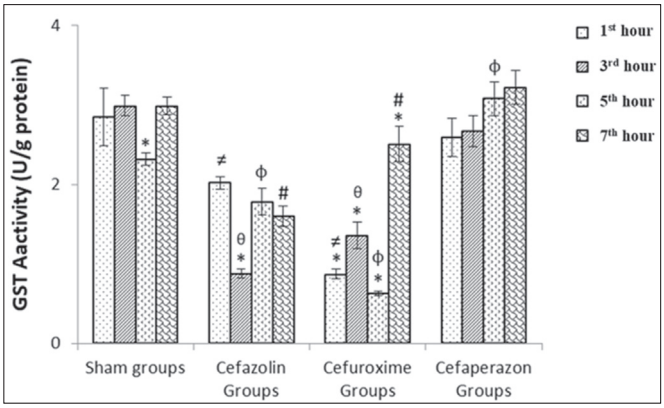

Fig. 5. Comparison of the effect of different drugs on the activity of GST enzyme in eye tissue *p: The same groups in different time intervals exhibit meaningful values ($p < 0.05$), ≠p: 1st h exhibited meaningful values compared to the sham group ($p < 0.05$), θp: 3th h exhibited meaningful values compared to the sham group ($p < 0.05$), Φp: 5th h exhibited meaningful values compared to the sham group ($p < 0.05$), #p: 7th h exhibited meaningful values compared to the sham group ($p < 0.05$).

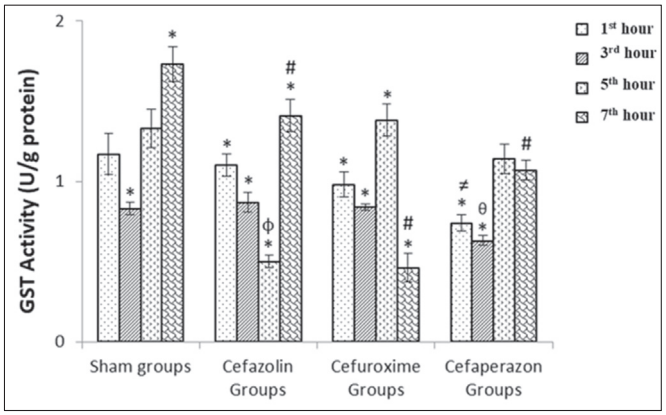

Fig. 7. Comparison of the effect of different drugs on the activity of GST enzyme in muscle tissue *p: The same groups in different time intervals exhibit meaningful values ($p < 0.05$), ≠p: 1st h exhibited meaningful values compared to the sham group ($p < 0.05$), θp: 3th h exhibited meaningful values compared to the sham group ($p < 0.05$), Φp: 5th h exhibited meaningful values compared to the sham group ($p < 0.05$), #p: 7th h exhibited meaningful values compared to the sham group ($p < 0.05$).

vivo and *in vitro* for the effect of antibiotics on GSTs and they have obtained similar findings to this study [37]. In this study, the cefuroxime showed activator property on the brain GSTs activity during the first 5 h time-dependently. However, other drugs showed the opposite effect. In addition, cefuroxime and cefoperazone exhibit activator effect on muscle and eye GSTs levels, whereas cefazolin exhibited inhibition effect depending on time.

The function of GR enzyme is crucial to the balance of intracellular redox reaction, the protection of macromolecular and the damaging of protein against the oxidations [38]. Upon the discovery of GR enzyme, the defining of studies has been performed, thence, the characterization of GR revealed the importance and its functions in metabolism. So far, some animal tissue, erythrocytes, and various different drugs have been tested on the inhibition of GR enzyme [39]. Our results showed that the tested drugs cefazolin, cefuroxime, and cefoperazone inhibit brain and eye GR enzyme activity the first during 5 h. However, cefuroxime and cefaperazon induced muscle GR activity in the same time period.

In another study, we reported that the tested drugs have different activities in different tissues on the studied enzyme levels and MDA levels. In addition, these drugs had an inducing effect on the levels of other studied enzymes and GST activity in some tissues, while causing inhibition in some tissues time-dependently. The results of this study reveal different and complex effects of the drugs *in vivo* time-dependently [40].

This report contains an extensive experimental study of *in vivo* to determine the inbition effects of cefazolin, cefuroxime, and cefoperazone drugs on GSTs and GR enzymes in brain eye and muscle of rats time-dependently. The results obtained from *in vivo* experiments are crucial to explane the physiological properties of GSTs and GR enzymes. In summarize, the antibiotics tested in this study showed different inhibition effects on GSTs and GR enzymes in brain, eye and muscle tissues in different time intervals. Especially, the interaction of enzyme the drug is a key factor to highlight the toxicological mechanism.

5. Conclusion

Cefazolin, cefuroxime, and cefoperazone drugs exhibited some strong inhibition effects on the activity of GR in the brain and eye tissue over the first 5 h, and their inhibitions effects were eliminated in the other time interwals. But, the tested drugs showed activation effect on

GR activity in muscle tissue time-dependently. In addition, test drugs exhibited activation profile on GSTs activity compared to the sham groups in the brain tissue time-dependently, while they showed generally inhibitory effect in the other tissues. These results revealed the complex effects of the tested substances on GR and GSTs activity at different time intervals and in different tissues. This indicated that the tested substances could be exposed to different interactions *in vivo*. These interactions should be detailed in further studies. Also, the dosage and use of these drugs should be adjusted correctly.

Disclosure statement

All the authors have no conflict for the publication of this article.

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