



Effect of different doses of the catecholamine epinephrine on antioxidant responses of larvae of the flesh fly *Sarcophaga dux*

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**Effect of different doses of the catecholamine epinephrine on antioxidant responses of
larvae of the flesh fly *Sarcophaga dux***

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Abstract

The production and use of pharmaceutical products have increased over the past decades, and several are considered potential or proved hazardous wastes. When contaminating the environments, they can severely impact biodiversity. The catecholamine epinephrine (adrenaline) is no exception. Epinephrine can be administered as growth promoter in cattle, and is used for anaphylaxis treatment in human. While a range of studies have examined the effects of this catecholamine on vertebrates tissues, and evidenced that it can disrupt the oxidative stress status, the effects epinephrine could have on insects have remained poorly considered. Here, we examined the physiological effects of different concentrations (0, 25, 50 and 100 µg/mL) of epinephrine on larvae of the flesh fly *Sarcophaga dux*. Following experimental treatments, levels of H₂O₂, GSH, CAT, GPx, and CEH were measured from the fat body, cuticle, gut, and hemolymph of 3rd instars. Significant differences are reported for these physiological endpoints among the considered body compartments, and epinephrine concentrations. Epinephrine treatments did not increase reactive oxygen species production (H₂O₂ amounts), except for gut tissues. Increased levels of GSH suggest that epinephrine may have enhanced glucose metabolism and flux towards the pentose phosphate pathway, while reducing glutamine oxidation. CAT activity was slightly increased when the concentration of epinephrine was higher. The decreased GPx activity in the fat body was consistent with GSH variations. In sum, the injection of epinephrine seemed to elicit the antioxidant response in *S. dux* larvae, in turn attenuating ROS production.

Keywords

Adrenaline, Oxidative stress, Hydrogen peroxide, Glutathione, Catalase, Glutathione peroxidase, Sarcophagidae

Highlights

- The effects of different doses of epinephrine on flesh fly larvae were examined
- Levels of H₂O₂, GSH, CAT, GPx, and CEH were measured from different body parts
- Increased levels of GSH were found in epinephrine-treated larvae
- CAT activity was slightly increased when the concentration of epinephrine was higher
- Epinephrine can reduce the production of reactive oxygen species

1. Introduction

Environmental pollution is becoming a significant issue worldwide, severely impacting biodiversity in general (Edwards 2002; de Souza Machado et al. 2018; Miller et al. 2018). In particular, pharmaceutical residues can reach high amounts (Grenni et al. 2018) in soils fertilized with wastewater effluents, sewage sludge, or waste manure from livestock (Kaczala & Blum 2016). When released into the environment, these drugs can be biomagnified along with the food web (Kelly et al. 2007; Fremlin et al. 2020), increasing the threats to top predators and higher vertebrates (Shore et al. 2014), as well as for those species thriving on decaying cadavers of top predators. Among the different drugs administered to humans and cattle, antibiotics, antidepressants, or growth promoting factors are frequently reported from natural environments (Küster and Adler 2014). These pollutants, which can persist over the long term, can greatly threaten biodiversity (Santos et al. 2010; Richmond et al. 2016). For instance, in a recent study, Renault et al. (2018) experimentally evidenced the deleterious effects of antibiotics on the development and survival of the blow fly *Chrysomya albiceps*. In mosquitoes, chronic exposure of the parental generation to the anti-inflammatory ibuprofen had consistent effects on the progeny by accelerating their development and increasing their capacity to handle starvation periods (Prud'homme et al. 2018).

Nowadays, many pharmaceuticals are listed as potential or proven hazardous wastes by the federal Resource Conservation and Recovery Act (RCRA), including the catecholamine epinephrine (adrenaline). This compound is used to treat anaphylaxis episodes in human, *i.e.* limiting the severity of venom, food or medication-induced allergies. This compound, also used as growth promoter in cattle, can be further found in the environment, with unknown consequences for wildlife. Epinephrine can also be detected in high amounts in plants cultivated in industrial areas, bio-indicating plants' exposure to environmental stress (Azzazy 2020). As a result, environmental risks, owing to the management of the produced wastes, the potential transfer of part of the drug into the environment *via* urine, or for herbivorous and saprophagous organisms developing on rich epinephrine-containing organisms, can be significant.

In mammals, epinephrine (Adrenaline: ((R)-4-(1-hydroxy-2-(methylamino)ethyl)-benzene-1,2-diol) is a naturally produced catecholamine having a neurotransmitter function. Epinephrine is excreted by the sympathetic nervous system and the adrenal medulla, and it is known to help in the flight or fight process of vertebrate organisms by regulating blood pressure, blood sugar levels and cardiac activity (Cahova et al. 2012; Wu et al. 2013). When administered as a growth promoter or for anaphylaxis treatment, epinephrine can reach high amounts that can have cascading physiological consequences for the organism. Specifically, large concentrations of epinephrine have been reported to provoke deleterious effects on several biological functions and macromolecules, in particular proteins, lipids and DNA, increasing the production of protein carbonyls, lipid peroxides, and DNA strand breaks due to its auto-oxidation and pro-oxidant activity (Tichy 2011; Gavrilović et al. 2012; Radaković et al. 2018). While epinephrine can contribute to reduce oxidative stress (Álvarez-Diduk and Galano 2015), excessive contents of this catecholamine enhance the production of reactive oxygen species, as demonstrated in mammals (Djelić et al. 2015; Radaković et al. 2018; Pal et al. 2020). Thus, according to the physiological situation, epinephrine can be protector or molecular target of free radicals (Álvarez-Diduk and Galano 2015).

Oxidative stress is the consequence of imbalanced amounts in the levels of reactive oxygen species (ROS), such as superoxide, peroxide, hydroxyl radical or singlet oxygen, and non-enzymatic and enzymatic ROS-scavenging compounds in the organism. To reduce the negative effects of ROS (which includes DNA strand breaks, production of protein carbonyls and lipid peroxides), enzymatically-based mechanisms, involving catalase (CAT), glutathione peroxidase (GPx), and carboxyl esterase hydroxylase (CEH), as well as non-enzymatically based mechanisms, such as reduced glutathione (GSH), are acting in the organisms as the first defense line. Antioxidant responses have been characterized from several arthropods exposed to a diversity of stressful abiotic factors (Lalouette et al. 2011; Lawniczak et al. 2013; Renault et al. 2016; Abdelfattah et al. 2017; Yousef et al. 2017; Abdelfattah et al. 2021).

While they can be subjected to epinephrine exposure in their environments, the effects of this catecholamine have poorly examined in arthropods. In crustaceans (crabs), it has been reported that epinephrine injections increased the activity of the myogenic heart (Yamagishi 2003) and the amounts of glucose in the hemolymph, while decreasing glycogen levels in the hepatopancreas of the individuals (Model et al. 2019). In insects, Naidu (1955) reported an acceleration of the heartbeat in epinephrine-injected cockroaches. Chowanski et al. (2017) experimentally demonstrated that epinephrine stimulates the contraction of muscles of hindgut and oviduct in the beetle *Tenebrio molitor*. To further improve our understanding of the effects of this catecholamine on insects, we assessed the physiological effects of different concentrations of epinephrine on the flesh fly *Sarcophaga dux*. This insect, of prominent importance for the decomposition of cadavers (Pape 1996), is also frequently used in ecotoxicological researches aiming at monitoring the impacts of various drug concentrations on the development and physiology of insects. While epinephrine may protect the organism against the deleterious effect of oxidative stress at physiological doses, by acting as antioxidant and free radical scavengers (Jhang et al. 2014), high doses may become at risk for living organisms (Zarow et al. 2003; Weinshenker et al. 2008). Here, we evaluated if different epinephrine concentrations (injections of 0, 25, 50 or 100 µg/mL) could affect the levels of H₂O₂, GSH, CAT, GPx, and CEH from different body compartments (fat body, cuticle, gut, and hemolymph) of 3rd instar larvae of the flesh fly *Sarcophaga dux* (Supplementary Information 1).

2. Material and Methods

2.1. Insects rearing and epinephrine treatments

For the purpose of the study, freshly emerged 3rd instars of the flesh fly *Sarcophaga dux* were obtained from the Entomology Department of the Faculty of Science of Cairo University (Cairo, Egypt). The insects were reared in plastic squared boxes of 30x30x15 cm (LxLxh) at 37 °C (RH, 12:12h Light: Dark), and supplied with beef meat and water *ad libitum*. In this species, the duration of the 3rd instar ranges from 44 to 151h at 38 °C (Chakraborty et al. 2014). Before the larvae were used for the experiments, they were starved for 24h in order to standardize the feeding status among individuals.

Different concentrations of the catecholamine epinephrine, *i.e.* 0, 25, 50 and 100 µg/mL, were freshly prepared (obtained from Sigma Aldrich, CAS number 51-43-4). The desired concentrations were obtained by dissolving epinephrine in deionized water. Then, a volume of 3 µL of one of the

prepared epinephrine solutions was injected to each larva. The larvae that received deionized water only (3 μ L) served as controls. All injections were made with a microsyringe between the third and fourth segment of the abdomen. Twenty minutes post-injection, the cuticle of the larvae was punctured with a fine sterile dissecting needle, and hemolymph of the larvae was collected with a glass capillary tube under a binocular. Immediately after collection, hemolymph was transferred to sterile and chilled microtubes which were kept at -20 °C until subsequent analyses.

For the dissection of gut, cuticle, and fat body, the larvae were transferred to a Petri dish containing 1X phosphate buffered saline solution and kept on cold ice. The delay between injection and dissection was fixed to ensure that epinephrine could diffuse within the body and has potential physiological effects. The dissected parts of the body and hemolymph, hereafter referred to as body samples, were stored at - 20 °C until use. For each experimental group, pools of 20 individuals of the 3rd instar of *S. dux* were prepared.

2.2. Measurements of the amounts of hydrogen peroxide

For each sample, hydrogen peroxide (H₂O₂) concentration was measured spectrophotometrically following the method described by [Junglee et al. \(2014\)](#). Briefly, 150 mg of each body sample (or 150 μ L for hemolymph) was homogenized at 4 °C for 10 min into 1 mL of a solution containing 0.25 mL Trichloroacetic acid (TCA) (0.1% (v:v)), 0.5 mL KI (1 M) and 0.25 mL potassium phosphate buffer (10 mM, pH 7.0). This procedure combined the extraction of H₂O₂ and the colorimetric reaction; samples were quickly covered with aluminum foil to limit their alteration by light. The blank was obtained by replacing KI by 0.5 mL of H₂O. The homogenates were then centrifuged at 12,000 $\times g$ for 15 min at 4 °C. For each sample, an aliquot of 200 μ L of the supernatant was transferred to the well of an UV-microplate, and incubated at 20 °C for 20 min before the absorbance was measured at 240 nm. A calibration curve, obtained with H₂O₂ standard solutions prepared in 0.1% TCA, was used for quantification. H₂O₂ concentration was expressed as ppm. All samples and blanks were analyzed in triplicates.

2.3. Measurements of the amounts of reduced glutathione

The concentration of glutathione (GSH), which has a non-enzymatic antioxidant activity, was conducted by colorimetric methods according to the procedure of [Allen et al. \(1984\)](#). Briefly, 200 mg of each body sample (200 μ L for hemolymph) was homogenized with 2 mL of 5% (w/v) TCA in 1 mM EDTA before being centrifuged at 10,000 $\times g$ for 20 min at 4 °C. A volume of 1 mL of the reaction mixture, which contained 150 μ L of extract, 800 μ L of 0.1 M phosphate buffer (pH 8.0), and 50 μ L (0.01% in 0.1M phosphate buffer, pH 8.0) of 5, 5'-Dithio bis-2-nitrobenzoic acid (DTNB), was mixed thoroughly, and then incubated at 25 °C for 20 minutes. The absorbance of the reaction mixture was measured at 412 nm. The GSH content was determined from a GSH standard curve, and the GSH concentration was expressed in μ g GSH/ mg protein.

2.4. Measurements of the catalytic activities of catalase, glutathione peroxidase and carboxyl esterase hydrolase

For each replicate, 200 mg of body sample (200 μ L for hemolymph) was grinded into 2 mL of PBS (pH= 7.0; 10mM). The samples were centrifuged at 10,000 $\times g$ for 20 min at 4 °C, and supernatants were collected for the measurements of the catalytic activity of the enzymes. The enzymatic activity of catalase (CAT) was determined by colorimetric methods, as formerly described by [Aebi \(1984\)](#).

The reaction mixture contained 0.9 mL of a potassium phosphate buffer (50 mM, pH 7.0), 60 μ L of the supernatant, and 40 μ L of freshly prepared H₂O₂ (10 mM). The change in absorbance was measured at 240 nm over 1 min. The CAT activity was further expressed as OD/mg protein/min.

Glutathione peroxidase (GPx) antioxidant activity was measured according to the method described by Mazhoudi et al. (1997), with minor modifications. The reaction mixture contained 0.5 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.2 mL of 1% (m/v) guaiacol, 0.2 mL OF 0.4% (v/v) H₂O₂, and 0.1 mL of the supernatant. Absorbance was measured at 470 nm over 3 min, and GPx activity was expressed as OD/mg protein/min.

The activity of carboxyl esterase hydrolase (CEH) was determined following the method described by Gomori (1953) and modified by Bunyan et al. (1968). The reaction mixture contained 18 mg of fast blue B salt dissolved in 10 mM potassium phosphate buffer (pH 7.0), 600 μ L of 0.113 M α -naphthyl acetate which was dissolved in 50 % acetone. A volume of 240 μ L of this solution was mixed with 10 μ L of each body sample extract, and the absorbance was measured at 450 nm over 3 min. CEH activity was expressed as OD/mg protein/min.

2.5. Total protein amount

Total protein concentration was quantified for each body sample with Bradford's method (Bradford 1976), using Coomassie Brilliant Blue (COBB). A volume of 0.9 mL of the dye reagent (10 mg COBB mixed with 5 mL methanol and 10 mL of 85% O-phosphoric acid, completed to 100 mL with distilled water) was mixed with 0.1 mL of body sample, then left for 2 min before measuring the optical density of the samples at 595 nm. The blank consisted in distilled water instead of the protein sample. Bovine serum albumin (BSA) fraction V (Sigma-Aldrich) dissolved in 0.15 M NaCl was used as drawing the calibration curve.

2.6. Statistical analysis

As a first step, a Generalized Estimating Equation (GEE) was computed to examine if the concentrations of epinephrine (0, 25, 50 or 100 μ g/mL), the type of body sample (fat body, gut, cuticle, hemolymph), and the interaction of these terms affected the physiological endpoints we measured (H₂O₂, GSH, CAT, GPx, and CEH). These models were preferred over generalized linear models, as it is very likely that the variations of H₂O₂, GSH, CAT, GPx, and CEH measured from the body samples of the larvae were not independent. Wald Chi-square tests were run to assess the statistical validity of the models. In a second step, the effect of the different concentrations of epinephrine on the levels of H₂O₂, GSH, CAT, GPx, and CEH in the hemolymph, gut, cuticle and fat body of 3rd instars of *S. dux* was assessed by performing a one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* tests when necessary. The comparison of the amounts of H₂O₂, GSH, CAT, GPx, and CEH among the different body samples was also conducted by performing a one-way ANOVA. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.).

3. Results and Discussion

The interaction analysis obtained from the computation of Generalized Estimating Equation (GEE) revealed that the different concentrations of epinephrine (0, 25, 50, 100 µg/mL), the nature of the body samples (fat body, cuticle, gut, hemolymph), and the interaction of these terms, significantly influenced the physiological endpoints we measured (H₂O₂, GSH, CAT, GPx & CEH) in 3rd instars of the flesh fly *S. dux* (**Table 1, Supplementary Information 2 & 3**).

Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), are formed in the cells during metabolic processes. Several exogenous and endogenous factors contribute to increase ROS production, with, for instance, studies having reported that the catecholamine epinephrine increases the production of H₂O₂ in animals (Maral et al. 1977; Díaz-Cruz et al. 2007). Yet, our results revealed that epinephrine treatment did not affect the production of H₂O₂ in the assayed body tissues of the fly *S. dux* larvae, and even decreased its amounts, except in the gut tissues of the individuals injected with epinephrine at 50 µg/mL (**Figure 1a, Supplementary Information 3**). Importantly, the highest epinephrine concentration (100 µg/mL) resulted in a drastic reduction of H₂O₂ in the cuticle and hemolymph of the larvae of *S. dux*, with levels being about 10 times lower as compared with their control relative (SS=2268.2, 1005.1; df=3, 3; MS= 756, 335, $p<0.001$ for cuticle, and $F=1124.9, 192, p<0.001$ for hemolymph) (**Figure 1a**). Levels of H₂O₂ were also significantly lower in the cuticle, gut, and hemolymph of larvae injected with 3 µL of epinephrine at 100 µg/mL as compared with their relatives that received half of this dose. This result suggests the existence of a concentration threshold before any significant epinephrine effect could be observed on H₂O₂ production.

There is little information on the effects of epinephrine on insects' physiology in the literature. Yet, the studies conducted on crustaceans (Yamagishi 2003; Model et al. 2019) and rats (Garcia et al. 1999; Wu et al. 2013) showed that injections of epinephrine had similar effects on the cardiac activity and metabolism of these organisms. For instance, in the burrowing crab *Neohelice granulata*, epinephrine increased the amounts of glucose circulating in the body fluids, while reducing glycogen stores in the fat body (Model et al. 2019). In rats, it has been reported that epinephrine concentrations of 50µM increased the metabolism of glucose (Garcia et al. 1999). Thus, the similarities in the reported effects of epinephrine suggest that the knowledge gathered from other animal taxa can be considered for interpreting our results.

In rats, epinephrine deviated the glucose flux from the pentose phosphate pathway, with cascading consequences on the ability of the organisms to handle oxidative stress (Garcia et al. 1999). Impaired redeployment of glucose towards the pentose phosphate pathway might be problematic in animals facing oxidative stress. Indeed, this mechanism represents a prominent way for the regeneration of NADPH, allowing the glutathione system to work properly (Christodoulou et al. 2018). While glucose deviation towards the pentose phosphate pathway may have been impaired in the gut of *S. dux* larvae injected with epinephrine at 50 µg/mL, as depicted by the high increase in H₂O₂ production, we did not find signs in favor of this hypothesis for most experimental treatments and body samples (**Figure 1a**).

Garcia et al. (1999) reported that glutamine oxidation was reduced in epinephrine-treated cells of rats, suggesting that higher amounts of this amino acid might have been made available for the synthesis of glutathione. A similar pattern may have occurred in the larvae of the flesh flies, at least

in the hemolymph, as supported by the significant increase in the levels of GSH in larvae that received the 100 µg/mL epinephrine concentration, in parallel to showing reduced amounts of H₂O₂ (**Figures 1a and b, Supplementary Information 3**). In the gut, GSH contents were progressively decreased when the concentration of epinephrine was increased (**Figure 1b**). This pattern may additionally be interpreted in light of the results obtained by Sugiyama and Natori (1994) on *Sarcophaga peregrina* flies. These authors demonstrated that GSH levels were decreased in flies whose tegument was injured. The changing GSH amounts we are reporting for the body samples may have resulted from the combined effects of altered glutamine oxidation and injured tegument of the pricked flies. In future studies, it would be interesting to have measures of GSH amounts from non-pricked flies to obtain information regarding the basal GSH levels of intact individuals. Finally, GSH levels were significantly decreased in the gut of flies treated with epinephrine (**Figure 1b**), and this may have permitted the expression of insect defense proteins (Sugiyama and Natori 1994), as formerly evidenced in *Drosophila* flies (Engstrom et al. 1993).

The little, yet significant, increase in the activity of catalase (CAT) observed in body samples of the larvae treated with 50 or 100 µg/mL epinephrine as compared with controls (**Figure 1c, Supplementary Information 3**), except in the cuticle, is consistent with the measured changes in H₂O₂ levels. CAT is well known for its breakdown capacities of H₂O₂ in a range of animals (Storey 1996). With CAT, GPx represents another important antioxidant enzyme of the defense system against ROS, in particular for controlling both intra- and extracellular amounts of H₂O₂ (Giorgio et al. 2007). Often, it has been suggested that CAT catalyzes the hydrolysis of H₂O₂ when it is at high concentration, while GPx catalyzes this reaction at low H₂O₂ concentration inside cells (Fridovich 1995). The little increase activity of CAT may suggest that the enzyme has played a role in controlling the production of ROS in treated larvae, with epinephrine treatments being known to protect from oxidative stress or to induce it, depending on the body sample and physiological situation (Álvarez-Diduk and Galano 2015).

In our study, the activity of GPx, an enzyme which needs GSH as the electron donor and allows converting H₂O₂ to H₂O, was similar in the fat body of control *S. dux* flies as in those treated with epinephrine at 25 µg/mL (**Figure 1d, Supplementary Information 3**). GPx activity then decreased in this tissue when the epinephrine concentrations were higher, paralleling the variation pattern reported for GSH. It is suggested that the reduction in the levels of GSH may inhibit GPx activity (Kazek et al. 2020), and that H₂O₂ might have been preferentially scavenged by CAT in this tissue. Former studies evidenced the importance of CAT in the control of H₂O₂ when low glutathione levels limit GPx activity in insects (Paes et al. 2001). Conversely, the activity of GPx enzyme was slightly increased in the hemolymph at the two higher epinephrine treatments (**Figure 1d**), and very high amounts of GSH were measured at the highest epinephrine treatment. These findings suggest that GSH is certainly not the only factor regulating GPx activity, as observed at epinephrine concentrations of 50 µg/mL in hemolymph; at this epinephrine concentration, GPx activity may have remained high to counteract the high amounts of H₂O₂.

The activity of CEH was decreased gradually along with increased concentrations of epinephrine (25 - 100 µg/mL) in all body samples of the 3rd instars of the flesh fly *S. dux* (**Figure 1e**). The lowest values were measured from larvae injected with epinephrine concentrations of 100 µg/mL. CEH are

essential enzymes processing the biotransformation of drugs, endogenous compounds, or pollutants (Di 2018). By hydrolyzing endogenous esters, this enzyme has important role in lipid metabolism. In rats, it has been demonstrated that epinephrine may have different effects on tissue metabolism, by either stimulating glycogen breakdown in parallel to reducing triacylglycerol levels, or by stimulating lipid hydrolysis over glycogen breakdown (Peters et al. 1998). While epinephrine treatment was found to increase lipid metabolism in the fat body of cockroaches (Bhakthan and Gilbert 1968), our results suggest that this might not have happened in our flies. Here, epinephrine treatment may have rather stimulated glycogen breakdown in flesh flies.

In this study, we have examined the effects of the catecholamine epinephrine (**Supplementary Information 1**), a compound used as a growth promoter for cattle, and for anaphylaxis treatments in human. When used as a drug, epinephrine can be further transferred to the environment, with potential consequences for wildlife. We demonstrated that larvae of the fly *S. dux* that received the highest epinephrine concentration were characterized by highly reduced amounts of H_2O_2 , suggesting that epinephrine injections may have had a protective effect against ROS production, as formerly reported from other animal taxa. The changing patterns we measured for CEH, combined with former evidence reporting increased glucose metabolism in epinephrine-treated tissues, suggested that higher amounts of glucose were diverted towards the pentose phosphate pathway. Increased glucose metabolism and redeployment over the pentose phosphate pathway would have helped containing ROS production by enhancing regeneration of NADPH. This hypothesis will have to be explored in future studies.

Table 1. Statistical results of the effects of different concentrations of epinephrine (0, 25, 50 or 100 µg/mL), the nature of the body samples (fat body, cuticle, gut, hemolymph), and interaction of these terms, on the measured physiological endpoints, i.e. the categorical variables, in 3rd instars of the flesh fly *Sarcophaga dux*.

Categorical variables	Chi-square (χ^2)	df	QIC	p value
Intercept				
H ₂ O ₂	4089.0	1	104	<0.0001
GSH	2018.7	1	38	<0.0001
CAT	3618.3	1	1844	<0.0001
GPx	4468.6	1	36	<0.0001
CEH	1255.0	1	39	<0.0001
Effect of epinephrine concentration				
H ₂ O ₂	3330.3	3	104	<0.0001
GSH	401.1	3	38	<0.0001
CAT	143.4	3	1844	<0.0001
GPx	1627.4	3	36	<0.0001
CEH	301.5	3	39	<0.0001
Effect of body samples				
H ₂ O ₂	2910.4	3	104	<0.0001
GSH	469.7	3	38	<0.0001
CAT	164.9	3	1844	<0.0001
GPx	702.6	3	36	<0.0001
CEH	654.3	3	39	<0.0001
Effect of the interaction epinephrine concentration – body samples				
H ₂ O ₂	4064.9	9	104	<0.0001
GSH	3742.0	9	38	<0.0001
CAT	285.9	9	1844	<0.0001
GPx	4889.3	9	36	<0.0001
CEH	434.6	9	39	<0.0001

Generalized Estimating Equation (GEE) were performed to test the effects of different concentrations of epinephrine.

The categorical variables are: reactive oxygen species concentration H₂O₂, non-enzymatic antioxidants concentration GSH, and antioxidant enzymes activity CAT, GPx and CEH.

QIC is the quasi-likelihood under the independence model criterion and is a derivation of Akaike's information criterion for GEE

Figure legends

Figure 1. Effects of epinephrine on the amount (mean \pm SE) of **(a)** hydrogen peroxide (H_2O_2), **(b)** glutathione reduced (GSH), and catalytic activity of **(c)** catalase (CAT), **(d)** glutathione oxidase (GPx) and **(e)** carboxyl esterase hydrolase (CEH), measured in tissue homogenates (fat body, cuticle and gut) and hemolymph of 3rd instars of the flesh fly *Sarcophaga dux*. Larvae were injected with different concentrations of epinephrine: 0, 25, 50 or 100 $\mu\text{g}/\text{mL}$. For each tissue homogenate, bars with different letters report significant differences among the experimental treatments (epinephrine concentration of 0, 25, 50 or 100 $\mu\text{g}/\text{mL}$) (ANOVA, *Tukey's-b* test, $p < 0.05$).

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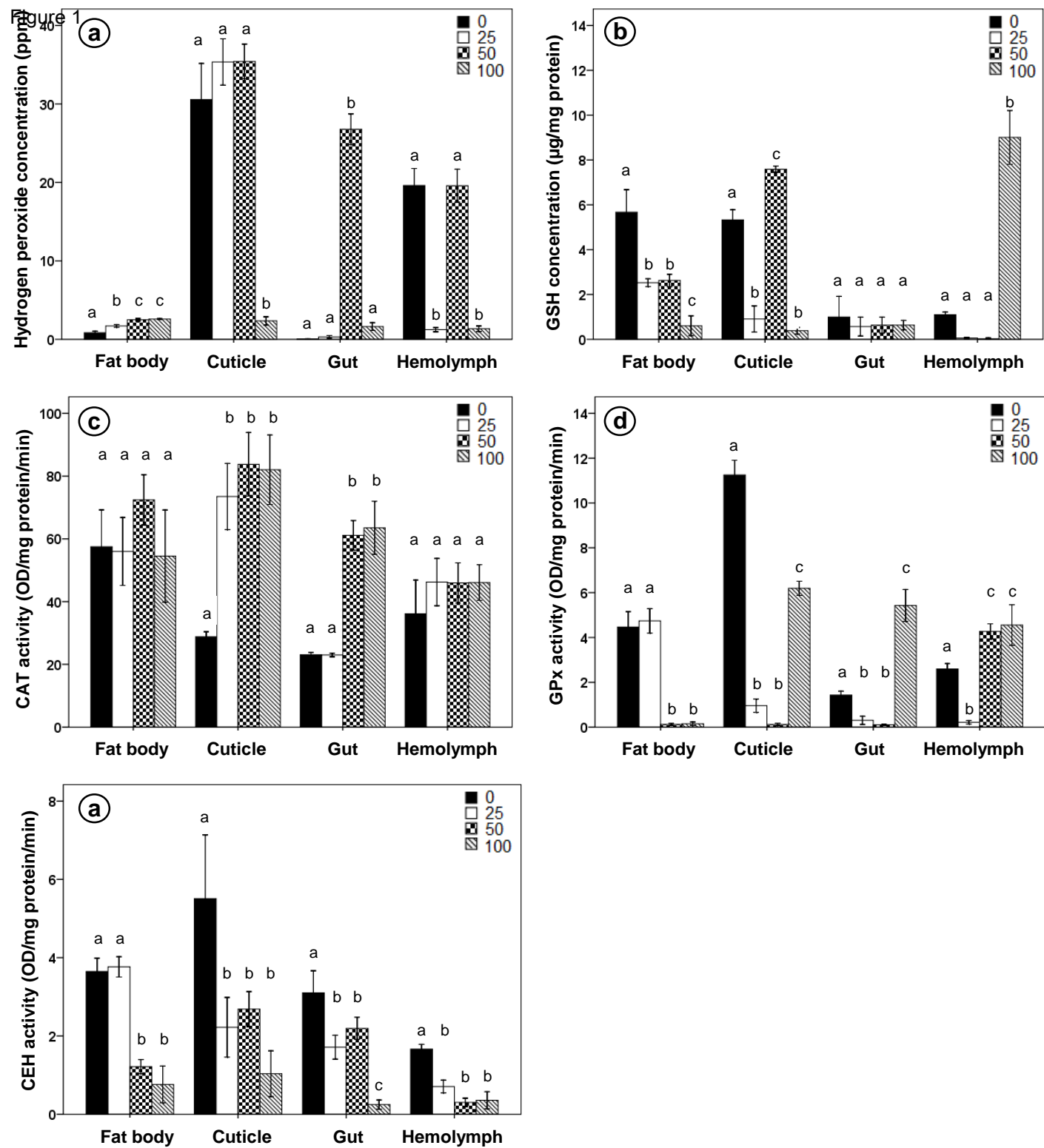


Figure 1

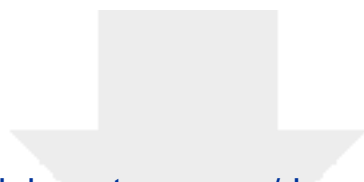


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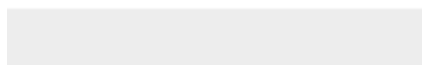
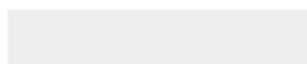


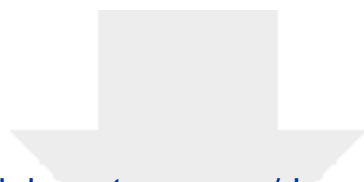


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