# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities




WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

## Interested in publishing with us?
## Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Chapter 11

# Substrate Specificities and Kinetic Parameters of Recombinant *Drosophila melanogaster* Glutathione *S*-Transferases E6 and E7

Vennobaashini Venu and Zazali Alias

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72970

**Abstract**

*D. melanogaster* glutathione transferases E6 and E7 (DmGSTE6 and DmGSTE7) were successfully cloned, purified, and biochemically characterized. The recombinant proteins were readily purified using the combination of both anionic and BSP/GSH-agarose affinity chromatography. Although both GSTs have significant identity in their amino acid sequence, each enzyme displayed unique biochemical characteristics. Both recombinant proteins were only active toward 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), and *p*-nitrobenzyl chloride (*p*-NBC) with significant difference in catalytic activities. The findings have shown that neither GSTE6 nor GSTE7 was able to counter oxidative stress. Comparatively, GSTE7 was a more efficient enzyme at turning over DCNB and *p*-NBC, based on its kcat/Km values which were of 0.183 and 2.25 $min^{-1}$ $mM^{-1}$, respectively. Thin-layer chromatography analysis showed that both isoforms were not able to conjugate several tested insecticides. The inhibition kinetics of natural products and dyes toward GSTs in vitro revealed that phenol red possessed inhibition effects only on GSTE6 while rose bengal and cardiogreen inhibit significantly on both GSTE6 and GSTE7. In contrast, methylene blue dye and *trans*-chalcone have been shown to stimulate GSTE7 activity toward CDNB.

**Keywords:** detoxification, *D. melanogaster*, glutathione transferases, insecticide resistance, kinetic parameters

## 1. Introduction

One of the most popular classes of detoxification enzymes that constitute in almost all living organisms is the glutathione transferases (GSTs). GSTs conjugate the thiol groups of reduced glutathione (GSH) toward the negative charge center of lipid soluble compounds (xenobiotics)

INTECH
open science | open minds

© 2018 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

to make it water soluble and easy to excrete out. These enzymes have wide distribution in nature and are found ubiquitously in almost all living things including plants, animals, and even bacteria. GSTs are expressed in sex, age, tissue, organ, species, and tumor-specific patterns of expression, and their composition differs significantly [1]. Insect GSTs can be categorized into six classes including Delta, Epsilon, Theta, Omega, Sigma, and Zeta, but it is the Delta and Epsilon classes that are most commonly associated with resistance [2, 3]. Several studies also reported that Epsilon classes in Dipteran organisms are to confer insecticide resistance, and their catalytic diversity would likely promote their role in detoxification [4–7]. Another study [8] also suggested that the expression of the epsilon class GSTs, SlGSTE2, and SlGSTE3 genes in *Spodoptera litura,* a Lepidoptera detoxifies carbaryl, DDT, RH5992, malathion, and deltamethrin, which is a synthetic chemical insecticide.

A work in housefly isozymes suggested that MdGST6A and MdGST6B which belong to the epsilon class function as key enzymes in the detoxification of insecticides such as methyl parathion and lindane [9]. It was demonstrated that the expression of GSTE6 and GSTE7 in *D. melanogaster* significantly increased by more than 50% upon exposure to paraquat (1,1-dimethyl-4,4`-bipyridylium) and phenobarbital (PhB) [10]. Besides that, acute insecticide exposure of methyl parathion results in significant increase in expression of GSTE6 (100%) and GSTE7 (72%) [11]. This suggested their immediate involvement in insecticide metabolism.

Therefore, the current study wanted to investigate the behavior of two epsilon class *D. melanogaster* GSTs, namely, GSTE6 and GSTE7, against varieties of xenobiotics and therefore characterizes their biochemical contribution. This may give insight the potential role the GSTs could have played during xenobiotic metabolism. This could help anticipate the behavior of insecticides related GSTs in other dipterans of economic importance.

## 2. Materials and methods

### 2.1. Chemicals and source of insect

Unless otherwise stipulated, chemicals employed were of the highest grade obtainable. Buffer components, pesticides, dyes, and GST substrates used were purchased from Sigma-Aldrich. *D. melanogaster* obtained from the Genetic Department of the University of Malaya was reared on oats and glucose-based diet at room temperature. Only 5 days post emerged, flies were used for the experiments.

### 2.2. DNA and protein analysis

Nucleotide and deduced amino acid sequences of *DmGSTE6* and *DmGSTE7* obtained from sequencing were compared to existing sequences in Gene Bank by BLAST searching (http://www.ncbi.nlm.nih.gov). The software used for sequence analysis, matrix table of percentage amino acid identities sequence and alignment with CLUSTAL W, was BioEdit software version 7.2.0.

### 2.3. Cloning, expression, and purification of recombinant proteins

Total DNA was isolated from adult tissues using DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instruction. The primers for amplifying GSTE6 were forward primer containing Nde1 site: 5′-GGAATTC CATATG gtgaaattgactttatac-3′ and reverse primer containing EcoR1 site: 5′-CG GAATTC tcatgcttcgaatgtgaa-3′; the primers for amplifying GSTE7 were forward primer containing Nde1 site: 5′-GGAATTC CATATGcccaaattgatactgtac-3′ and reverse primer containing Xho1 site: 5′-CCG CTCGAGttaattcgatgcgaaagt-3′. Genomic DNA was denatured at 95°C for 3 min, followed by 32-cycle amplification (95°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 min) with final extension at 72°C for 7 min. PCR products were analyzed using 1.0% agarose gel and stained with ethidium bromide. These two PCR products were cloned into pET 30a(+) expression vector (Novagen). The recombinant plasmids were analyzed by sequencing and transformed into *E. coli* BL21 (DE3) pLysS (Invitrogen) independently and grown at 37°C in 400 mL Luria-Bertani (LB) media containing 30 μg/mL kanamycin. Protein expression was induced by the addition of isopropyl β-D-thiogalactoside (IPTG) at final concentration of 1 mM. Incubation was continued for further 4 h at 37°C, after which the cells were harvested by centrifugation at 4°C (5000 rpm for 15 min) and resuspended in 5 mL binding buffer (25 mM sodium phosphate, pH 7.4). 100 μL of lysozyme (10 mg/mL) (Sigma-Aldrich) was added and incubated at room temperature for 1 h. The cell suspension was homogenized; the cell debris was palleted by centrifugation at 100,000× *g* for 1 h (4°C). The clear obtained supernatant was used as source for recombinant purification.

Chromatography was carried out using an ÄKTA Purifier FPLC equipped with UNICORN software Version 5.1 and a fraction collector (Frac900) for greater automation of the purification process. The recombinant proteins were purified using Hi-Trap™ Q HP column (5 ml, GE Healthcare) followed by BSP-GSH-Sepharose column (packed in TriconTM, 1 ml) (BSP-GSH-Sepharose matrix was a gift from Dr. AG Clark, Victoria University of Wellington). The BSP (2 mM) used to elute the proteins from affinity matrix was removed from eluate using Hi-Trap™ Desalting column (GE Healthcare).

The Hi-Trap™ Q HP column was equilibrated with 25 mM phosphate buffer and pH 7.4, and 5 mL lysate was applied to the column. Both GSTE6 and GSTE7 enzymes did not bind to the Hi-Trap™ Q HP column, so the flow through was collected and loaded to a BSP-GSH-Sepharose column which was pre-equilibrated with 25 mM phosphate buffer and pH 7.4. The column was washed with 1 M NaCl, and the proteins were eluted with 2 mM BSP in 25 mM phosphate buffer and pH 7.4. Purified enzymes were desalted using Hi-Trap™ Desalting column (15 ml) (GE Healthcare). Proteins were concentrated using Vivaspin 20 (10,000 MWCO, Sartorius). The pooled purified enzymes from subsequent purification were freeze-dried and stored in −20°C for further analysis.

### 2.4. Protein quantification (Bradford assay)

Protein concentration was determined using Coomassie Brilliant Blue G-250, and Bovine serum albumin was used as standard [12].

### 2.5. Molecular weight estimation

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a 12% polyacrylamide gel [13] using BioRad Mini-PROTEAN system. Mark 12™ unstained standard (Invitrogen, USA) was used as the protein marker, and the gels were stained with Colloidal Coomassie Blue G-250 [14]. Stained gels were scanned with Image Scanner III (GE Healthcare) and visualized and analyzed with Image Master Software.

### 2.6. Enzymatic assay and kinetic evaluation

All assays were performed using a Jasco V-630 spectrophotometer equipped with temperature controller. Enzymatic assays with 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid (EA), sulfobromophthalein (BSP), *p*-nitrobenzyl chloride (NBC), and *trans*-4-phenyl-3-buten-2-one (PBO) [15] and 1,2-dichloro-4-nitrobenzene (DCNB) [16] were determined accordingly. The ability to conjugate trans-2-octenal, hexa-2,4-dienal, *trans*-hex-2-enal, and *trans*,*trans*-2,4-heptadienal were performed accordingly [17]. The peroxidase activities were determined using cumene hydroperoxide and hydrogen peroxide as substrates as previously described [18].

For the determination of kinetic parameters, initial velocity data was obtained by varying the concentration of hydrophobic substrates (CDNB, NBC, and DCNB) at concentration range of 0.01 to 2.5 mM at fixed GSH concentration (1 mM). Kinetic data were evaluated by non-linear regression analysis with Michaelis Menten equation ($\upsilon = Vmax \times [S]/Km + [S]$), using SigmaPlot 12.0 graph and analysis software. The catalytic constant, *K*cat, was calculated using the equation Kcat = Vmax/[E], where [E] is the total enzyme concentration. The effect of inhibitors on the catalytic activity was studied by analyzing the reaction rate (nmol/min) in the presence (concentration varied) and the absence of inhibitors. Experiments were repeated at least three times. The IC50 and EC50 were determined by fitting sigmoid concentration-response curves using GraphPad Prism 6.00 software.

### 2.7. Pesticide conjugation

The capability of both recombinant proteins to conjugate selected pesticides (Clodinafop-propargyl, Fenoxaprop-ethyl, Propoxur, isoproturon, and methyl parathion) was evaluated using thin-layer chromatography. An aliquot of 8 μL of reaction products was loaded on a 0.2 mm thick, 8 cm × 10 cm thin-layer chromatography silica gel 60 F2 s4 plate (Merck) and developed using butan-1-ol/acetic acid/water (12,3,5, by vol.) for 1 h. The air-dried plate was stained with ninhydrin (0.25%, w/v, in acetone) [19]. Control reactions using CDNB substrate were performed in a similar manner using CDNB and GSH.

## 3. Results

In the study both Dm*GSTE6* and *DmGSTE7* were amplified using PCR where genomic DNA was used as the template. Their intron-free coding sequences [20] were used directly for

http://dx.doi.org/10.5772/intechopen.72970

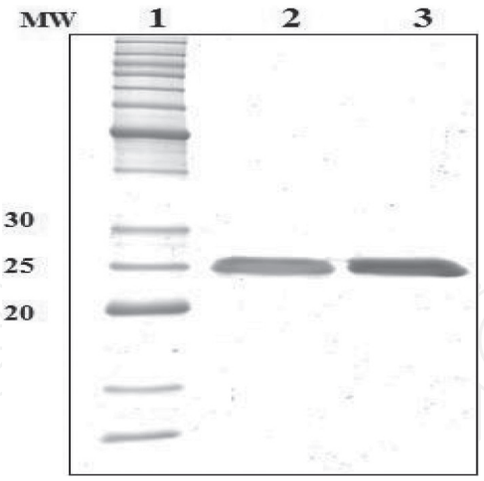



**Figure 1.** SDS-PAGE of purification of GSTE6 and GSTE7 using BSP/GSH-agarose matrix. The bound recombinant proteins were eluted using 2 mM BSP solution in 25 mM phosphate buffer, pH 7.4. Lane 1, BenchMark™ protein ladder; lane 2, purified recombinant GSTE6; and lane 3, purified recombinant GSTE7.

| **Substrates** | **Substrate specificity (nmol/min/mg)** | |
|---|---|---|
| | **GSTE6** | **GSTE7** |
| 1-Chloro-2,4-dinitrobenzene | 80.67 ± 4.42 | 740.33 ± 15.04 |
| 1,2-Dichloro-4-nitrobenzene | 18.11 ± 1.04 | 37.04 ± 2.11 |
| *trans*-Hex-2-enal | ND | ND |
| Hexa-2,4-dienal | ND | ND |
| *trans*-Oct-2-enal | ND | ND |
| *trans*-4-Phenyl-butene-2-one | ND | ND |
| *trans,trans*-Hepta-2,4-dienal | ND | ND |
| Ethacrynic acid | ND | ND |
| *p*-Nitrobenzyl chloride | 3.66 ± 0.58 | 249.67 ± 9.61 |
| Bromosulfophthalein | ND | ND |
| Cumene hydroperoxide | ND | ND |
| Hydrogen peroxide | ND | ND |

Means ± SD of three experiments, each with triplicate determinations. *ND denotes not detected

**Table 1.** Substrate activities of GSTE6 and GSTE7.

expression of the proteins in *E. coli* under induction of IPTG. Proper orientation of the GSTE6 and GSTE7 expression constructs was confirmed by PCR analysis using the constructs as a template, DNA sequencing, and CDNB conjugation activity assay of the extracted bacteria lysate. The proteins were highly expressed and isolated using combination of an anion exchange and BSP/GSH-agarose matrix which has been shown to capture a number of Epsilon class GSTs from *D. melanogaster* [10, 11]. The subunit size of GSTE6 and GSTE7 is predicted to

be 25.0146 kDa and 25.5101 kDa, respectively, based on their amino acid compositions. The SDS-PAGE demonstrated that both recombinants migrated at 25 kDa (**Figure 1**).

The work later proceeded into looking at the activities of the recombinants toward different common GST substrates as seen in **Table 1**. The comparison indicated that both recombinants reacted toward the same types of substrates but of different degree of specific activities. Our kinetic analysis as shown in **Table 2** indicates the variation of kinetic parameters of both isoforms on DCNB and NBC. Comparatively GSTE6 has shown to have higher affinity toward DCNB than GSTE7.

To further investigate both isoforms' functional differences, several natural products and dyes were acted upon the enzymes as seen in **Table 3**. Each dye appeared to have different inhibitory effects on each GST isoform. Comparatively, cardiogreen and rose bengal had inhibited the activities of GSTE6 and GSTE7, respectively, at IC50 less than 10 nM.

| **Enzyme** | **Substrate** | **$V_{max}$ (nmol/min)** | **$K_m$ (mM)** | **$K_{cat}$ ($min^{-1}$)** | **$K_{cat}/K_m$ ($min^{-1}mM^{-1}$)** |
|---|---|---|---|---|---|
| GSTE6 | DCNB | 0.029 ± 0.008 | 0.167 ± 0.001 | 0.007 | 0.042 |
| | *p*-NBC | 0.209 ± 0.013 | 0.278 ± 0.005 | 0.051 | 0.183 |
| GSTE7 | DCNB | 0.296 ± 0.033 | 0.415 ± 0.002 | 0.043 | 0.104 |
| | *p*-NBC | 1.311 ± 0.051 | 0.060 ± 0.002 | 0.135 | 2.25 |

Means ± SD of three experiments, each with triplicate determinations.

**Table 2.** Kinetic parameters of GSTE6 and GSTE7 when DCNB and ***p***-NBC were used as substrates.

| **Compound** | **Compound concentration (mM)** | **GSTE6** | **GSTE7** | |
|---|---|---|---|---|
| | | **$IC_{50}$ (nM)** | **$IC_{50}$ (nM)** | **$EC_{50}$ (nM)** |
| Sebacic acid | 0–100 | NE | NE | |
| *trans*-chalcone | 0–100 | 86.79 | | $2.958 \times 10^5$ |
| Cardiogreen | 0–3 | 4.21 | 9.22 | |
| Crystal violet | 0–10 | 32.24 | 50.59 | |
| Methylene blue | 0–100 | 76.66 | | $1.747 \times 10^5$ |
| Rose bengal | 0–3 | 3.68 | 1.07 | |
| Phenol red | 0–10 | 7.29 | 30.36 | |
| Cibacron blue | 0–10 | 82.64 | 210.56 | |

The data are mean value of at least three independent experiments. *NE denotes no effect.

**Table 3.** Effect of selected dyes and natural products on the activities of GSTE6 and GSTE7.

## 4. Discussions

The molecular weights of purified enzymes were approximately 25 kDA, respectively, which corresponded to the calculated molecular masses (**Figure 1**) and were in agreement with data previously reported [6].

It was observable (**Table 1**) that both recombinants have the same affinity toward certain substrates notably of compound containing aromatic ring. However GSTE7 seemed to have higher specific activities toward each conjugated substrate as compared to GSTE6. GSTE7 was reacted 9-, 4-, and 68-fold higher toward CDNB, DCNB, and NBC, respectively, as compared to GSTE6. Both were unable to conjugate lipid peroxidation products, and neither could it have shown peroxidase activities. This implied that neither GSTE6 nor GSTE7 could have directly participated in countering oxidative stress in fruit flies. In another instance, substrate specificity variation was observed when other substrates are used. It was previously reported that GSTE6 reacted toward 4-hydroxynonenal, adrenochrome, phenethyl isothiocyanate, 5-hydroperoxyeicosatetraenoic acid, and hydroxyethyldisulfide, while GSTE7 reacted only to 4-hydroxynonenal, 5-hydroperoxyeicosatetraenoic acid, and 2-hydroxyethyldisulfide [6].

In comparison to *Musca domestica*, DmGSTE6 is 79 and 77% similar and also 62 and 59% identical to MdGST6A and MdGST6B, respectively. Both MdGST6A and MdGST6B showed more or less 40% identity with other *Drosophila* Epsilon class proteins. MdGST6A and MdGST6B were both known involved in insecticide metabolism in houseflies [9]. Further assumption on both isoforms (DmGSTE6 and DmGSTE7) that could have been participating in insecticide conjugation was much supported by the induction study performed previously [10]. Through proteomic analysis, the expression of GSTE6 and GSTE7 was shown to increase in the methyl parathion-challenged adult flies. The authors however cautiously suggested any direct relationship of the isoforms increase expression to metabolism of the tested insecticide. There were reports suggested that only Epsilon class GSTs were able to react with DCNB [21] and the detoxification ability of GSTs against insecticides is correlated to its ability to react with DCNB [9].There was also instances of which GSTE1-1z of a reportedly high specific activity toward DCNB and yet does not confer insecticide resistance in *Anopheles gambiae* [22].

The rate of conjugation reaction, $V_{max}$, for GSTE7 toward DCNB was however ten times faster than GSTE6. The behavior could have implied the differences in the stabilization of the GSH in the hydrophilic pocket that lead to the conjugation of DCNB. This has remarkably affected the Kcat and Kcat/Km values of both isoforms. The turnover and the catalytic efficiency of GSTE7 were, respectively, sixfold and twofold higher than those of GSTE6. GSTE7 has lower $K_m$ value to suggest its higher affinity toward NBC. The behavior of GSH conjugation to NBC could probably differ to what has been with DCNB where the speed of reaction was very much higher in GSTE7 when NBC was the second substrate. The catalytic efficiency, Kcat/Km, of the reaction catalyzed by GSTE7 was shown 12 times higher than of the GSTE6. These

observations proposed that GSTE7 was a better isoform for conjugation of the selected substrates than GSTE6.

These differences indicated that GSTE6 and GSTE7 have considerable variations in their secondary structural organization. Such variations in structure may form the basis of differences in their corresponding substrate specificities and in catalytic efficiency [23] although both originated from same cluster and located next to each other on the genomic DNA.

Our preliminary investigative attempt to show that both isoforms could conjugate insecticides was performed using thin-layer chromatography. Pesticides such as temephos, malathion, DDT, fenthion, fenitrothion, permethrin, bromophos, chlorpyrifos, clodinafop-propargyl, fenoxaprop-ethyl, propoxur, isoproturon, and methyl parathion were used in the test. None of the pesticides appeared conjugated by both isoforms. The test suggested both recombinant GSTE6 and GSTE7 did not react and involve in detoxification of insecticides and herbicides directly. Thus so far, the role of GSTE6 and GSTE7 in detoxification of insecticides remains unclear as there is no any promising evidence to prove its involvement in detoxification process.

A comprehensive microarray-based atlas of adult gene expression in multiple *Drosophila* tissues available (http://flyatlas.org) reported that GSTE6 expressed in adult crop, midgut, tubule, hindgut, ovary, and larval hindgut while GSTE7 expressed in adult crop, midgut, tubule, hindgut, virgin spermatheca and larval midgut, and hindgut and fat body. Other reports also indicated that GSTE6 was found abundant in hindgut, while GSTE7 was found abundant in Malpighian tubules [24]. The co-expression of GSTE6 and GSTE5 was suggested to play a role in male reproductive fitness and success [25]. It was identified that a potential DNA transcription factor binding motifs (TFBMs) of cytochrome $P_{450}$, GSTs, and carboxylesterases is expressed in the *D. melanogaster* third instar larval midguts [26].

GSTE6 was reported to have GRE-like, Fox-like, NF-kappaB-like, and E47-like TFBMs while GSTE7 to have GRE-like and E47-like TFBMs. The four mentioned TFBMs are known to have mammalian function and were observed to be linked to the oxidative stress response [26]. The author reported that GSTE6 and GSTE7 responded different levels of dietary hydrogen peroxide. However, the author concluded that there is no solid evidence to prove if some or all of the potential TFBMs are functional or response of the midgut-associated GSTs to the oxidative stressor, dietary H2O2. They may simply be associated with these genes with limited or no role in response to this oxidative stressor. In another study, GSTE7 also appeared to be involved in activation of survival program through immune deficiency (IMD) pathway as it was reported being expressed in a strongly infected airway epithelium of *D. melanogaster* [27].

Exposure of *Drosophila* to toxins evokes coordinated response by the Malpighian tubules, involving both alterations in detoxification pathways as well as enhanced transport through DHR96, the *Drosophila* ortholog of the vertebrate PXR/CAR family of nuclear receptors [21]. In relation with that statement, a study [28] stated that in insects, either two distinct receptors have evolved

http://dx.doi.org/10.5772/intechopen.72970

the ability to regulate a very similar set of genes. More than one receptor pathway exists to regulate similar sets of genes. This suggests the possibilities of induction of GSTE6 and GSTE7 together with other genes. Apart from that, basal expression and induction were detected in the key metabolic tissues, namely, sections of the midgut and the Malpighian tubules.

However, the difference in the expression of both GSTE6 and GSTE7 and its inability to detoxify possibly due to cis-regulatory elements controlling the expression of genes may not be acting independently whereby the substrate models may be acting solely to increase the transcriptional output of the tissue-specific modules [28], and the fact that these two genes are found sequentially on the chromosome may support a model of coordinated regulation [7]. In another instance, reports have indicated that DmGSTE6 was strongly co-expressed with DmGSTE7, DmGSTE8, DmGSTE5, DmGSTE3, DmGSTE9, and DmGSTD1, while DmGSTE7 was strongly co-expressed with DmGSTE6, DmGSTE8, DmGSTE3, and DmGSTE9 [29]. These gave insights of possible role of a selective protein to be the key regulator of sets of genes.

Rose bengal was known to inhibit several drug-metabolizing enzymes such as cytochrome P450 and UDP-glucuronosyltransferase [30]. Phenol red however inhibited GSTE6 much effective than on GSTE7.

Methylene blue dye and *trans*-chalcone had shown significant enhancement of GSTE7 activity toward CDNB with EC50 ranging from 1 to 2 × 105 nM. Chalcones are open chain flavonoids that are widely biosynthesized in plants. A study [31] revealed the pharmacological properties of natural and synthetic chalcones as antioxidant, cytotoxic, anticancer, antimicrobial, antiprotozoal, antiulcer, antihistaminic, and anti-inflammatory activities but mechanism of action of *trans*-chalcone as an inhibitor to GSTE6 while as a stimulator for GSTE7 is remaining unclear. It was reported that certain haloalka(e)nes including ethylene bromide and methylene chloride form a highly reactive episulfonium ion intermediates that catalyze GST activation reactions [32]. The intermediate effect as an inhibitor to GSTE6 while as a stimulator for GSTE7 activity is remaining unclear. Basic triphenylmethane dyes such as crystal violet have been shown to inhibit glutathione *S*-transferases from both insect sources [33] and rat liver [34]. The mode of inhibition of crystal violet appeared to involve competition by the free dye with the electrophilic substrate [35].

## 5. Conclusions

In conclusion, the study has highlighted that both DmGSTDE6 and DmGSTDE7 behave catalytically different toward same substrates, despite their high sequence similarity. DmGSTDE7 appeared to demonstrate high specific activities toward mentioned substrates and catalytically stimulated by *trans*-chalcone and methylene blue. None of the isoforms appeared to be able to conjugate pesticides in vitro and hence contradictory to previous work that may cautiously suggest their involvement in methyl parathion metabolism. Many other related findings may lead to assumption that the increase in expression of both isoforms could have been a result of co-expression with other related genes, and yet they have not involved directly in

pesticide metabolism. The work has also indicated that both isoforms were not participating in oxidative stress and its functional role thus far was of the normal detoxification xenobiotic taken by the organism. This may thus generate further interest in their functional roles, and a more suitable approach could be adapted to realize the goal.

## Acknowledgements

The work has been funded by the University of Malaya Postgraduate Research Fund (PPP: PV034/2012A) and the Ministry of Science, Technology, and Innovation (12-02-03-2041).

## Conflict of interest

There is no conflict of interests that could be perceived as prejudicing the impartiality of the research reported.

## Author details

Vennobaashini Venu and Zazali Alias*

*Address all correspondence to: alias@um.edu.my

Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

## References

[1] Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: Implications for classification of non-mammalian members of an ancient enzyme superfamily. The Biochemical Journal. 2001;**360**(1):16

[2] Ding Y, Ortelli F, Rossiter LC, Hemingway J, Ranson H. The Anopheles Gambiae glutathione transferase supergene family: Annotation, phylogeny and expression profiles. BMC Genomics. 2003;**4**:35-50

[3] Ranson H, Rossiter L, Ortelli F, Jesen B, Wang X, Roth CW, Collins FH, Hemingway J. Identification of a novel class of insect glutathione *S*-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. The Biochemical Journal. 2001;**359**:295-304

[4] Enayati AA, Ranson H, Hemingway J. Mini review: Insects glutathione transferases and insecticide resistance. J. Insect Molecular Biology. 2005;**14**:3-8

[5] Ketterman AJ, Saisawang C, Wongsantichon J. Insect glutathione transferases. Drug Metabolism Reviews. 2011;**43**:253-265

[6] Saisawang C, Wongsantichon J, Ketterman AJA. Preliminary characterization of the cytosolic glutathione transferase proteome from *Drosophila melanogaster*. The Biochemical Journal. 2011;**442**:181-190

[7] Lumjuan N, Rajatileka S, Changsom D, Wicheer J, Leelapat P, Prapanthadara LA, Somboon P, Lycett G, Ranson H. The role of the *Aedes aegypti* epsilon glutathione transferases in conferring resistance to DDT and pyrethroid insecticides. Insect Biochemistry and Molecular Biology. 2011;**41**:203-209

[8] Deng H, Huang Y, Feng Q, Zheng S. Two epsilon glutathione *S*-transferase cDNAs from the common cutworm, *Spodoptera litura*: Characterization and developmental and induced expression by insecticides. Journal of Insect Physiology. 2009;**55**:1174-1183

[9] Wei SH, Clark AG, Syvanen M. Identification and cloning of a key insecticide metabolizing glutathione *S*-transferase (MdGST-6A) from a hyper insecticide resistant strain of the housefly *Musca domestica*. Insect Biochemistry and Molecular Biology. 2001;**31**:1145-1153

[10] Alias Z, Clark AG. Studies on the glutathione *S*-transferase proteome of adult *Drosophila melanogaster*. Responsiveness to chemical challenge. Proteomics. 2007;**7**:3618-3628

[11] Alias Z, Clark AG. Adult *Drosophila melanogaster* glutathione *S*-transferases: Effects of acute treatment with methyl parathion. Pesticide Biochemistry and Physiology. 2010; **98**:94-98

[12] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;**72**:248-254

[13] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;**227**:680-685

[14] Neuhoff V, Arnold N, Taube D, Ehrhardt W. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 R-250. Electrophoresis. 1988;**9**:255-262

[15] Habig WH, Pabst MJ, Jakoby WB. Glutathione *S*-transferases: The first enzymatic step in mercapturic acid formation. The Journal of Biological Chemistry. 1974;**249**:7130-7139

[16] Motoyama N, Dauterman WC. Purification and properties of housefly glutathione S- transferase. Insect Biochemistry. 1977;**7**:361-369

[17] Brophy PM, Southan C, Barrett J. Glutathione transferases in the tapeworm *Moniezia expansa*. The Biochemical Journal. 1989;**262**:939-946

[18] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine. 1967;**70**:158-169

[19] Ben-Arie N, Khen M, Lancet D. Glutathione *S*-transferases in rat olfactory epithelium: Purification, molecular properties and odorant biotransformation. The Biochemical Journal. 1993;**292**:379-384

[20] Sawicki R, Singh SP, Mondal AK, Benes H, Zimniak P. Cloning, expression and biochemical characterization of one epsilon-class (GST-3) and ten Delta-class (GST-1) glutathione *S*-transferase from *Drosophila melanogaster*, and identification of additional nine members of the epsilon class. The Biochemical Journal. 2003;**370**:661-669

[21] Wang Y, Qiu L, Ranson H, Lumjuan N, Hemingway J, Setzer WN, Meehan EJ, Chen L. Structure of an insect epsilon class glutathione *S*-transferase from the malaria vector *Anopheles gambiae* provides an explanation for the high DDT-detoxifying activity. Journal of Structural Biology. 2008;**164**:228-235

[22] Ortelli F, Rossiter J, Vontas H, Ranson J, Hemingway J. Heterologous expression of four glutathione transferase genes genetically linked to a major insecticide-resistance locus from the malaria vector *Anopheles gambiae*. The Biochemical Journal. 2003;**272**:957-963

[23] Tang AH, Biochemical TCP. Characterization of Drosophila glutathione *S*- transferases D1 and D21. The Journal of Biological Chemistry. 1994;**269**:27876-27884

[24] Yang J, McCart C, Woods DJ, Terhzaz S, Greenwood KG, Ffrench-constant RH, Dow JAT. A *Drosophila* systems approach to xenobiotic metabolism. Physiological Genomics. 2007;**30**:223-231

[25] Drnevich JM, Reedy MM, Ruedi EA, Rodriguez-Zas S, Hughes KA. Quantitative evolutionary genomics: Differential gene expression and male reproductive success in *Drosophila melanogaster*. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2004;**271**:2267-2273

[26] Li HM, Buczkowski G, Mittapalli O, Xie J, Wu J, Westerman R, Schemerhorn BL, Murdock LL, Pittendrigh BR. Transcriptomic profiles of *Drosophila melanogaster* third instar larval midgut and responses to oxidative stress. Journal of Insect Molecular Biology. 2008;**17**:325-339

[27] Mizutani T. Toxicity of xanthene food dyes by inhibition of human drug-metabolizing enzymes in a noncompetitive manner. Journal of Environmental and Public Health. 2009; **2009**:1-9

[28] Willoughby L, Chung H, Lumb C, Robin C, Batterham P, Daborn PJ. A comparison of *Drosophila melanogaster* detoxification gene induction responses for six insecticides, caffeine and phenobarbital. Insect Biochemistry and Molecular Biology. 2006;**36**:934-942

[29] Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, Doerks T, Julien P, Roth A, Simonovic M, Bork P. von Mering C. STRING 8—A global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Research 2009;**37**:412-416

[30] Uesugi N, Furumiya K, Mizutani T. Inhibition mechanism of UDP–Glucuronosyltransferase 1A6 by xanthene food dyes. Journal of Health Science. 2006;**52**:549-557

[31] Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. Current Clinical Pharmacology. 2010;**5**:1-29

[32] Armstrong RN. Glutathione *S*-transferases: Reaction mechanism, structure, and function. Chemical Research in Toxicology. 1997;**4**:131-140

[33] Balabaskaran S, Smith JN. The inhibition of 1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane (DDT) dehydrochlorinase and glutathione S-aryl transferase in grass-grub and housefly preparations. The Biochemical Journal. 1970;**117**:989-996

[34] Debnam P, Glanville S, Clark AG. Inhibition of glutathione *S*-transferases from rat liver by basic triphenylmethane dyes. Biochemical Pharmacology. 1993;**45**:1227-1233

[35] Glanville SD, Clark AG. Inhibition of human glutathione *S*-transferases by basic triphenylmethane dyes. Journal of Life Sciences. 1997;**60**:1535-1544

IntechOpen

IntechOpen

IntechOpen