Pakistan J. Zool., vol. 49(6), pp 2279-2289, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.6.2279.2289

Cloning, Expression and Molecular Characterization of Glutathione Transferase P1-1 Gene from the Camel, *Camelus dromedarius*

Farid S. Ataya,[1,2,*] Dalia Fouad,[3,4] Ajamaluddin Malik,[1] Nikolaos E. Labrou,[5] Mohamed S. Daoud[1,6] and Hesham M. Saeed[7]

[1]*Department of Biochemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*
[2]*Molecular Biology Department, Genetic Engineering Division, National Research Centre, 33 El-Bohouth St. (former El-Tahrir St.), P.O. 12622, Dokki, Giza, Egypt*
[3]*Department of Zoology, College of Science, King Saud University, P.O. 22452, Riyadh 11459, Saudi Arabia*
[4]*Department of Zoology and Entomology, Faculty of Science, Helwan University, Ein Helwan, Cairo, Egypt*
[5]*Laboratory of Enzyme Technology, Department of Biotechnology, School of Food, Biotechnology and Development, Agricultural University of Athens, 75 Iera Odos Street, GR-11855-Athens, Greece*
[6]*King Fahd Unit Laboratory, Department of Clinical and Chemical Pathology, Kasr Al-Ainy University Hospital, Cairo University, El-Manial, Cairo 11562, Egypt*
[7]*Department of Biotechnology, Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt*

Article Information
Received 15 December 2016
Revised 01 March 2017
Accepted 06 April 2017
Available online 06 December 2017

Authors' Contribution
FSA, FD, HMS and AM performed the experiments. MD canalyzed the data. FSA wrote the manuscript while NEL revises and edited it.

Key words
One-humped camel, GST-pi, Gene expression, Molecular modelling, Cloning, Phylogenetic analysis.

ABSTRACT

In this study, we report the cloning, expression and characterization of the glutathione transferase isoenzyme P1-1 gene from *Camelus dromedarius* (*Cd*GSTP1-1). The coding sequence was cloned using RT-PCR. Sequence analysis demonstrated significant differences between amino acid sequence of *C. dromedarius* and other mammalian GSTP1-1 enzymes. Phylogenetic relationship was studied with different organisms belonging to animal kingdom and revealed that *Cd*GSTP1-1 is grouped with the enzyme from *S. scrofa*. The 3D homology model of *Cd*GSTP1-1 showed similar fold and topology with the porcine GSTpi enzyme. Gene expression analysis in five camel tissues was examined employing real-time PCR. The highest level of transcripts was found in the camel testis, followed by liver, spleen, kidney and lung. *Cd*GSTP1-1 was heterologously expressed in *Eschericia coli* BL21(DE3) as a ~24 kDa soluble protein and showed to be catalyticly active towards the model substrate 1-chloro-2,4-dinitrobenzene. The results of the present study provide new information into camelid evolution and give further insights into the diversity and complex enzymatic functions of GST superfamily.

INTRODUCTION

Living cells are exposed to many intrinsic and extrinsic genotoxic factors like xenobiotics and reactive oxygen species (ROS). They have evolved specific detoxification mechanism through the action of enzymes responsible for the inactivation of such toxic compounds. The detoxification process involves both activation (phase I) and detoxification (phase II) reactions. Among phase II enzymes, glutathione transferases (GSTs; EC 2.5.1.18) form a large multifunctional family. They are classified into three main groups, soluble cytosolic GSTs (cGST), mitochondrial GSTs (mGST) and membrane-bound microsomal GSTs (MGSTs) (Hayes *et al*., 2005; Zimniak and Singh, 2006). In mammals, cGSTs are classified into seven distinct classes termed: alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), sigma (GSTS), theta (GSTT) and zeta (GSTZ). They catalyse the conjugation of the reduced glutathione (GSH; γ-Glu–Cys–Gly), via its -SH group, to a wide variety of electrophilic substrates (Zimniak, 2006; Allocati *et al*., 2009) converting them to less toxic and more water-soluble compounds readily excretable from the cell (Hayes and Pulford, 1995; Eaton and Bammler, 1999).

* Corresponding author: fataya@ksu.edu.sa
0030-9923/2017/0006-2279 $ 9.00/0

In mammals, GSTP1-1 is an important enzyme that exhibits many different biological functions and roles (Sánchez-Gómez *et al*., 2010; Wu *et al*., 2006; Townsend *et al*., 2009; Federici *et al*., 2009; Manevich *et al*., 2004; Sun *et al*., 2010). Although the detoxification reactions of GSTP1-1 have been the main research focus over the last years, now it has become apparent that GSTP1-1 plays diverse functional roles in cell survival, cell death and stress signalling mechanisms. For example, human GSTP1-1 is involved in the regulation of stress signalling pathways (Sánchez-Gómez *et al*., 2010; Wu *et al*., 2006) and the glutathionylation of cellular proteins (Townsend *et al*., 2009). In particular, through protein-protein interactions, human GSTP1-1 can sequester and inhibit the apoptotic c-jun N-terminal kinase (JNK) (Federici *et al*., 2009). It is well established that S-glutathionylation regulates the catalytic activity and biological function of a number of proteins. In addition, it is able to form complexes with other proteins that participate in redox regulation (Manevich *et al*., 2004). Moreover, human GSTP1-1 exhibits protective role mainly against the cytotoxic effects of some electrophilic agents, and their metabolites (Sun *et al*., 2010).

The domesticated Arabian camel is the most important animal in the Arabian Peninsula where Saudi camels comprise 16% of the animal biomass (Al-Swailem *et al*., 2010), as it represents the main source of meat, and has high cultural and economic value. Therefore, functional genomics of camelid genes that are involved in antioxidant and detoxification mechanisms and contribute to the animal stress response and adaptation is both of academic interest and practical importance.

In the present work, we describe the cloning and heterologous expression in *Eschericia coli* BL21 (DE3) of the enzyme *Cd*GSTP1-1 from *Camelus dromedarius*. In addition, its gene expression analysis in five camel tissues was examined employing real-time PCR. Phylogenetic analysis and homology modelling were also carried out aiming at shining light towards the structural and catalytic features of *Cd*GSTP1-1. This work is one in a series of research studies aimed at identifying and characterizing specific camelid genes (Ataya *et al*., 2012, 2014; Wang *et al*., 2012; Saeed *et al*., 2014, 2015) that may help to a better understanding of how the camel is adapted to live in harsh desert conditions.

MATERIALS AND METHODS

Tissues, strains and growth conditions

Tissues (testis, liver, spleen, kidney and lung) from three adult male camels were obtained from the Southern Riyadh Main Slaughterhouse, immediately after slaughtering, and submerged in RNAlater solution (Qiagen, France) to avoid RNA degradation. *E. coli* strains [JM109 and BL21 (DE3)] were used for cloning and expression of recombinant *Cd*GST-P1 in Luria-Bertani (LB) medium supplemented with either 100 µg/ml ampicillin or 25 µg/mL kanamycin, respectively depending on the vector used.

RNA extraction, cDNA synthesis and molecular cloning

Fifty mg of liver, kidney, spleen, lung or testis tissue were used to extract the total RNA using the E.Z.N.A. kit (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer's instructions. The integrity of extracted RNA was assessed via electrophoresis on formaldehyde agarose gel (1%) and spectrophotometrically quantified at 260 nm using a NanoDrop spectrophotometer (NanoDrop, Thermo Scientific, Wilmington, DE, USA). Two µg of total RNA was reverse transcribed to produce single-stranded cDNA using the ImProm-II Reverse Transcription System (Promega, Madison, WI, USA), as recommended by the manufacturer.

A gradient temperature PCR was performed (50 to 60°C) using a reaction mixture composed of 25 µL of GoTaq® Green Master Mix (Promega), 5 µL of cDNA, 3 µL of each forward (GSTpF) and reverse primer (GSTpR) (30 pmol) and nuclease-free water to a final volume of 50 µL. The amplification was done by denaturating the cDNA template at 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50–60°C for 45 s and 72°C for 60 s, with a final extension at 72°C for 5 min. The obtained PCR products were electrophoretically separated on 1.2% agarose gels, excised from the gels, purified using a gel extraction kit (Qiagen) and ligated into the pGEM-T-Easy vector (Promega) according to the instructions recommended by the manufacturer. Then, the ligation mixture was used to transform competent *E. coli* JM109 cells that was used to inoculate LB agar containing isopropyl 1-thio-β-galactopyranoside (IPTG), X-gal and ampicillin, according to Sambrook *et al*. (1989).

Oligonucleotide design

A series of oligonucleotide primers were designed from highly conserved regions of *GSTP1* genes identified in the GenBank database and from the available EST of camel genome project database (http:/camel.kacst.edu.sa/). The entire coding region was amplified using a primer pair: GSTpF, 5'-GGATCCATGCCGCCCTACAC-3' and GSTpR, 5'- CTCGAGAAGCCCTCACTGC-3'. Two other primers were designed to determine gene expression levels via qPCR: qPCRF, 5'- GGACGGAGACCTCACCCTGTA -3' and qPCRR, 5'- TCCTTGCCTGCCTCATAGTTGG-3'.

Gene expression study using qPCR

The expression of *Cd*GSTP1 transcripts was quantified

via qPCR in a 7500 Fast real-time PCR system (Applied Biosystems, Alameda, CA, USA) with the fluorescent dye SYBR Green. The reaction mixture included cDNA from camel liver, kidney, spleen, lung or testis, 5 pmol of each primer (qPCRF and qPCRR) and 10 µL of Fast-SYBR Green qPCR Master Mix (Applied Biosystems) in a final 20-µL. The qPCR reaction was started by initial denaturation at 95°C for 3 min and amplification for 40 cycles of serial heating at 95°C for 3 s and 60°C for 30 s.

DNA sequencing, alignment and phylogenetic study

The cloned *Cd*GSTP1 in pGEM-T-Easy vector was sequenced at KFSHRC, Riyadh, KSA, using a 3730XL DNA Analyzer (Applied Biosystems) using the universal T7 and SP6 primers. The sequences were analysed using the Seqman program, version 5.07 (2003) and the deduced amino acid sequence was predicted with the program PROTEAN, version 5.07 (2003). The deduced sequence was compared with the existing sequences in the NCBI Protein Database, then used as a template to identify homologous mammalian sequences through PSI-BLAST. Sequences from different mammals were aligned with the ClustalW program using the MAFFT Multiple Sequence Alignment, version 6.864 (2001), colour coded according to identity using Jalview features version 2.3 (2011) and the phylogenetic tree was constructed using BLOSUM62 in the same program package.

Biocomputing analysis

An *in silico* homology modeling of *Cd*GSTP1 (ref or Accession No. ADJ57597) were predicted using Swiss model server (de Beer *et al*., 2014). The homology modeling was done using as template the 2.1Å crystal structure of porcine pi class GST (PDB id 2GSR). The *Cd*GSTP1-1 3D structure was analyzed using PyMOL software (Biasini *et al*., 2014) (Delano Scientific). The secondary structure topology was generated using online PDBsum pictorial database. The superimposition of camel and porcine GSTP structure and G-site binding residues were analyzed by PyMOL Program (2006). The quality assessment of modeled *Cd*GSTP1-1 3D structure was done using online Protein Structure Validation Suite (PSVS).

CdGSTP1-1 expression in E. coli *BL21 (DE3), assay and electrophoretic separation*

The coding region corresponding to *Cd*GSTP1-1 was cloned in the expression vector pET30a(+) (Novagen, Inc. Madison, USA) between *Bam*HI and *XhoI* restriction sites under the control of T7 promotor and kanamycin resistance gene for selection. The recombinant plasmid was used to transform *E. coli* BL-21 (DE3) and the protein is induced for 4 h at 37°C by the addition of 1 mM IPTG to the cultured cells when its absorbance was about 0.6 at 600 nm wavelength. Cells were collected by centrifugation at 5,000 g for 5 min, resuspended in 1.5 ml of 50 mM potassium phosphate buffer, pH 8.0, containing 300mM NaCl and lysed by sonocation. The GST activity and protein concentration in the soluble fraction were assayed according to Habig *et al*., (1974) and Bradford (1976), respectively, and the expression of the recombinant protein was detected electrophoretically under denaturing conditions using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to Laemmli (1970).

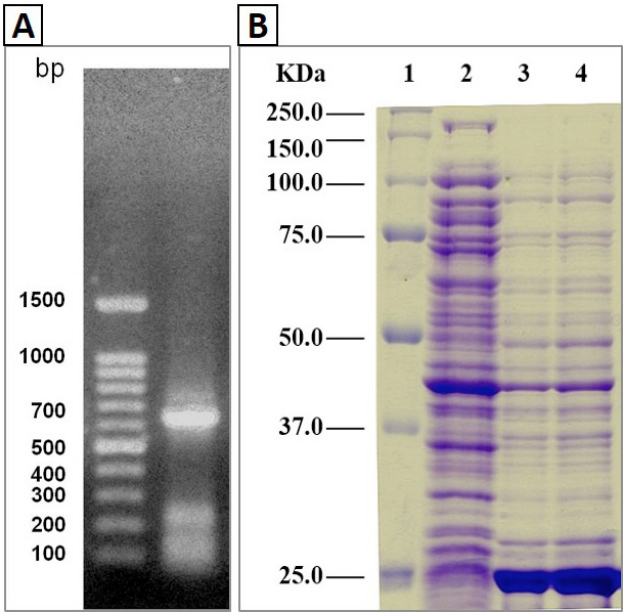

Fig. 1. A, Agarose gel (1.2% w/v) electrophoresis for RT–PCR products of CdGSTP1-1 using the specified primers and camel liver cDNA. Left, DNA ladder; Right, RT–PCR products; B, SDS-PAGE analysis of purified *Cd*GSTP1-1 enzyme. Lane 1, molecular weight markers; Lane 2, crude extract of *E. coli* BL21(DE3) uninduced; Lane 3 and 4, crude extract of *E. coli* BL21(DE3) induced with 1 mM IPTG after 2 and 4 h.

RESULTS AND DISCUSSION

cDNA cloning and bioinformatics analysis

The camel's *Cd*GSTP1-1 full coding sequence was obtained via RT-PCR at an annealing temperature of 54°C using specific primer pair (GSTpF/ GSTpR). The PCR amplicon showed a single band of the expected size after separation by electrophoresis on a 1.2% agarose gel (Fig. 1A). The purified band was cloned into the pGEM-T Easy vector and sequenced. Sequence analysis confirmed that *Cd*GSTP1-1 ORF consists of 627 bp, corresponding to 208

residues (Fig. 2). The nucleotide and amino acid sequences were submitted to NCBI GenBank (accession number HM132060 and ADJ57597, respectively). The theoretical molecular weight of the polypeptide is 23.3 kDa and the isoelectric point is 6.74.

```
1     ATG CCG CCC TAC ACC ATT GTC TAC TTC CCT GTT CGA GGG CGC TGC   45
1      M   P   P   Y   T   I   V   Y   F   P   V   R   G   R   C   15

46    GAG GCC ATG CGC ATG CTG CTG GCT GAC CAG GAC CAG AGC TGG AAG   90
16     E   A   M   R   M   L   L   A   D   Q   D   Q   S   W   K   30

91    GAG GAA GTG GTG ACC ATG GAG ACC TGG CCT GCA CTC AAA CCC TCC   135
31     E   E   V   V   T   M   E   T   W   P   A   L   K   P   S   45

136   TGT CTG TAT GGG CAG CTC CCC AAG TTC CAG GAC GGA GAC CTC ACC   180
46     C   L   Y   G   Q   L   P   K   F   Q   D   G   D   L   T   60

181   CTG TAC CAG TCC AAT GCC ATC CTC CGA CAC CTG GGC CGC TCA CTC   225
61     L   Y   Q   S   N   A   I   L   R   H   L   G   R   S   L   75

226   GGA CTG TAT GGG AAG GAC CAG CAG GAG GCA GCC CTT TTG GAC GTG   270
76     G   L   Y   G   K   D   Q   Q   E   A   A   L   L   D   V   90

271   GTG AAT GAT GGC GTG GAG GAC CTC CGT TGC AAA TAT GTC ACA CTC   315
91     V   N   D   G   V   E   D   L   R   C   K   Y   V   T   L   105

316   ATC TAC ACC AAC TAT GAG GCA GGC AAG GAG GGC TAT GTG AAG GCA   360
106    I   Y   T   N   Y   E   A   G   K   E   G   Y   V   K   A   120

361   CTG CCC GAG CAT CTG AAG CCT TTT GAG ACT CTG CTG TCC CAG AAC   405
121    L   P   E   H   L   K   P   F   E   T   L   L   S   Q   N   135

406   CAG GGG GGC CAG GCC TTC ATC GTG GGC AAC CAG ATC TCC TTC GCA   450
136    Q   G   G   Q   A   F   I   V   G   N   Q   I   S   F   A   150

451   GAC TAC AAC CTG CTG GAC TTG CTG CTG AAT CAC CAG GTC CTG GCC   495
151    D   Y   N   L   L   D   L   L   L   N   H   Q   V   L   A   165

496   CCT GGC TGC CTG GAC TCC TTC CCC CTG CTC TCA GCC TAC GTG GCT   540
166    P   G   C   L   D   S   F   P   L   L   S   A   Y   V   A   180

541   CGC CTC AGT GCC CGG CCC AAG CTC AAG GCC TTC TTG GCC TCC CCT   585
181    R   L   S   A   R   P   K   L   K   A   F   L   A   S   P   195

586   GAG CAC GTG AAG CGC CCT ATC AAC GGC AAC GGG AAG CAG TGA ggg   630
196    E   H   V   K   R   P   I   N   G   N   G   K   Q   *   G   210

631   ctt   633
```

Fig. 2. The nucleotide and deduced amino acid sequences of the cloned *CdGSTP1-1* (GenBank accession number HM132060 and ADJ57597, respectively.

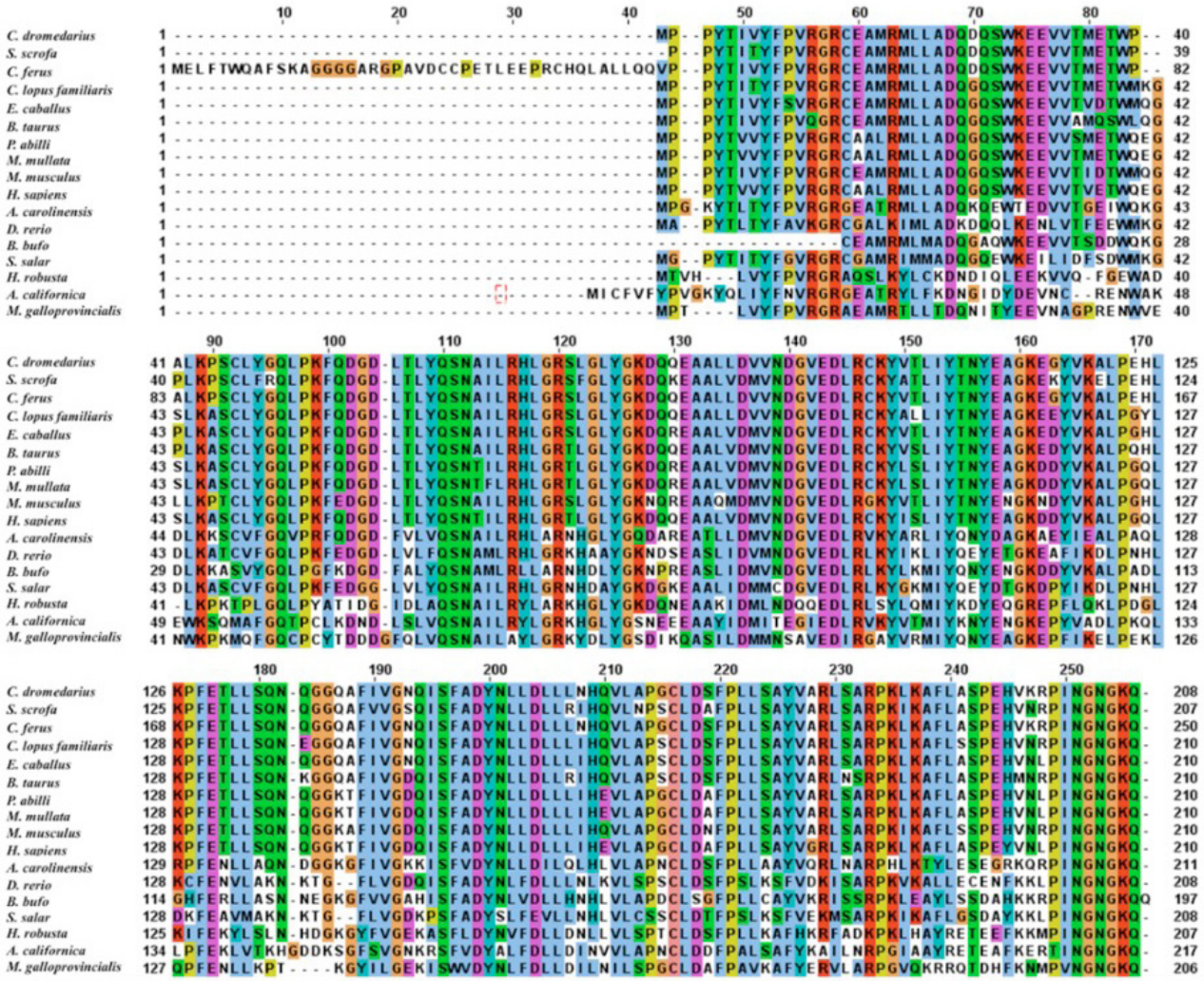

Fig. 3. Amino acid sequence alignment of *Cd*GSTP1-1 with homologues enzymes. The alignment was generated with the JalView program. The sequences that were used and their accession numbers are: *Sus scrofa,* 2GSR_A; *Camelus ferus*, EPY87515.1; *Canis lupus familiaris*, NP_001239096; *Equus caballus*, XP_001498156; *Bos taurus*, NP_803482.1; *Pongo abilli*, NP_001127471.1; *Macaca mullata*, NP_001036141.1; *Mus musculus*, NP_038569.1; *Homo sapiens*, NP_000843.1; *Anolis carolinensis*, XP_003215129.1; *Cyprinus carpio*, ABD67510.1; *Danio rerio*, NP_571809.1; *Bufo bufo*, AAN04480.1; *Salmo salar*, ACI70112.1; *Helobdella robusta*, ESO09543.1; *Aplysia californica*, XP_005099401.1; *Mytilus galloprovincialis*, AAM91994.1.

The predicted aminoacid sequence was used to *in silico* screen orthologues from different organisms (Fig. 3, Table I). *Cd*GSTP1-1 showed high identity with mammals and lower with reptiles, birds, fish and mollusks. The highest identity was found with other pi class GSTs from: dog, *Canis lupus familiaris* (92%); horse, *Equus caballus;* (91%); porcine, *Sus scrofa* (90%); cattle, *Bos taurus* (88%); and human, *Homo sapiens* (86%). Less identities was found with reptiles, fish, amphibians, worms, and mollusks; lizard; *Anolis carolinensis* (67%), zebra fish *Danio rerio* (61%), frog; annelid; *Helobdella robusta* (51%), mollusk; *Aplysia californica* (50%), and mussel; *Mytilus galloprovincialis* (49%). Surprisingly, camel and porcine GSTs lost two important aminoacids during evolution (K41, E41 or Q41 and G42) that are present in all other organisms (Fig. 3). Other noteworthy difference is that *Cd*GSTP1-1 from the Arabian camel *C. dromedarius* differs from the two humped camel *C. ferus* GST-Pi, as the later has 42 aminoacid insertion at the N-terminal.

Analysis of the aminoacid sequence of *Cd*GSTP1-1 reveals the presence conserved motifs that are characteristics in GST classes. For example, the SNAIL motif (Pemble *et al*., 1996) in the N-terminal domain, that form part of the GSH binding site, is located at position 64-68 (*Cd*GSTP1-1

numbering, Fig. 2). The C-terminal domain of cytosolic GSTs contains a conserved N-capping box motif (Ser/Thr-Xaa-Xaa-Asp) at the beginning of H6 helix that forms a hydrogen bond interaction of the hydroxyl group of Ser/Thr with Asp (Aceto *et al*., 1997; Cocco *et al*., 2001). *Cd*GSTP1-1 possesses an N-capping box motif (Ser-Phe-Ala-Asp) that is found between amino acids 148-151 (Fig. 2 and 3). This motif is conserved among all pi class GSTs and it is involved in the H6-helix formation, playing a crucial structural and functional roles on GST folding.

The main interaction that provides the driving force for GSTs dimerization is the hydrophobic 'lock-and-key' motif (Hegazy *et al*., 2004). This 'lock-and-key' motif affect significant role the catalytic activity and structural stability. In this motif, the 'key' is an aromatic residue (Tyr48, Figure 2 and 3 in one monomer and the 'lock' is a cluster of hydrophobic residues from the other interacting subunit (Hegazy *et al*., 2004)). This motif is conserved in *Cd*GSTP1-1.

In order to examine the genetic relationship between this enzyme and other GSTs from pi classes, a phylogenetic analysis was achieved. The phylogenetic relationship between *Cd*GSTP1-1 and the deduced aminoacid sequence from 17 different animal species is shown in Figure 4. The analysis showed that *Cd*GSTP1-1 apparently evolved from an ancestral GST-Pi gene that predated the appearance of vertebrates, and it grouped with pig, cattle, dog, horse, human and monkey enzymes.

Expression analysis of CdGSTP1-1 in camel tissues

Expression analysis of stress-related and detoxifying enzymes, such as GSTs, within tissues allow for better understanding and predictions of potential sites of toxicity and metabolism in response to exposure to particular stress and/or environmental pollutants (Mitchell *et al*., 1997). Therefore, the expression of *Cd*GSTP1-1 in five camel tissues (testis, liver, spleen, kidney and lung) was examined employing real-time PCR (Fig. 5). The constitutive levels of expression of xenobiotic-metabolizing enzyme in a tissue determine its ability to detoxify xenobiotic compounds and endogenous metabolic stress. The highest level of *CdGSTP1-1* transcripts was found in the camel testis followed by the liver and spleen. The mean expression level of *Cd*GSTP1-1 in testis was about 1.9-fold higher than in liver and more than 6-fold higher than that in spleen. In the other two tissues (kidney and lung), *Cd*GSTP1-1 expression was also observed but at significant lower level (Fig. 5).

Table I.- Sequences that were employed in phylogenetic analysis.

Organism	NCBI accession No.	Amino acid residues	Total score	Identity (%)	Positive (%)	e- value
Camelus dromedarius	---	208	---	100	100	00
Canis lupus familiaris	NP_001239096	210	394	92	93	6e-137
Equus caballus	XP_001498156	210	389	91	93	2e-135
Bos taurus	NP_803482	210	384	88	94	5e-133
Camelus ferus	EPY87515	250	428	99	100	7e-150
Pongo abilli	NP_001127471	210	376	86	93	3e-130
Macaca mullata	NP_001036141	210	376	86	92	6e-130
Mus musculus	NP_038569	210	379	88	93	3e-131
Homo sapiens	NP_000843	210	374	86	92	2e-129
Anolis carolinensis	XP_003215129	211	290	67	77	3e-96
Cyprinus carpio	ABD67510	208	277	62	77	4e-91
Danio rerio	NP_571809	208	273	61	77	1e-89
Bufo bufo	AAN04480	197	259	65	77	2e-84
Xenopus laevis	NP_001082252	212	223	53	66	7e-70
Salmo salar	ACI70112	208	258	59	75	8e-84
Helobdella robusta	ESO09543	207	207	51	66	1e-63
Aplysia californica	XP_005099401	217	196	50	65	2e-59
Mytilus galloprovincialis	AAM91994	206	194	49	65	9e-59

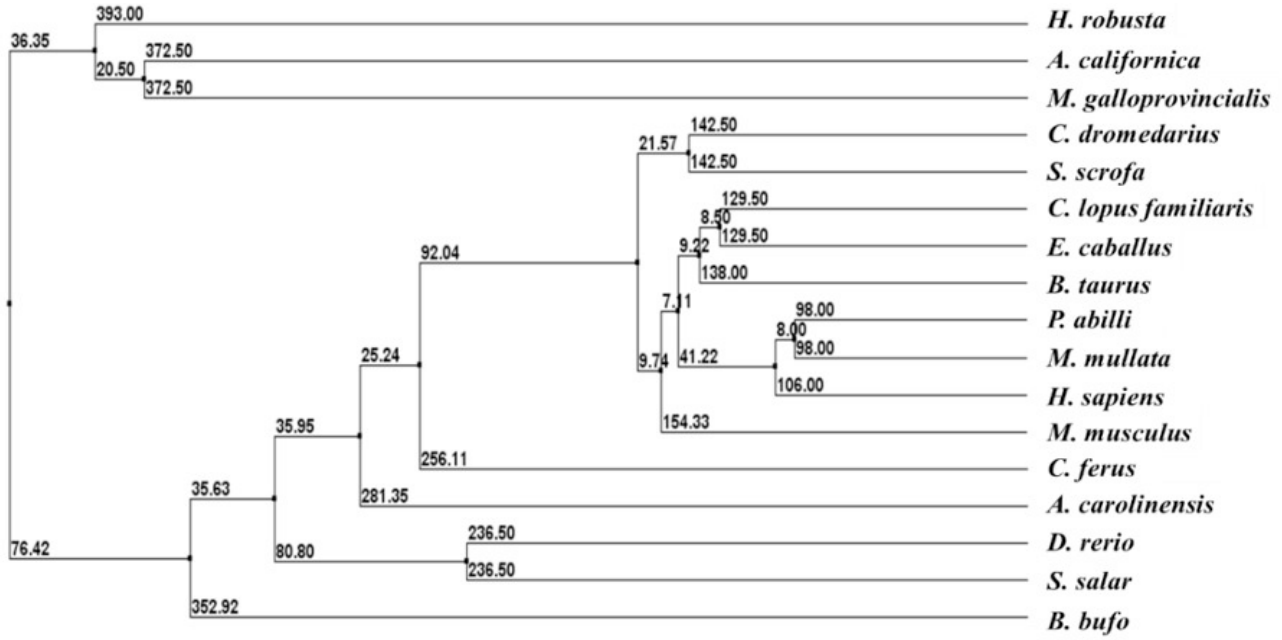

Fig. 4. The phylogenetic tree of *Cd*GSTP1-1 with homologues enzymes shown in Figure 3.

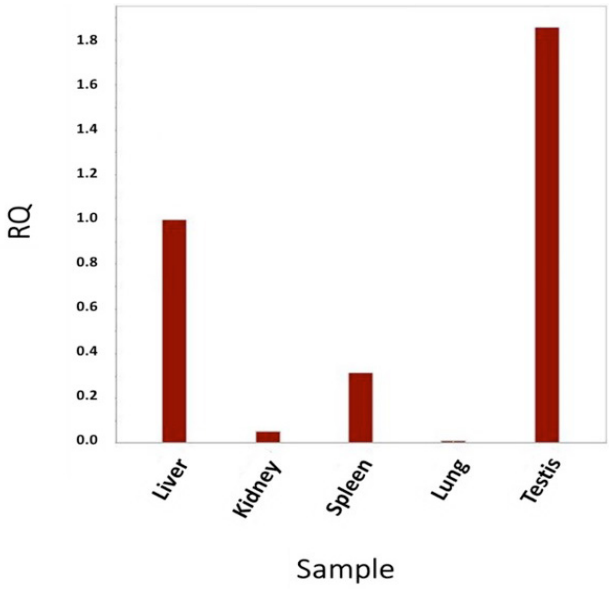

Fig. 5. Relative mRNA expression levels of *Cd*GSTP1-1 transcript in different camel tissues using the 18S ribosomal subunit as the housekeeping gene.

Gupta *et al*. (1990) studied the expression of the alpha, mu, and pi classes GSTs in mouse brain, heart, kidney, spleen, liver, and muscle. In agreement with the results of the present study they found that GST isoforms were variably expressed in different mouse tissues, suggesting that their expression was tissue specific. In mice, the pi class GST was found to be expressed in various organs. In this case, the tissue with the higher expression was liver. The expression profiles described by Coles *et al*. (2002) in humans have some similarities as well as differences from that found in mice and in the present study.

Expression of CdGSTP1-1 in E. coli

The coding sequence of *Cd*GSTP1-1 was cloned and expressed as a soluble protein in *E. coli* BL21 (DE3) under T7 promotor of pET-30a(+) vector. GST activity was assayed in the soluble fraction. Considerable activity was detected using 1-chloro-2,4-dinitrobenzene (CDNB) (4.3 unit/mg protein). A dense recombinant protein band was detected using SDS-PAGE and the density of the band increased with induction time following induction by 1mM IPTG at 37C (Fig. 1B).

Molecular modeling

To understand the structural and catalytic properties of *Cd*GSTP1-1, the enzyme sequence was subjected to structure prediction using homology modeling. The model was built using as template the available porcine GST pi (90% sequence identity, PDB ID 2GSR, 2.1Å resolution). As expected, the overall fold is similar to that of GSTs and exhibits the usual alpha helical rich dimeric protein (Fig. 6). Each subunit consists of two domains; the smaller, thioredoxin-like, N-terminal domain (1-74 residues) is composed of 4 beta-sheets and 4 alpha helices and the C-terminal domain that consists of seven alpha helices.

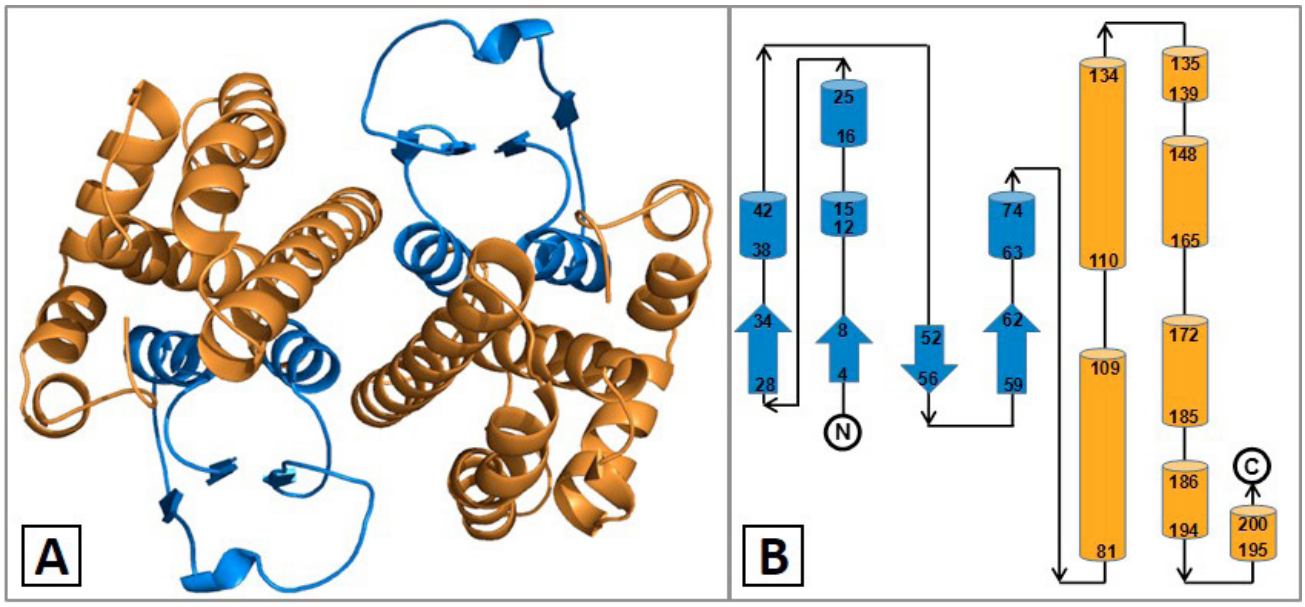

Fig. 6. Homology model of *Cd*GSTP1-1. A, N-terminal domain (blue) and the C-terminal domain (orange); B, topology diagram for *Cd*GSTP1-1. The N-terminal domain is colored blue and the c-terminal colored orange.

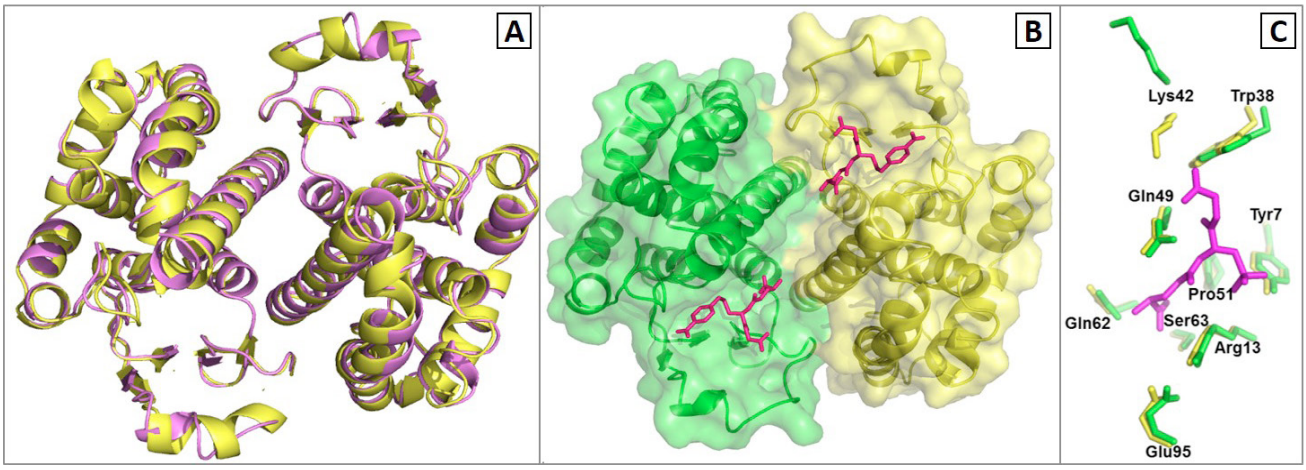

Fig. 7. A, Superimposed modeled CdGSTP1-1 (violet) on template porcine GSTpi (yellow). The modeled CdGSTP1-1 indicated very high similarity in folding pattern with porcine GSTpi; B, surface view of the G- and H-site of CdGSTP1-1. The two subunits are shown in green and yellow. The bound inhibitor, glutathione sulfonate, is shown with magenta color; C, Comparison of the G-site binding residues and other important residues in camel (green) and porcine GSTpi (yellow).

The GSH binding region, known as G-site, is located on each subunit (Fig. 7). The N-terminal domain of each subunit provides major framework support for each G-site region (Reinemer *et al*., 1991; Dirr *et al*., 1994). The interactions at the G-site appear to be conserved. Glutathione sulfonate at the G-site adopts an extended conformation with its γ-L Glu moiety near the subunit interface. The sulphonyl moiety of glutathione sulfonate positioned towards the C-terminal domain of the same subunit and the Gly moiety of the glutathione sulfonate located above third beta sheet (β3) (Fig. 7). Residues of G-site involved in GSH binding are superimposed very well in *Cd*GSTP1-1 and porcine GST pi, except Lys42 (Fig. 7C). The γ-L glutamyl moiety of glutathione sulfonate docks into polar pocket formed by four side chains (Arg13, Gln 49, Gln62 and Ser63). In the porcine GSTpi, glutathione sulfonate makes 15 polar contact and four water mediated contacts at the G-site (Dirr *et al*., 1994).

The hydroxyl group of Tyr7 makes hydrogen bonds with the sulphonyl group of the inhibitor. Tyr7 is the catalytic residue that plays major role in the catalytic mechanism (Kong *et al*., 1992; Dirr *et al*., 1994; Karshikoff *et al*., 1993). Electrostatic potential analysis indicate that the G-site is positive due to the presence of positive charged residues and the partial positive charge associated with the N-terminus of H1. This positive electrostatic field in the G-site is characteristic of all GSTs and has been suggested to promote GSH binding and -SH ionisation (Axarli *et al*., 2009). The gross conformation and key residues involved in the interaction between glutathione sulfonate and GST pi are very similar to the 3D structure of the mu class GST complexed with reduced glutathione (Ji *et al*., 1992) and also to the alpha class GST bound with S-benzyl-glutathione (Sinning *et al*., 1993).

CONCLUSION

In conclusion, in the present work we report the cloning, gene expression analysis and structural characterization of *Cd*GSTP1-1 using homology modeling. Gene expression analysis showed differential expression in different tissue types suggesting a differential role and regulation of *Cd*GSTP1-1 in camel tissues. Molecular modeling studies of *Cd*GSTP1-1 showed similar overall fold and domain organization, however major variations were identified in C-terminal helix that may affect xenobiotic substrate recognition and catalytic mechanism. The results advance our understanding of camelid detoxification enzymatic system and provide new information on evolution of the humped camels.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group project number RGP-VPP-173.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Aceto, A., Dragani, B., Melino, S., Allocati, N., Masulli, M., di Ilio, C. and Petruzzelli, R., 1997. Identification of an N-capping box that affects the alpha 6-helix propensity in glutathione S-transferase superfamily proteins: a role for an invariant aspartic residue. *Biochem. J.,* **322**: 229-234. https://doi.org/10.1042/bj3220229

Al-Swailem, A.M., Shehata, M.M., Abu-Duhier, F.M., Al-Yamani, E.J., Al-Busadah, K.A., Al-Arawi, M.S., Al-Khider, A.Y., Al-Muhaimeed, A.N., Al-Qahtani, F.H., Manee, M.M., Al-Shomrani, B.M., Al-Qhtani, S.M., Al-Harthi, A.S., Akdemir, K.C., Inan, M.S. and Out, H.H., 2010. Sequencing, analysis, and annotation of expressed sequence tags for *Camelus dromedaries*. *PLoS One,* **5**: e10720. https://doi.org/10.1371/journal.pone.0010720

Allocati, N., Federici, L., Masulli, M. and di Ilio, C., 2009. Glutathione transferases in bacteria. *FEBS J.,* **276**: 58–75. https://doi.org/10.1111/j.1742-4658.2008.06743.x

Ataya, F.S., Al-Jafari, A.A., Daoud, M.S., Al-Hazzani, A.A., Shehata, A.I., Saeed, H.M. and Fouad, D., 2014. Genomics, phylogeny and in silico analysis of mitochondrial glutathione S-transferase-kappa from the camel *Camelus dromedarius*. *Res. Vet. Sci.,* **97**: 46-54. https://doi.org/10.1016/j.rvsc.2014.04.004

Ataya, F.S., Fouad, D., Malik, A. and Saeed, H.M., 2012. Molecular cloning and 3D structure modeling of APEX1, DNA base excision repair enzyme from the camel, *Camelus dromedarius*. *Int. J. mol. Sci.,* **13**: 8578–8596. https://doi.org/10.3390/ijms13078578

Axarli, I., Dhavala, P., Papageorgiou, A.C. and Labrou, N.E., 2009. Crystallographic and functional characterization of the fluorodifen-inducible glutathione transferase from glycine max reveals an active site topography suited for diphenylether herbicides and a novel L-site. *J. mol. Biol.,* **385**: 984-1002. https://doi.org/10.1016/j.jmb.2008.10.084

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., Kiefer, F., Cassarino, T.G., Bertoni, M., Bordoli, L. and Schwede, T., 2014. SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information. *Nucl. Acids Res.,* **42**: W252-258. https://doi.org/10.1093/nar/gku340

Bradford, M.M., 1976. A rapid and sensitive method for the quanti- fication of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.,* **72**: 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cocco, R., Stenberg, G., Dragani, B., Rossi Principe, D., Paludi, D., Mannervik, B. and Aceto, A., 2001. The folding and stability of human alpha class glutathione transferase A1-1 depend on distinct roles of a conserved N-capping box and hydrophobic staple motif. *J. biol. Chem.,* **276**: 32177-32183. https://doi.org/10.1074/jbc.M104057200

Coles, B.F., Chen, G., Kadlubar, F.F. and Radominska-

Pandya, A., 2002. Interindividual variation and organ-specific patterns of glutathione S-transferase alpha, mu, and pi expression in gastrointestinal tract mucosa of normal individuals. *Arch. Biochem. Biophys.,* **403**: 270-276. https://doi.org/10.1016/S0003-9861(02)00226-6

Dirr, H., Reinemer, P. and Huber, R., 1994. Refined crystal structure of porcine class Pi glutathione S-transferase (pGST P1-1) at 2.1 A resolution. *J. mol. Biol.,* **243**: 72-92. https://doi.org/10.1006/jmbi.1994.1631

Eaton, D.L. and Bammler, T.K., 1999. Concise review of the glutathione S-tarnsferases and their significance to toxicity. *Toxicol. Sci.,* **49**: 156–164. https://doi.org/10.1093/toxsci/49.2.156

Federici, L., Lo Sterzo, C., Pezzola, S., di Matteo, A., Scaloni, F., Federici, G. and Caccuri, A.M., 2009. Structural basis for the binding of the anticancer compound 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol to human glutathione s-transferases. *Cancer Res.,* **69**: 8025-8034. https://doi.org/10.1158/0008-5472.CAN-09-1314

Gupta, S., Medh, R.D., Leal, T. and Awasthi, Y.C., 1990. Selective expression of the three classes of glutathione S-transferase isoforms in mouse tissues. *Toxicol. appl. Pharmacol.,* **104**: 533-542. https://doi.org/10.1016/0041-008X(90)90175-T

Habig, W.H., Pabst, M.J. and Jakoby, W.B., 1974. Glutathione-S-transferase. A first enzymatic step in mercapturic acid formation. *J. biol. Chem.,* **294**: 7130-7139.

Hayes, J.D. and Pulford, D.J., 1995. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. mol. Biol.,* **30**: 445–600. https://doi.org/10.3109/10409239509083492

Hayes, J.D., Flanagan, J.U. and Jowsey, I.R., 2005. Glutathione S-transferases. *Annu. Rev. Pharmacol. Toxicol.,* **45**: 51–88. https://doi.org/10.1146/annurev.pharmtox.45.120403.095857

Hegazy, U.M., Mannervik, B. and Stenberg, G.J., 2004. Functional role of the lock and key motif at the subunit interface of glutathione transferase p1-1. *J. biol. Chem.,* **279**: 9586–9596. https://doi.org/10.1074/jbc.M312320200

JALVIEW, 2011. *Version 2.3; Multiple sequence alignment*. University of Dundee, Scotland, UK.

de Beer, T.A., Berka, K., Thornton, J.M. and Laskowski, R.A., 2014. PDBsum additions. *Nucl. Acids Res.,* **42**: D292-296. https://doi.org/10.1093/nar/gkt940

Ji, X., Zhang, P., Armstrong, R.N. and Gilliland, G.L., 1992. The three-dimensional structure of a glutathione S-transferase from the mu gene class. Structural analysis of the binary complex of isoenzyme 3-3 and glutathione at 2.2-A resolution. *Biochemistry,* **3**: 10169-10184. https://doi.org/10.1021/bi00157a004

Karshikoff, A., Reinemer, P., Huber, R. and Ladenstein, R., 1993. Electrostatic evidence for the activation of the glutathione thiol by Tyr7 in pi-class glutathione transferases. *Eur. J. Biochem.,* **215**: 663-670. https://doi.org/10.1111/j.1432-1033.1993.tb18077.x

Kong, K.H., Takasu, K., Inoue, H. and Takahashi, K., 1992. Tyrosine-7 in human class Pi glutathione S-transferase is important for lowering the pKa of the thiol group of glutathione in the enzyme-glutathione complex. *Biophys. Res. Commun.,* **184**: 194-197. https://doi.org/10.1016/0006-291X(92)91177-R

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**: 680–685. https://doi.org/10.1038/227680a0

MAFFT, 2011. *Version 6.864, Multiple sequence alignment program*. Computational Biology Research Center (CBRC), Tokyo, Japan.

Manevich, Y., Feinstein, S.I. and Fisher, A.B., 2004. Activation of the antioxidant enzyme 1-CYS peroxiredoxin requires glutathionylation mediated by heterodimerization with pi GST. *Proc. natl. Acad. Sci. U.S.A.,* **101**: 3780-3785. https://doi.org/10.1073/pnas.0400181101

Mitchell, A.E., Morin, D., Lakritz, J. and Jones, A.D., 1997. Quantitative profiling of tissue-and gender-related expression of glutathione S-transferase isoforms in the mouse. *Biochem. J.,* **325**: 207-216. https://doi.org/10.1042/bj3250207

Pemble, S.E., Wardle, A.F. and Taylor, J.B., 1996. Glutathione S-transferase class Kappa: characterization by the cloning of rat mitochondrial GST and identification of a human homologue. *Biochem. J.,* **319**: 749-754. https://doi.org/10.1042/bj3190749

PROTEAN, 2003. *Version 5.07, Molecular analysis of protein*. DNASTAR Inc., Madison, WI, USA.

PYMOL, 2006. *PYMOL*. S. N. Y., NY, USA.

Reinemer, P., Dirr, H.W., Ladenstein, R., Schäffer, J., Gallay, O. and Huber, R., 1991. The three-dimensional structure of class pi glutathione S-transferase in complex with glutathione sulfonate at 2.3 A resolution. *EMBO J.,* **10**: 1997-2005.

Saeed, H., Shalaby, M., Embaby, A., Ismael, M., Pathan, A., Ataya, F.S., Alanazi, M. and Bassiouny, K.,

2015. The Arabian camel *Camelus dromedarius* heat shock protein 90α:cDNA cloning, characterization and expression. *Int. J. Biol. Macromol.,* **81**: 195–204. https://doi.org/10.1016/j.ijbiomac.2015.07.058

Saeed, H.M., Alanazi, M.S., Shalaby, M.A., Alshahrani, O., Ataya, F.S., Pathan, A.A. and Abduljaleel, Z.A., 2014. Molecular cloning and cDNA characterization of *Camelus dromedarius* putative cytochrome P450s 1A, 2C, and 3A. *Genet. Mol. Res.,* **13**: 2886-2905. https://doi.org/10.4238/2014.March.17.1

Sambrook, J., Fritsch, E. and Manaiatis, T., 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, New York, NY, USA.

Sánchez-Gómez, F.J., Díez-Dacal, B., Pajares, M.A., Líorca, O. and Pèrez-Sala, D., 2010. Cyclopentenone prostaglandins with dienone structure promote cross-linking of the chemoresistance-inducing enzyme glutathione transferase P1-1. *Mol. Pharmacol.,* **78**: 723-733. https://doi.org/10.1124/mol.110.065391

SEQMAN, 2003. *Version 5.07, Matching nucleotide sequences*. DNASTAR Inc., Madison, WI, USA.

Sun, N., Sun, X., Chen, B., Cheng, H., Feng, J., Cheng, L. and Lu, Z., 2010. MRP2 and GSTP1 polymorphisms and chemotherapy response in advanced non-small cell lung cancer. *Cancer Chemother. Pharmacol.,* **65**: 437-446. https://doi.org/10.1007/s00280-009-1046-1

Sinning, I., Kleywegt, G.J., Cowan, S.W., Reinemer, P., Dirr, H.W., Huber, R., Gilliland, G.L., Armstrong, R.N., Ji, X., Board, P.G., Olin, B., Mannervik, B. and Jones, T.A., 1993. Structure determination and refinement of human alpha class glutathione transferase A1-1, and a comparison with the Mu and Pi class enzymes. *J. mol. Biol.,* **232**: 192-212. https://doi.org/10.1006/jmbi.1993.1376

Townsend, D.M., Manevich, Y., He, L., Hutchens, S., Pazoles, C.J. and Tew, K.D., 2009. Novel role for glutathione S-transferase pi. Regulator of protein S-Glutathionylation following oxidative and nitrosative stress. *J. biol. Chem.,* **284**: 436-445. https://doi.org/10.1074/jbc.M805586200

Wang, Z., Ding, G., Chen, G., Sun, Y., Sun, Z., Zhang, H., Wang, L., Hasi, S., Zhang, Y., Li, J., Shi, Y., Xu, Z., He, C., Yu, S., Li, S., Zhang, W., Batmunkh, M., Ts, B., Narenbatu, S., Unierhu, Bat-Ireedui, H., Gao, B., Baysgalan, Q., Li, Z., Jia, Turigenbayila, Subudenggerile, Narenmanduhu, Z., Wang, J., Wang, L., Pan, Y., Chen, Y., Ganerdene, Dabxilt, Erdemt, Altansha, Altansukh, Liu, T., Cao, M., Aruuntsever, Bayart, Hosblig, He, F., Zha-ti, A., Zheng, G., Qiu, F., Sun, Z., Zhao, L., Zhao, W., Liu, B., Li, C., Chen, Y., Tang, X., Guo, C., Liu, W., Ming, L., Temuulen, A., Cui, Y., Li, J., Gao, J., Wurentaodi, LI, Niu, S., Sun, T., Zhai, Z., Zhang, M., Chen, C., Baldan, T., Bayaer, T., Li, Y. and Meng, H., 2012. Bactrian camels genome sequencing and analysis consortium, jirimutu, Genome sequences of wild and domestic bactrian camels. *Nat. Commun.,* **3**: 1202-1208. https://doi.org/10.1038/ncomms2192

Wu, Y., Fan, Y., Xue, B., Luo, L., Shen, J., Zhang, S., Jiang, Y. and Yin, Z., 2006. Human glutathione S-transferase P1-1 interacts with TRAF2 and regulates TRAF2-ASK1 signals. *Oncogene,* **25**: 5787-5800. https://doi.org/10.1038/sj.onc.1209576

Zimniak, P. and Singh, S.P., 2006. Families of glutathione transferases. In: *Toxicology of glutathione transferases* (ed. Y.C. Awasthi). CRC Press, Boca Raton, FL, pp. 11–26. https://doi.org/10.1201/9781420004489.ch2

Zimniak, P., 2006. Substrate and reaction mechanism of GSTs. In: *Toxicology of glutathione transferases* (ed. Y.C. Awasthi). CRC Press, Boca Raton, FL, pp. 71–102. https://doi.org/10.1201/9781420004489.ch5