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# Purification and Characterization of Glutathione-S-Transferase from Rat's Liver: Effect of Carbon Tetrachloride and Camel's Milk

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## Abstract

Glutathione-*S*-transferases (GSTs) constitute a multifunctional family of dimeric and mainly cytosolic biotransformation enzymes which play an important role in protecting tissues from oxidative stress. They catalyzed the conjugation of intracellular glutathione with great varieties of chemicals possessing electrophilic centre and the final GSH-conjugates had increased hydrophilicity which facilitate their further metabolism and elimination. Our study was carried out to purify GST enzyme from rat's liver using one step affinity chromatography and investigate the inhibitory effect of carbon tetrachloride and ameliorating effect of camel's milk administration. The purification of GST enzyme revealed that the protein concentration decreased in GSH affinity filtrate than the crude extract while the specific enzyme activity increased indicating that the single purification step cut off the other proteins and conserve the protein of GST enzyme. $CCL_4$ decrease the $V_{max}$ and increase $K_m$ for both GSH and CDNB substrates than control indicating that $CCL_4$ may be uncompetitive inhibitor of GST enzyme while camel's milk increase the $V_{max}$ and decrease $K_m$ for both GSH and CDNB substrates indicating that camel's milk increase the affinity of enzyme to both substrates. The activity of GST increased by increasing the substrate concentration till reaching the maximum activity after which the activity of GST forming a plateau. Regarding the effect of temperature on GST activity, the maximum GST activity was observed between 25-35ºC and inactivated over 40ºC. The GST activity increased when pH increased progressively till reach the maximum activity at pH value 7.1 and decreased when the pH increased. Our conclusion indicating that $CCL_4$ is uncompetitive inhibitor of GST enzyme while camel's milk increases the activity and affinity of enzyme toward its both substrates.

**Keywords**: $CCL_4$; GST; Affinity chromatography; Camel's milk

## Introduction

The glutathione-*S*-transferases (GSTs) belonged to a multigene enzyme superfamily which catalyzes the nucleophilic addition of Thiol of reduced glutathione to varieties of electrophiles [1]. The GSTs are homodimers or heterodimers comprised of pairings of seven different subunits. Five main classes of GSTs exist; each containing more than one isozymes based on substrate affinity and inhibitor properties. The cytosolic classes have been named alpha (α), mu (μ), pi (π) and theta (θ) based on their subunit composition, substrate/inhibitor specificity, primary and tertiary structure similarities and immunological identity [2]. The fifth class is the microsomal form of the enzyme; specific GST subunits are induced by various xenobiotics and are expressed in a tissue specific manner [3]. The enzyme contains two binding sites within the active site, a G-site for the binding of GSH and a H-site for the binding of an electrophils. Electrophiles have a slow spontaneous rate of reaction with GSH which is greatly enhanced in the presence of GST. Electrophilic substrate for GST includes xenobiotics such as carcinogens and their metabolites, herbicides and mutagens. In addition, GSTs bind with varying affinity to a variety of hydrophobic compounds such as heme, bilirubin, polycyclic aromatic hydrocarbons and dexamethasone [1]. Most conjugated products of GSTs are cytotoxic and therefore must be eliminated, glutathione *S*-conjugated products are exported from cells (in particular from liver cells where cytotoxins are concentrated) via a membrane ATP-dependent pump known as the glutathione *S*-conjugate export pump [4].

Glutathione-*S*-transferases were isolated and purified from rat brain and testes according to [5]. Briefly the tissues were homogenized in buffer containing 25 mmol/L Tris-HCl pH 8.0 in a Potter Elvehjen homogenizer with a Teflon pestle, this homogenate was centrifuged at 78000 xg for 1 h at 4ºC. The supernatant was collected and dialyzed against the same buffer. The dialysate was loaded into a pre-equilibrated affinity chromatography column (swollen *S*-hexylglutathione linked agarose-4B affinity matrix, 2cm x 7cm) with the same buffer at flow rate of 60 ml/h. the column was washed with the same buffer containing 0.2 mol/L KCL until the absorbance of protein in the elute was less than 0.005 OD at 280 nm. Bound protein was eluted with the same buffer containing 5 mmol/L *S*-hexyl GSH, 2.5 mmol/L GSH and 0.2 mol/L KCL. The eluent was collected in 3 ml fractions. In the present study, we examined the kinetic properties of purified GST enzyme from rat liver subjected to carbon tetrachloride and camel's milk.

## Materials and Methods

### Animals

The study was carried out on 180 male albino rats of 120-150 g body weight each and about 80 ± 5 days old. The animals were purchased from the medical research institute of Alexandria University. They were housed in plastic cages (8-10 rats/cage) with soft-wood chips for bedding. They were given a commercial basal diet which consisted of bread, corn and soybean and water *ad libitum*. All animals were housed

under the mentioned environmental conditions and the basal diet for a minimum of two weeks before experiments for acclimatization and to ensure normal growth and behavior.

## Camel's milk

Two types of camel's milk were used in this study; farm camel's milk was obtained from the centre of camel's development and studies (fed on commercial ration) at Marsa Matrouh governorate. Desert camel's milk was obtained from camels grazing in desert of Al-Dabaa city Marsa Matrouh governorate.

All camel's milk were collected every week from different multiparous she-camels 4-7 years old and packaged in plastic bottle of 1 liter capacity then transported to laboratory and kept freezing at -20°C till used.

## Experimental design

The principle of this experiment was to evaluate the hepatoprotective effect of both types of camel's milk during chronic toxicity of carbon tetrachloride. 180 rats were used in this experiment and divided into 6 groups (30 rats each) as follows:

**Group (1):** Rats were fed on basal diet and water *ad libitum*.

**Group (2):** Rats were fed on basal diet and water *ad libitum* and subcutaneous injected with carbon tetrachloride at dose of 2 ml/kg b.wt diluted with paraffin oil (1:4) twice weekly according to [6].

**Group (3):** Rats were fed on basal diet and farm camel's milk *ad libitum*.

**Group (4):** Rats were fed on basal diet and farm camel's milk *ad libitum* and subcutaneous injected with carbon tetrachloride at dose of 2 ml/kg b.wt diluted with paraffin oil (1:4) twice weekly.

**Group (5):** Rats were fed on basal diet and desert camel's milk *ad libitum*.

**Group (6):** Rats were fed on basal diet and desert camel's milk *ad libitum* and subcutaneous injected with carbon tetrachloride at dose of 2 ml/kg b.wt diluted with paraffin oil (1:4) twice weekly.

## Collection of rat livers for the biochemical studies

After decapitation of rats, they were eviscerated, the livers were removed from the carcasses and washed by ice-cold saline buffer to remove the blood and then blotted in filter papers and finally kept frozen at -70°C for biochemical analysis.

**Glutathione-S-transferase assay:** Glutathione-*S*-transferase activity using chlorodinitrobenzene (CDNB) or dichloronitrobenzene (DCNB) as substrates was assayed spectrophotometrically essentially as described by [7]. The cuvettes (final volume of 3.0 ml) contained 0.1 M phosphate buffer (pH 6.5 for assaying chlorodinitrobenzene and pH 7.8 for dichloronitrobenzene activity), 1 mM GSH and 1 mM of either substrate or suitable aliquots (usually 20 μl) of appropriately diluted enzyme from the different sources. Change in absorbance at 340 nm (for chlorodinitrobenzene) and 344 nm (for dichloronitro-benzene) was followed against a blank containing all reactants excepting enzyme protein, specific activity was expressed as nmol conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 and 8.5 for CDNB and DCNB respectively.

**Purification of liver glutathione-*S*-transferase enzyme:** Glutathione-*S*-transferase was purified according to [8].

**Preparation of crude extract**: Rat livers were homogenized at 4°C in a ratio of 1:4 (w/v) with phosphate buffer saline pH 7.3. The homogenate was centrifuged at 10.000 g/10 minutes and the pellet was discarded. The supernatant was filtered through glass wool and the cytosolic glutathione-*S*-transferase was obtained. The enzyme solution was stored in the cold -20°C until needed.

- **Purification of GST using glutathione agarose affinity chromatography:** The enzyme containing solution was applied to affinity column (GSTrap FF column, 71-5016-96 AK, GE health care). The column is $0.7 \times 2.5$ cm (1 ml volume) packed with glutathione and 10-carbon linker arm as ligand at concentration 120-320 μmole GSH/ml medium. The bead structure is highly crossed linked 4% agarose with average particle size of 90 μm. The tube or syringe was filled with binding buffer, and then the column was connected to syringe using the adaptor supplied or pump tubing to avoid introducing air into the column. The snap off end was removed at the column outlet. The column was equilibrated with 5 column volumes with binding buffer. The sample was applied using a syringe pumping it into the column using a flow rate of 0.2-1 ml/minute. Washing with 5-10 column volumes of binding buffer or until no materials appeared in the effluent at flow rate of 1-2 ml/minute. Elution was done with 5-10 column volumes of elution buffer at flow rate of 1-2 ml/minute. All fractions were determined for GST activity according to [7] and protein level was determined by [9]. The fractions with GST activities were pooled and subsequently used for kinetic and electrophoretic studies.

- **Estimation of protein content during purification:** The protein content was determined by folin-lowry colorimetric method [9].

## Kinetic properties of purified glutathione-S-transferase enzyme

**Effect of substrate concentration**: The kinetic values of GST for GSH and CDNB were determined by the procedure as follows: different concentration of GSH (0.25, 0.5, 1, 2, 3 and 4 mM) or CDNB (0.25, 0.3, 0.4, 0.5, 0.65, 0.75, 0.85, 1, 1.5 and 2 mM) was varied while the concentration of other substrate was fixed at 1 mM. 50 ul of purified enzyme was added to 250 ul distilled water, 1500 ul of working reagent (reduced GSH) and the reaction was initiated by adding 50 ul of starting reagent (CDNB) and degree of absorbance at 340 nm were recorded every minute for 4 minutes, enzyme concentration was calculated as previously mentioned. $K_m$ and $V_{max}$ were calculated from the line weaver-Burk plot of the results.

**Effect of temperature**: The enzyme activity was monitored at 25, 35, 45, 50, 60 and 70°C. 50 ul of purified enzyme was added to 250 ul distilled water, 1500 ul of working reagent (reduced GSH) and the reaction was initiated by adding 50 ul of starting reagent (CDNB) and degree of absorbance at 340 nm were recorded every minute for 4 minutes, enzyme concentration was calculated as previously mentioned.

**Effect of pH**: The pH dependence of the enzyme activity and stability was determined by measuring the activity of the enzyme in 0.1M sodium phosphate buffer (pH 4-10). 50 ul of purified enzyme was added to 250 ul distilled water, 1500 ul of working reagent (reduced GSH) and the reaction was initiated by adding 50 ul of starting reagent (CDNB) and degree of absorbance at 340 nm were recorded every minute for 4 minutes, enzyme concentration was calculated as previously mentioned.

**Figure 1:** Elution profile of rat liver GSTs enzyme from glutathione affinity column.
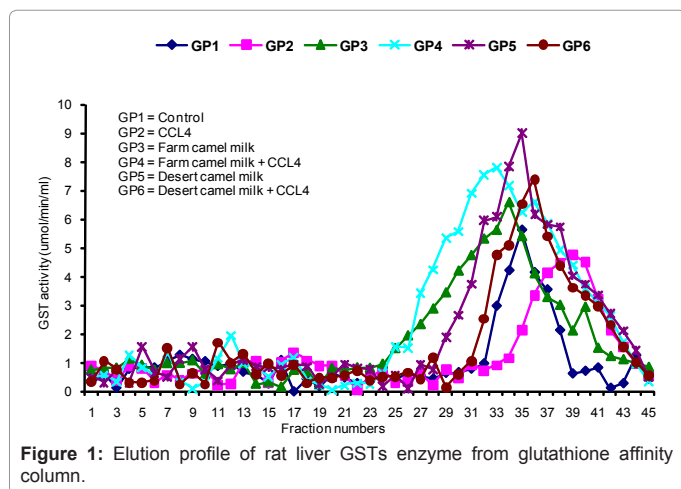
## Results

### Purification and elution profile of rat liver glutathione-S-transferase enzyme in different groups

Figure 1 showed the elution profile of GST enzyme from glutathione affinity chromatographic column. It was found that GST enzyme was purified and eluted in a single step as showed by one peak for each group.

**Control group:** The GST specific activity was increased from 0.305 to 12.995 mmol/min/ml/mg with a purification factor of 42.61. On the other hand, the total protein decreased from 572.29 to 8.61 mg as shown by the purification profile in table 1.

**CCL$_4$ group:** The GST specific activity was increased from 0.231 to 10.112 mmol/min/ml/mg with a purification factor of 43.76. On the other hand, the total protein decreased from 461.20 to 6.71 mg as showed by the purification profile in table 1.

**Farm camel's milk group:** The GST specific activity was increased from 0.531 to 15.645 mmol/min/ml/mg with purification factor of 29.46. On the other hand, the total protein decreased from 585.91 to

10.71 mg as showed by the purification profile in table 1.

**Farm camel's milk and CCL$_4$ group:** The GST specific activity was increased from 0.501 to 14.524 mmol/min/ml/mg with purification factor of 28.99. On the other hand, the total protein decreased from 577.14 to 10.11 mg as showed by the purification profile in table 1.

**Desert camel's milk group:** The GST specific activity was increased from 0.579 to 46.221 mmol/min/ml/mg with purification factor of 28.01. On the other hand, the total protein decreased from 592.36 to 11.24 mg as showed by the purification profile in table 1.

**Desert camel's milk and CCL$_4$ group:** The GST specific activity was increased from 0.562 to 15.451 mmol/min/ml/mg with purification factor of 27.49. On the other hand, the total protein decreased from 589.66 to 11.44 mg as showed by the purification profile in table 1.

### Kinetic studies of purified rat glutathione-S-transferase enzyme

**Effect of substrate concentration on enzyme activity:**

- **Effect of CDNB concentration on enzyme activity**: The relationship between CDNB and the activity of GST was shown in figure 2. When the initial velocity of GST is plotted versus substrate concentration, the curve is a right rectangular hyperbola. The activity of the enzyme increased progressively in a concentration dependent manner up to a rather high substrate concentration after which a plateau with saturation was attained. The initial velocity of GST reaction was measured as a function of substrate concentration and plotted as double reciprocal in accordance with the Line weaver-Burk analysis. The plot gives $K_m$ value and $V_{max}$ as shown in figure 6.

- **Effect of GSH concentration on enzyme activity**: The relationship between GSH and the activity of GST was shown in figure 3. When the initial velocity of GST is plotted versus substrate concentration, the curve is a right rectangular hyperbola. The activity of the enzyme increased progressively in a concentration dependent manner up to a rather high substrate concentration after which a plateau with saturation was attained. The initial velocity of GST reaction was measured as a function of substrate concentration and plotted as

| Purification step | Group | Total protein (mg) | Total activity (mmol/min) | Specific activity (mmol/min/ml) | Yield (%) | Purification fold |
|---|---|---|---|---|---|---|
| 1- Crude extract | Group (1) | 572.29 | 174.54 | 0.305 | 100 | 1 |
| 2- GSH affinity | | 8.61 | 111.88 | 12.995 | 64.11 | 42.61 |
| 1- Crude extract | Group (2) | 461.2 | 106.53 | 0.231 | 100 | 1 |
| 2- GSH affinity | | 6.71 | 67.87 | 10.112 | 63.67 | 43.76 |
| 1- Crude extract | Group (3) | 585.91 | 311.11 | 0.531 | 100 | 1 |
| 2- GSH affinity | | 10.71 | 167.55 | 15.645 | 53.83 | 29.46 |
| 1- Crude extract | Group (4) | 577.14 | 289.14 | 0.501 | 100 | 1 |
| 2- GSH affinity | | 10.11 | 146.83 | 14.524 | 50.78 | 28.99 |
| 1- Crude extract | Group (5) | 592.36 | 342.97 | 0.579 | 100 | 1 |
| 2- GSH affinity | | 11.24 | 182.32 | 16.221 | 53.15 | 28.01 |
| 1- Crude extract | Group (6) | 589.66 | 331.38 | 0.562 | 100 | 1 |
| 2- GSH affinity | | 11.44 | 176.75 | 15.451 | 53.33 | 27.49 |

i- Specific activity = Total activity / Total protein.
ii- Total activity = Specific activity x Total protein.
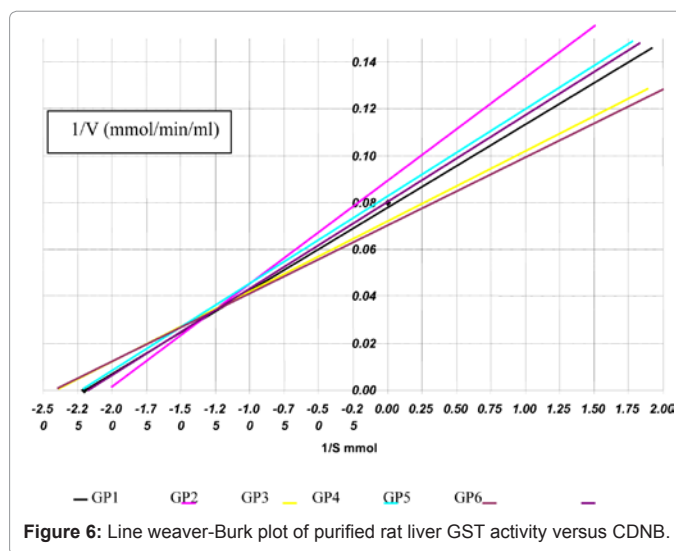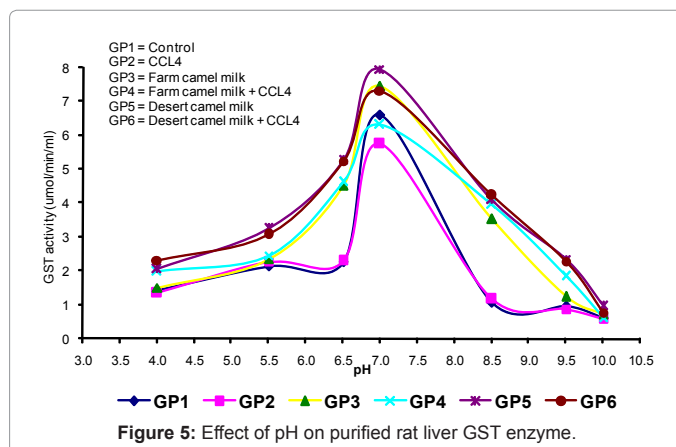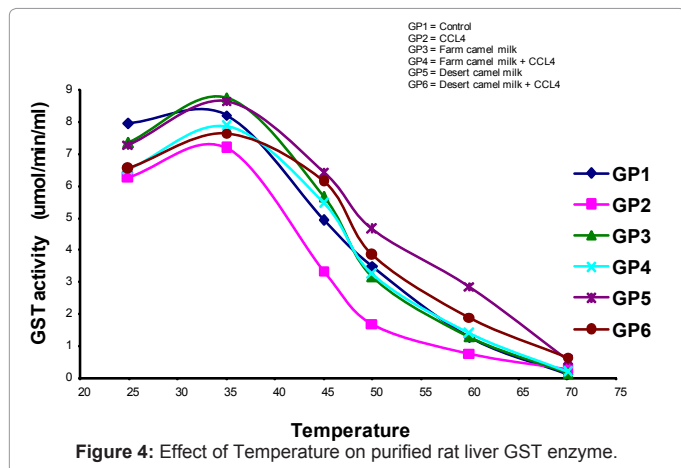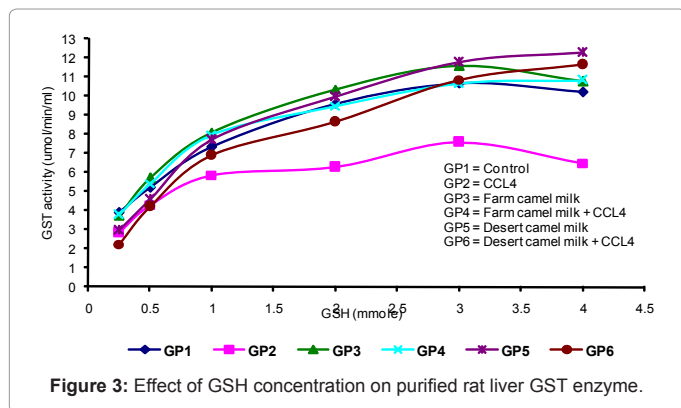iii- Purification fold = Specific activity of purified enzyme / Specific activity of crude enzyme.
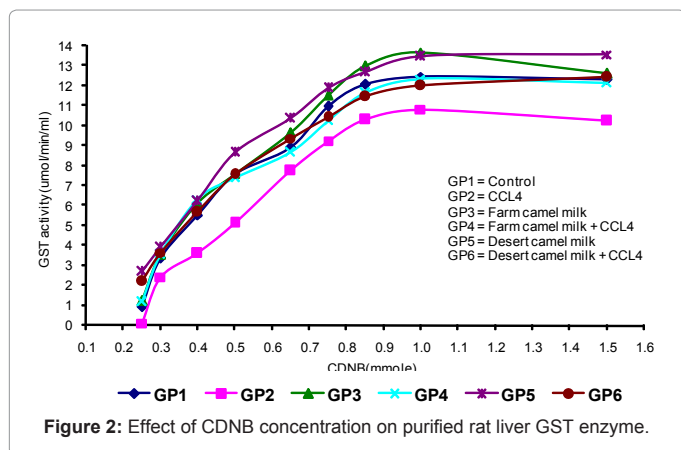iv- Yield (%) = (Total activity of purified enzyme / Total activity of crude enzyme) x 100.

**Table 1:** Purification profile of Glutathione-S-transferase enzyme in different groups.

double reciprocal in accordance with the Line waver-Burk analysis. The plot gives K$m$ value and V$max$ as shown in figure 7.

**Effect of temperature on enzyme activity**: Figure 4 showed that the maximum activity of purified rat GST occurred at 35°C. However, the enzyme activity declined sharply at higher temperature than 35°C.

**Effect of pH on enzyme activity**: Figure 5 showed that the velocity of purified rat GST enzyme increased gradually till pH 7.0 which seem to be the optimum pH for the enzyme activity at which a maximum activity was obtained. However, the enzyme activity was decreased at higher pH.



**Figure 2:** Effect of CDNB concentration on purified rat liver GST enzyme.



**Figure 3:** Effect of GSH concentration on purified rat liver GST enzyme.



**Figure 4:** Effect of Temperature on purified rat liver GST enzyme.



**Figure 5:** Effect of pH on purified rat liver GST enzyme.



**Figure 6:** Line weaver-Burk plot of purified rat liver GST activity versus CDNB.

## Discussion

The GST enzyme was purified from chronic $CCL_4$ intoxicated rat's liver. The crude extracts of rat's liver were loaded onto S-hexyl-GSH linked-4B affinity chromatographic column and on elution the bound protein was eluted as indicated by a single sharp peak which was similar in all groups as showed in figure 1. The pooled active fractions were assayed for GST activity using CDNB as substrate. The specific activity in normal rat's liver was found to be 12.995 μmol/min/ml. This activity was decreased when rats intoxicated with $CCL_4$ to 10.112 μmol/min/ml, however, the activity increased when the normal rats treated with farm camel's milk to 15.645 μmol/min/ml, administration of $CCL_4$ intoxicated rats with farm camel's milk the activity restored to 14.524 μmol/min/ml. Moreover, the activity was increased when the normal rats treated with desert camel's milk to 16.221 μmol/min/ml and administration of desert camel's milk to $CCL_4$ intoxicated rats the activity restored to 15.451 μmol/min/ml. Our results were in accordance with [5] who mentioned that the cytosolic extracts of rat's testis and brain were loaded onto S-hexyl GSH linked agarose-4B affinity column and on elution the bound protein was eluted as showed by a single sharp peak. The pooled active fractions were assayed for GST activity using CDNB, the specific activity was found to be 6.5 and 8.6 μmol/min/mg protein for testis and brain respectively. The yield of GST protein was 39% in testis and 32% in brain. Also, [10] purified

GST of lactuca sativa; GSH-conjugating activity toward CDNB in the crude extract was very low following chromatography on DEAE-sephacel of the crude extract a single peak of GST activity was eluted between 100 and 150 mM NaCl with an increase in specific activity of approximately 7 times. After passage through GSH sepharose affinity column the specific activity further increased to approximately 403 times with an overall recovery of around 9.6% of the initial activity. Moreover, the single step purification of pseudomonas GSTs with GSH affinity chromatography yielded a single protein and a 43 fold purification of GST enzyme. The purified protein showed GST activity toward GSH and CDNB as substrates the specific activity was 10.37 µmol/min/mg proteins [11].

In our study of rat's liver GST purification, the GSH agarose matrix of affinity chromatographic column retained about 50-60% of the GST activity from liver crude extract and about 40-50% was not fixed to the column matrix this may be attributed to that not all GST isozymes can bind to the affinity column matrix. This explanation came in agreement with [12] who purified GST from 100.000 xg supernatant of adult sativa cervi by affinity chromatography on GSH agarose column with a similar elution pattern for control, diethylcarbamazine (DEC) and Butylated hydroxyanisole (BHA) treated worms, a portion of GST activity did not bind to the GSH agarose affinity column in all samples. Furthermore, GSTs from Adalia bipuntata adults were purified by affinity chromatography on a sepharose column, the elution profile showed a single peak of enzyme and more than 77% of the total GST activity toward CDNB was bound to the column [13]. Also, [14] purified the GSTs from lamprey liver on affinity chromatography, the GSH sepharose matrix retained over 80% of the GST activity from liver cytosol when measured with CDNB, the existence of a 14-16% of GST activity which did not fixed to the affinity column matrix could be explained because certain GST isozymes for example as some alpha or theta isozymes were poorly linked to these column [15]. The purification of GSTs from the silk worm was summarized by [16], the GST specific activity (CDNB conjugation) was increased 6.33 fold after separation by GSH affinity chromatography from the crude homogenate. A large portion of material with CDNB conjugating activity passed through the GSH affinity column, the specific activity of the purified GST was 4.61 µmol/min/mg proteins. Furthermore, monopetrus albus GST was purified about 300 fold by GSH affinity chromatography with about 14% recovery of total activity using CDNB as substrate, the purified GST showed an enzymatic activity of 13.07 µmol/min/mg protein towards CDNB substrate [17]. It was reported that several factors had caused the low recovery rates of glutathione transferases such as excessive amount of the enzyme applied to each column, aging of the GSH agarose and inactivation of the enzyme during chromatography [18]. However, the glutathione S-transferase of carp's liver was purified from 100.000 g supernatant, about 85% of the total activity did not bind to DEAE sephacel but all of the unbound activity bind to S-hexyl glutathione sepharose 6B affinity matrix after washing the column was eluted with 10 mM hexyl-S-glutathione and the enzyme appeared as single sharp peak [19].

The difference in specific activity and protein percentage could depended on a great number of factors such as the biological species, the isolation and purification methods and the protein quantification method [15].

The purified GSTs of rat's liver showed maximal specific activity in desert camel's milk group followed by farm camel's milk while $CCL_4$ intoxication decreased the specific activity of GST. These results were agreed with administration of $CCL_4$ significantly decreased the activity of enzymatic antioxidant GST in liver tissue of rats [20]. The $V_{max}$ and $K_m$ values for CDNB and GSH were calculated from Line weaver-Burk analysis as summarized in Figure 6 and 7. $CCL_4$ decreased V*max* but increased $K_m$ of purified GST for both CDNB and GSH which indicated that $CCL_4$ decreased the affinity of enzyme to both substrates; $CCL_4$ or its free radicals may be an uncompetitive inhibitor of the enzyme due to conformational changes induced by pro-oxidant of $CCL_4$ or binding of $CCL_4$ or its free radicals to the enzyme substrate complex. This hypothesis is confirmed by [21] who reported that $H_2O_2$ leads to the formation of intra-or intersubunit disulphide bonds between particular cysteine residues within GST amino acid sequence leading to inactivation of the cytosolic enzyme. Also, the pro-oxidant activity of copper toxicity decreased $V_{max}$ of GST and increased $K_m$ for glutathione without any apparent changes for CDNB. The GSH (G site) and the xenobiotic substrate (H site) binding sites are constituted principally by hydrophobic amino acid residues. However, oxidative inactivation of some GST involves some cysteine residues which are in disulphide formation these cysteinyl residues seem to be located in the G site. The farm and desert camel's milk increased $V_{max}$ but decreased $K_m$ of purified GST for CDNB and GSH which potentiate the catalytic efficiency of the enzyme and increased the affinity of the enzyme to both substrates. Camel's milk contain high amount of selenium which act as antioxidant and perhaps increase the activity of GST enzyme, these explanation was in harmony with [22] who reported that repeated sodium selenite administration increased the activity of glutathione transferase enzyme in rats.

Our results for $K_m$ and $V_{max}$ of rat's liver GST were in accordance with [19] who reported that the $K_m$ values of carp GST for GSH as substrate was 0.5 mM which was very similar for all the isoenzymes and was in agreement with previous results on carp GSTs [23]. Moreover, these values were in general agreement with those reported in the literature for both vertebrate and invertebrate GSTs [24]. This may indicate that these enzymes bind GSH in a similar way. Also, [11] mentioned that the purified GST of pseudomonas sp. showed maximal activity toward CDNB with specific activity 10.6 µmol/min/mg. $K_m$ and $V_{max}$ values for CDNB calculated from Line weaver-Burk plot were 0.76 mM and 14.81 µmol/min/mg and those for GSH were 6.23 mM and 64.93 µmol/min/mg. Moreover, the lactuca sativa GST apparent $K_m$ value for GSH was calculated at 0.42 mM which was in general agreement with published $K_m$ values for GSH of other GSTs [25] in contrast the $K_m$ value of the



**Figure 7:** Line weaver-Burk plot of purified rat liver GST activity versus GSH concentration.
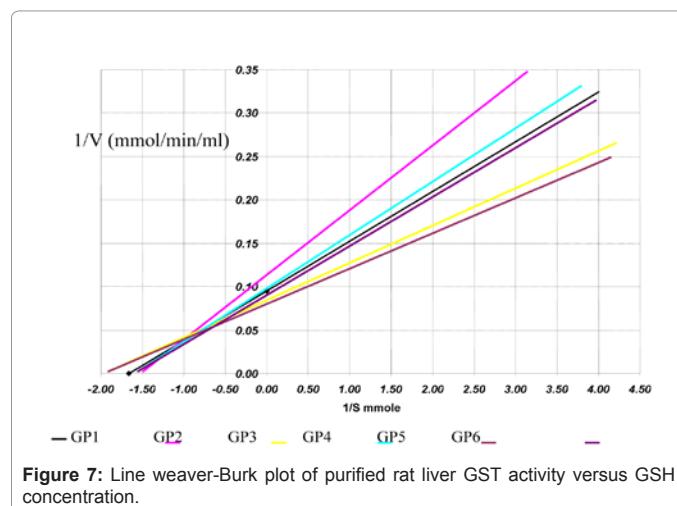
lactuca sativa GST for CDNB was 1.42 mM which was higher than $K_m$ value of rat liver GST [10].

Regarding to the effect of temperature on purified rat's liver GST enzymatic activity, enzymatic activity of purified rat's liver GST was highest at 35°C as showed in figure 4. These results were in harmony with [11] who mentioned that the enzymatic activity of pseudomonas GST was highest at 30°C and decreased above 40°C, the enzyme was stable below 35°C but was quickly inactivated above 45°C. But, the thermostability of lactuca sativa GST was investigated by incubation the enzyme at various temperature for 10 minutes, the midpoint of the temperature stability curve was approximately 53°C for the enzyme. The enzyme was stable to such incubation at temperature up to 45°C, above 50°C its activities declined rapidly as the temperature increased but the enzyme was not completely inactivated even at 80°C [10]. Moreover, the silkworm GST enzymatic activity was increased with increasing temperature up to 25°C then the activity started to decrease with increasing temperature [16].

Regarding to the effect of pH on purified rat's liver GST enzymatic activity, enzymatic activity of purified rat's liver GST was highest at pH 7.1 in phosphate buffer as shown in figure 5. These results were in agreement with [26] who recorded that the purified GSTs from aerobic turtle liver had an optimum pH at 7.2, the activity declined relatively slowly on the acidic side so that about 40% of the activity still remained at pH 6.0 whereas, the activity fell sharply at higher pH values with almost no activity remaining at pH 7.6 and above. Also, the optimum pH for silkworm GST with CDNB as a substrate was found to be 7.1 in phosphate buffer saline [16].

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