



Purification and characterization glutathione S-transferase enzyme from quail (*Coturnix, coturnix japonica*) heart and investigation the effect of some metal ions on enzyme activity

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Abstract. In this study glutathione S-transferase enzyme (EC: 2.5.1.18) from the heart of japonica quail was purified with 34.0 EU/mg specific activity, 10.44% purification yield and 78.29 purification folds and characterized. Purification processes are consist of three steps, firstly homogenate was prepared, and then ammonium sulfate precipitation was performed and finally glutathione-agarose gel affinity column chromatography was performed. To check the purity of GST enzyme used SDS-PAGE method. Then the M.W calculated at approximately 26.3 kDa by SDS-PAGE method. Enzymatic activity was determined spectrophotometrically according to Beutler's method at 340 nm. Also characterizations study carry out, and the results obtained are stability-pH = 9.0 in Tris/HCL buffer, optimum pH = 8.0 in Tris/HCl buffer, optimum temperature 60 °C, optimum ionic strength was 1.2 M in Tris/HCl buffer. And kinetic studies performed for GST enzyme purified from quail heart by used both glutathione and 1-chloro 2,4-dinitrobenzen as substrate. K_M and V_{max} values are determined as 1.642 mM and 0.502 EU/mL respectively for GSH substrate and 3.880 mM and 0.588 EU/mL respectively for CDNB substrate. In addition, the effect of some metal ions (Cu^{2+} , Cd^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} , Ag^+ , Co^{2+} , and Ti^{1+}) were investigated on the GST enzyme activity *in vitro*.

Keywords: Quail, Heart, Glutathione S-transferase, Purification, Characterizations, Metal Ions.

Glutasyon S-transferaz enziminin Japon bildircin (*Coturnix, coturnix japonica*) yürek dokusundan saflaştırılması, karakterizasyonu ve bazı metal iyonlarının enzim aktivitesi üzerine etkilerinin araştırılması

Özet. Bu çalışmada japon bildircin yürek dokusundan glutasyon S-transferaz enzimi (EC: 2.5.1.18) 34.0 EU/mg spesifik aktivite ile % 10.44 verimle ve 78.29 kat saflaştırılmıştır. Saflaştırma işlemi üç aşamada gerçekleştirilmiştir. Bu aşamalar homojenatın hazırlanması, amonyum sülfat çöktürmesi ve glutasyon-agaroz jel afinite kolon kromatografisinden oluşmaktadır. Bildircin yürek dokusu GST enziminin saflığını test etmek için SDS-PAGE yöntemi kullanılmıştır. Daha sonra SDS-PAGE yöntemi ile enzimin alt birimlerinin molekül kütlesi yaklaşık olarak 26.3 kDa olarak hesaplanmıştır. Enzimatik aktivite 340 nm'de Beutler yöntemine göre spektrofotometrik olarak belirlenmiştir. Yapılan karakterizasyon çalışmalarında enzime ait optimum pH Tris/HCl tamponu pH = 8.0, stabil pH Tris / HCL tamponu pH = 9.0, optimum sıcaklık 60 °C, optimum iyonik şiddet Tris/HCl tamponu 1.2 M olarak bulunmuştur. GST enzimi substrat olarak hem glutasyon hem de 1-kloro 2,4-dinitrobenzen kullanılmaktadır. Yapılan kinetik çalışmalarda GST enzimi için K_M ve V_{max} değerleri, GSH substratı için sırasıyla 3.880 mM ve 0.588 EU/mL, CDNB substrat için sırasıyla 1.642 mM ve 0.502 EU/mL

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bulunmuştur. Buna ek olarak bazı metal iyonlarının (Cu^{2+} , Cd^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} , Ag^+ , Co^{2+} ve Ti^{1+}) GST enzim aktivitesi üzerine *in vitro* etkileri araştırılmıştır.

Anahtar Kelimeler: Bildircin, Yürek, Glutatyon S-Transferaz, Karakterizasyon, Metal iyonları.

1. INTRODUCTION

Glutathione S-transferase (GST-EC: 2.5.1.18) isozymes consist of approximately 223 amino acids are ubiquitously distributed from the nature, can be detect in both prokaryotes and eukaryotes [1]. Being found in organism as diverse as microbes, insect, plants, fish, birds and mammals [2-3]. These cellular detoxification enzymes exist mostly from kidney and liver as well as intestine also present in other tissue such as heart, brain and erythrocyte. GST isoenzymes protect the cell against the harmful effects of toxic chemicals. GST isoenzymes are important antioxidant enzymes that protect cells from the toxic effects of reactive oxygen species (ROS) by detoxifying exogenous and endogenous substances [4]. Transferase enzymes catalyze different types of reactions. Some of these enzymes can catalyze the conjugation of the reduced glutathione (GSH) with compounds containing the electrophilic center. This reaction takes place with the formation of a thioether bond between the GSH and the xenobiotic sulfur atom [5]. A number of GST isoenzymes reveal some GSH-dependent catalytic activities, such as reduction of organic hydroperoxides⁶, and isomerization of different types unsaturated compounds [7].

GSTs can catalysis the conjugation of glutathione to different electrophiles and reverse conjugation of various electrophiles compounds with glutathione [8]. By lowering activation energy and increase speed of conjugation that participates in deprotonation of GSH to GS by a tyrosine residue in which as the base catalyst function. The first step from the mercapturic acid pathway is the glutathione conjugation due to elimination of xenobiotic compounds. Evolved GSH by GSTs and are abundant throughout most life forms. Because of GST response of detoxifying both endogenously and exogenously derived toxic compounds [9]. GST isoenzymes are divided into two distinct super-family members: the cytosolic family members and membrane bound microsomal based upon the similarity of amino acid sequence. In which five classes are cytosolic (designated α , μ , π , θ , and κ), so two are membrane-band. The cytosolic

family of GSTs are subject to genetic significantly polymorphisms in human populations [10].

Glutathione S-transferase GSTs have been purified and characterized from different sources such as rat brain cytosol [11], humane kidney [12], human placental tissues [13], and rainbow trout hepatocytes [14], liver of the freshwater fish *Monopterus albus* [15], rat liver [16], turkey liver [17], muscle tissue of Van Lake fish [18] and quail liver [19]. There have been used different techniques such as the affinity, ion exchange, hydrophobic, and gel filtration chromatography to purification.

Nowadays from the world our environmental was contaminated and polluted through the metal refuses, agriculture practice, industrials, commercials waste and human activity every day. In which any substances such as (drugs, metal ions, poisons, etc.) form *in vivo* and *in vitro* of the living organism cell can inhibition or activation of enzymes. Living organisms are exposed to heavy metals in nature. Nowadays, the toxic effects of heavy metals on living things have been intensively studied by researchers, and this has become a central research area in the toxicological field [20-22], So far, there were not found any encountered study on the purification and characterization glutathione S-transferase from the heart tissue of japanese quail and the effect of (Cu^{2+} , Cd^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} , Ag^+ , Co^{2+} , and Ti^+) metal ions on the quail heart GST activity.

The objective of this study was purification and characterization of GST enzyme from the quail heart and investigation of the any possible effects of metal ions on enzyme activity *in vitro*.

2. MATERIALS AND METHODS

2.1. Materials

Ammonium sulfate, sodium chloride, sodium hydroxide, potassium phosphate, EDTA ethylene diamine tetra acetic acid, Tris (Trihydroxy methyl amino methane), isopropanol, β -mercaptoethanol,

acrylamide, TEMED (N, N, N, N tetramethylethylenediamine), hydrochloric acid, phosphoric acid, glycerin, ethanol, methanol, acetic acid, GSH, glutathione-agarose gel (Sigma company), bromothymol blue, sodium acetate, potassium hydroxide, glycine amino acid and trichloroacetic acid (Merck company), SDS (sodium dodecyl sulfate), Commasie Brilliant Blue G-250, Commasie Brilliant Blue R-250 (Fischer Scientific), Ammonium persulfate (Chem Solute Bio).

2.2. Methods

2.2.1. Preparation of homogenate

The quail heart tissues were supplied fresh from the Bingol University application farm. It was frozen in deep freeze at -20°C . In the experiments, the frozen heart 4 g was cut into small pieces and suspended in 12 mL of homogenate buffer (50 mM Tris-HCl pH = 7.5) by homogenizer after that the suspension was centrifuged at 13,000 rpm for one hour and precipitate was discarded. These processes carry out at $+4^{\circ}\text{C}$.

2.2.2. Activity determination

Enzyme activity was determined according to Habig method [32].

2.2.3. Ammonium sulfate precipitation

Solid ammonium sulfate precipitation was performed at 0%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% to homogenate and the settling interval was determined. According to the method was performed by Green and Hughes [33].

2.2.4. Glutathione agarose affinity column chromatography

Weighted 1 gram of dry glutathione agarose gel and washed by distilled water for some times to remove impurities also dissolved gel in 200 mL distilled water or equilibration buffer and kept it overnight between 2°C and 4°C . The gel was swollen, the swollen gelatin air was removed by used water trap then put into the (1 x 10 cm) column and packed via closed system. The column was equilibrate with 10 mM KH_2PO_4 , 150 mM NaCl (pH = 7.4) and sample applied to the glutathione-agarose affinity column. Then the column was washed with 10 mM KH_2PO_4 , 0.1M

KCl, pH = 8.0, until absorbance difference 0.05 at 340nm by spectrophotometrically and then gradient elution was performed in 50 mM Tris-HCl and 1.25-10 mM GSH, pH = 9.5, finally the eluates were collected.

2.2.5. Protein determination

Quantitative protein was determined at 595 nm spectrophotometrically by used bovine serum albumin as a standard according to Bradford 1976 method [34].

2.2.6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

To control the purity of the GST enzyme, (SDS-PAGE) was performed at 3% to 15%. According to the Laemmli procedure. Also GST molar mass was determined according to the Laemmli method [35].

2.2.7. Determination optimum pH

To determine the optimal pH of the GST enzyme, two different buffers system were used; potassium phosphate with pHs 5.5 to 8.0 and Tris/HCl with pHs 7.5 to 9.0. With appropriate substrate solution, the GST enzyme activity was determined at 340 nm by spectrophotometrically.

2.2.8. Stable pH determination

To determine the GST enzyme pH-stable, two different buffers system were used; potassium phosphate with pHs 5.5 to 8.0 and Tris/HCl with pHs 7.5 to 9.0. The GST enzyme activity measured at 340 nm by spectrophotometrically. % activity were found as against time (days) at 24 hour intervals for 7 days under optimal conditions.

2.2.9. Optimum ionic strength determination

To determine the optimum ionic strength the GST enzyme, two different buffers system were used; KH_2PO_4 prepared with 0.1-1.0 M and Tris /HCl prepared with 0.1-1.4 M with the pH = 8.0 for all solutions. The GST enzyme activity was measured for each solution at 340 nm by spectrophotometrically.

2.2.10. Optimum temperature determination

To determine optimum temperature of GST enzyme was used water bath. The GST enzyme activity was measured increased temperatures at 10, 20, 30, 40, 50, 60, 70, 75 and 80°C , respectively.

2.2.11. Kinetic study

To determine the K_M and V_{max} values for GSH and CDNB substrates of GST enzyme, the enzyme activity was measured. In this process used at 5 different concentrations of CDNB and constant GSH concentration. Lineweaver-Burk graph plotted with the obtain data. The values of K_M and V_{max} for CDNB substrate were determined by Lineweaver-Burk graph. In the same way, activity measurement with 5 different concentrations of GSH and constant CDNB concentration. Activity measurements was performed under optimal condition [36].

2.2.12. In vitro effects of metal ions

The following concentrations of metal ions selected for this study; Ag^+ (0.05 - 1.0 mM), Cu^{2+} (0.1 - 1.0 mM), Cd^{2+} (1.0 - 7.5 mM), Fe^{2+} (0.05 - 1.5 mM), Fe^{3+} (0.001 - 0.007 mM), Co^{2+} (0.5 - 2.5 mM), Zn^{2+} (0.5 - 5.0 mM), Ti^+ (1 - 7 mM) and Pb^{2+} (0.25 - 1.5 mM) were added to the reaction medium, and GST enzyme activity was measured. The absence of metal ions was used as a control (100% activity). Activity (%) - metal ion concentration graph plotted, to determine the IC_{50} value which is the inhibitor concentration to reduce enzyme activity by half. To determine K_i values, 5 different (GSH) substrate concentrations (0.2, 0.5, 1.0, 2.0, 3.0 mM) and 3 different inhibitor (metal ions) solutions Cu^{2+} , Ag^+ and Cd^{2+} were added to the reaction cuvette. Lineweaver-Burk graphs ($1/V$ vs $1/[S]$) were drawn and K_i constant were calculated.

3. RESULTS

The GST enzyme was purified from the quail heart with the 34.0 EU/mg specific activity, 10.44% purification yield and 78.29 purification folds. The purity of the quail heart GST enzyme was checked by SDS-PAGE (Figure 1). The molecular weight of GST enzyme was determined as approximately 26.3 kDa by SDS-PAGE method (Figure 2).

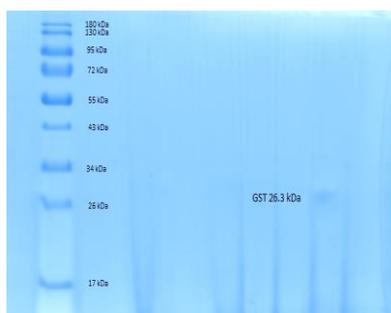


Figure 1. SDS-PAGE photograph: show a single band of GST enzyme.

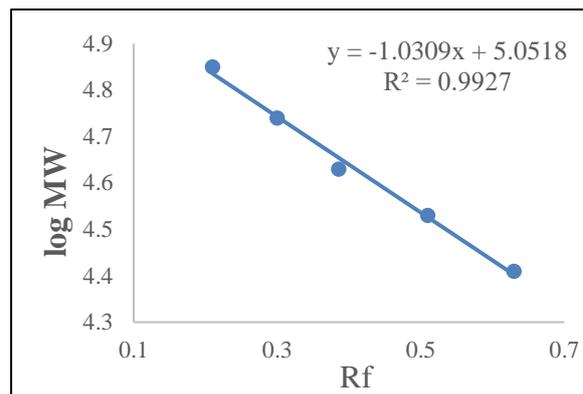


Figure 2. Rf-log M.W calibration curve to determine M.W of GST enzyme.

The pH stability for GST enzyme was determined as 9.0 in Tris-HCl buffer (Figure 3). The optimum pH for GST enzyme was determined as pH = 8.0 in Tris-HCl buffer (Figure 4). Optimum temperature for GST enzyme was determined by measuring the activity for GST enzyme in different temperature started from 0 °C to 80 °C. The obtained result showed the optimum temperature 60 °C (Figure 5). The effect of ionic strength on GST enzyme from the quail heart tissue was determined by the measured GST enzyme activity in 0.1 M to 1.0 M KH_2PO_4 and 0.1 M to 1.4 M Tris /HCl with pH = 8.0 for all solutions. The obtained result showed, the optimum ionic strength 1.2 M Tris/ HCl buffer (Figure 6).

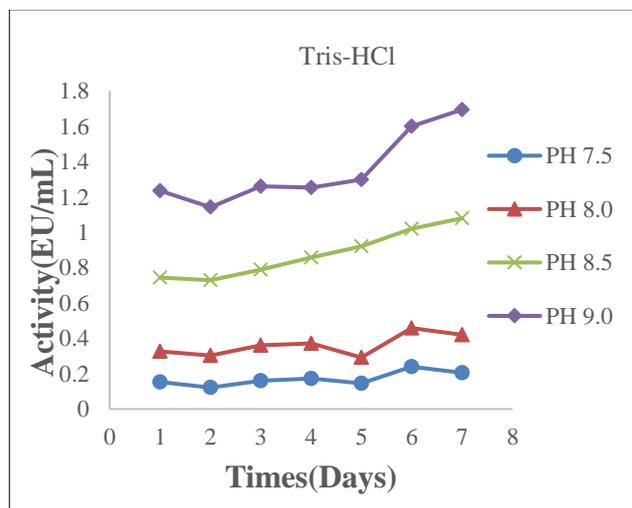


Figure 3. Graphic of stable pH studied with Tris-HCl

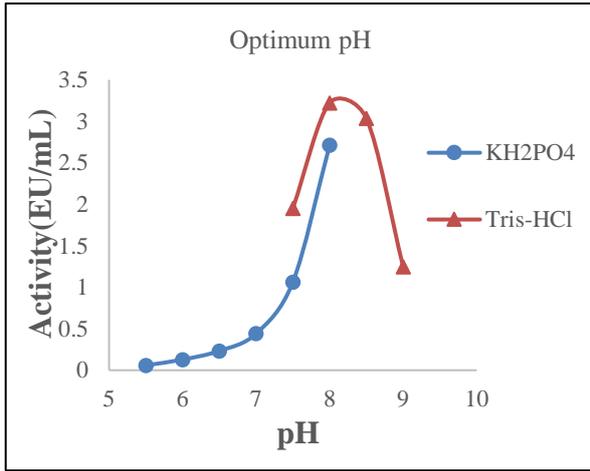


Figure 4. The graphic of optimum pH results.

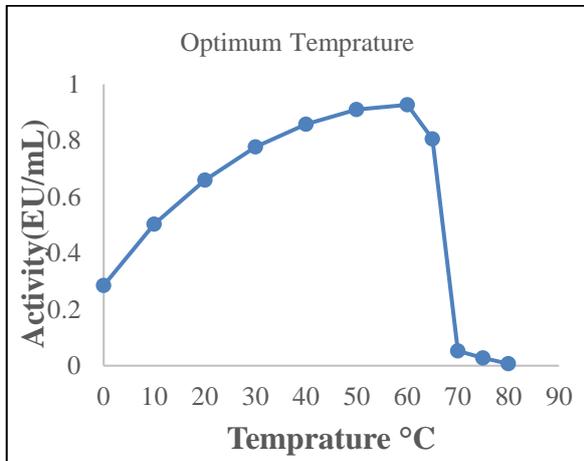


Figure 5. Result of optimum temperature.

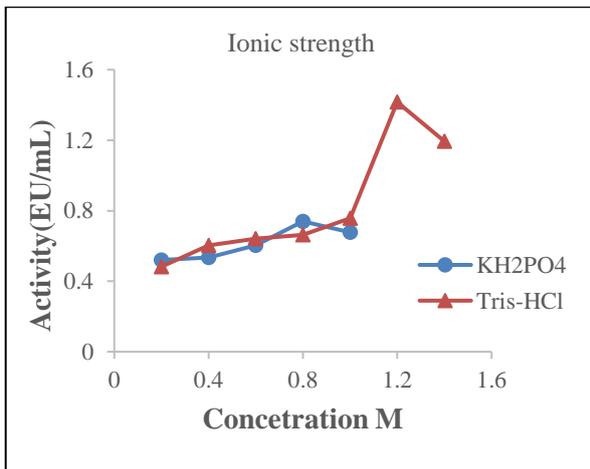


Figure 6. The results of optimum ionic strength (KH₂PO₄ and Tris-HCl buffer).

In addition enzyme kinetic study was performed to determine K_M and V_{max} for glutathione S-transferase purified from the quail heart tissues, and by using both GSH and CDNB as substrate. The results obtained are 1.642 mM and 0.502 EU/mL respectively for GSH substrate (Figure 7),

and 3.880 mM and 0.588 EU/mL respectively for CDNB substrate (Figure 8).

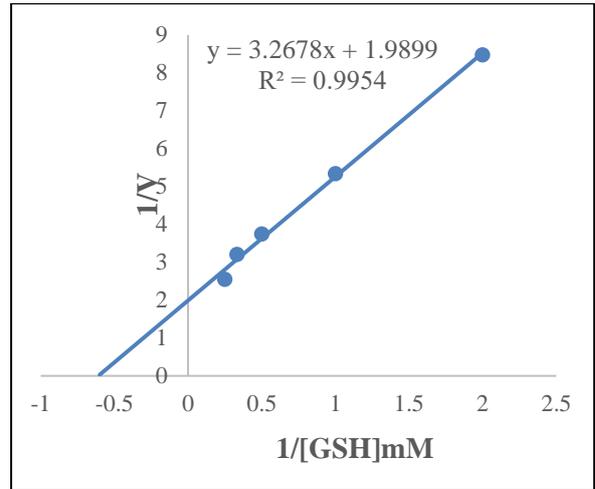


Figure 7. Lineweaver-Burk graphic with five different [GSH].

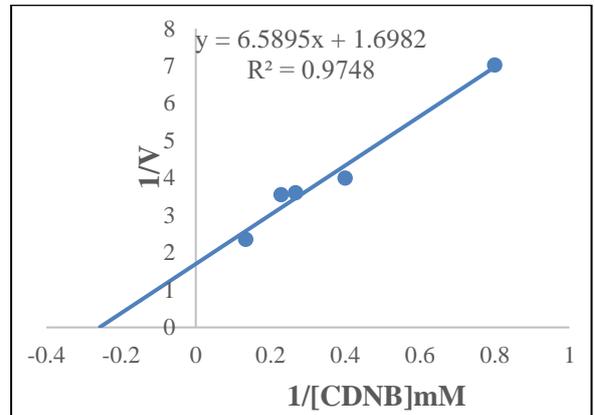


Figure 8. Lineweaver-Burk graphic with five different [CDNB].

The effect of metal ions on the GST enzyme activity were determined. Results show that Co^{2+} , Fe^{2+} , Fe^{3+} , Ti^+ , Zn^{2+} metal ions increased GST enzyme activity. Those metal ions indicated activator for GST enzyme, (an example Co^{2+} ion, Figure 9), But Cd^{2+} , Cu^{2+} , Ag^+ have negative effect on the GST enzyme cause the decreased enzyme activity, that indicated those are inhibitors for GST enzyme from quail heart (Figure 10, Figure 11).

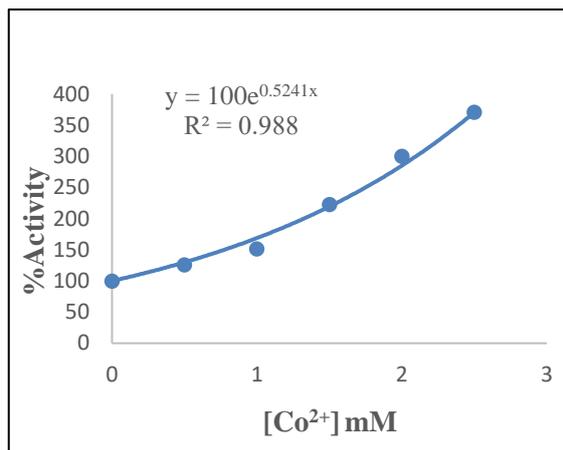


Figure 9. Effect of Co²⁺ on the GST enzyme activity.

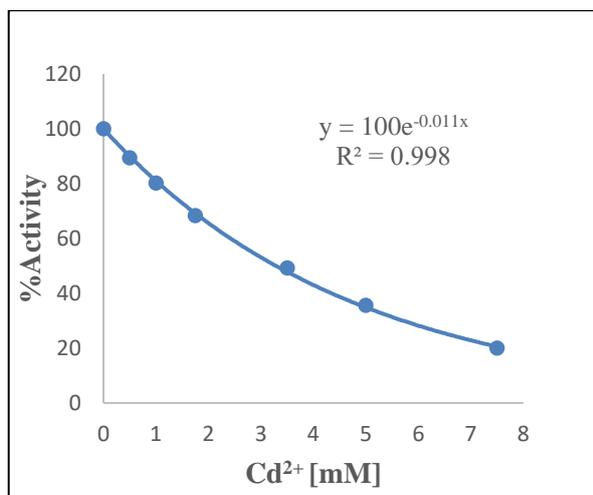


Figure 10. Effect of Cd²⁺ on the GST enzyme activity.

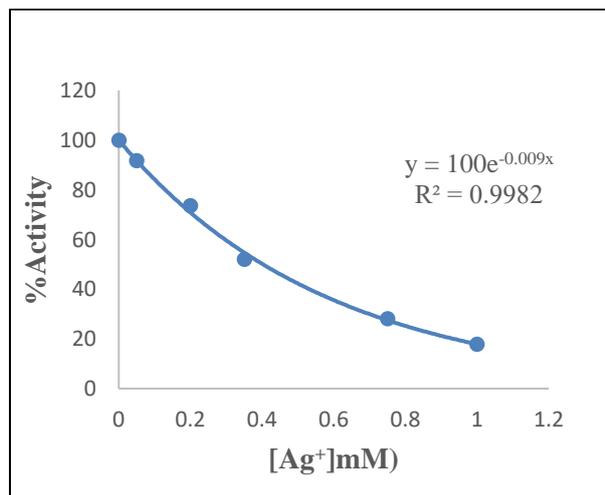


Figure 11. Effect of Ag⁺ on the GST enzyme activity.

4. DISCUSSION

In this study, glutathione S-transferase enzyme for the first time was purified from the Japanese quails heart tissue based on the protein purification process with three steps. After purification, characterization studies were carried out.

Glutathione agarose affinity column chromatography method was used to purify the GST enzyme. It is available and powerful method because it is an easy process to apply, low cost, less time, single step, potable for bulk amounts of enzymes purified and gives very good purification yields.

GST enzyme purified with specific activity 34.0 EU/mg, purification yield 10.439% and purification fold 78.296 folds in which compared with specific activity of 23.7 EU/mg, 11% yield, 1107-fold purification from the human placental tissues¹³, specific activity 1.250 x 10⁻⁶ unit/mg protein, 56.43% yield and 419.88 fold from the rat liver¹⁶, specific activity of 164.31 U/mg, a yield of 45%, with 252.7-folds from the turkey liver¹⁷, specific activity 29,304 EU / mg protein, purification fold 316,11 folds and purification yield 37.36% from the liver of the Van Lake fish²³, and specific activity of 35,583 EU/mg protein, purification fold 301.5-folds, a purification yield 19.07% from the muscle tissue of Van Lake fish¹⁸, in literature.

To check the enzyme purity, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method was used [35]. Then SDS-PAGE were stained and destained and single band was appeared on the gel, it is an evidence to purified enzymes. The same method were used in rat brain cytosol [11], human placenta [13], human hepatoma [25], rainbow trout hepatocytes [14], filarial worms *Setaria cervi* [26], rat liver [16], turkey liver [17], Van Lake fish liver [23], and muscle tissue of Van Lake fish [18]. SDS-PAGE method is suitable and powerful method to check purity of enzymes with good results by less time and easy to work. So calculate the molar mass of GST enzyme by took the R_f-value for standard protein bands and GST enzyme single band, the result value was drawn plot between R_f-value and logarithm molar mass of standard proteins. The molar mass was determined as approximately 26.3kDa for GST enzyme from the heart tissue of Japanese quail, in which compared with heterodimers of MW 26.5 kDa and MW 24.5 kDa subunit in human kidney [12], 25 kDa from the human placental tissues [13], 27 kDa in maize (*Zea mays* Pioneer Hybrid 3906) [27], 26 kDa from the sorghum cereal [28], 26.7 kDa from catfish intestinal mucosa [29], 26 kDa and 24 kDa from the turkey liver [17] and two subunits as 28 kDa and 33.8 kDa from liver of the Van Lake fish [23] in literature. This comparison illustrates that GST

isoenzymes have the different molar mass and ubiquitous distributed. In which GST molar mass from quail heart near to the GST in human kidney, turkey liver, sorghum cereal than human placental, liver of the Van Lake fish, maize (zea mays pioneer hybrid 3906), and catfish intestinal mucosa.

After purification of GST enzyme from quail heart tissues, characterization study was carry out to determine the optimum pH, optimum ionic strength, optimum temperature, pH-stability. Firstly, pH-stability was determined as pH = 9.0 in the Tris/HCl buffer. The result was compared with stable-pH = 7.5 in Down syndrome (DS) and normal children erythrocytes [30], stability-pH = 8.5 in turkey liver [17] and stable-pH = 5.5 liver of the Van Lake fish [23], result was closest to the stable pH in turkey liver in literature, pH-stability is most important point during the study on all enzymes because of keeping enzymes in stable pH help to have a best results and continues study for a longest interval on target enzymes, since enzymes are not denatured, loss activity and biological functions to a long time. By continuing the study optimum pH was determined as 8.0 in Tris-HCl buffer. Compare to optimum pH = 7.5 – 8.0 from the maize (zea mays pioneer hybrid 3906) [27], optimum pH = 7 from the Down syndrome (DS) and normal children erythrocytes [30], optimum pH = 7.3 from the turkey liver¹⁷ and optimum pH = 7.8 from the liver of the Van Lake fish [23] in the literature in which result was similar to the optimum pH of GST from the liver of the Van Lake fish and maize (zea mays pioneer hybrid 3906). In the living organism cell each enzyme works in specific pH called optimum pH in which it has maximum activity. Since pH is the important factor that have influence on enzyme activity at below and above optimum pH all enzymes loss activity and denatured especially in high acidic and basic medium.

Also temperature is another factor that have effect on enzyme activities, like all reaction enzyme activities increase with increase temperature but for enzyme increasing temperature is limited because at very high temperature all enzymes are denatured this is the lose biological function and lose enzymes activity. In this study optimum temperature was determined as 60 °C. In which above that degree GST loses activity until the 80 °C the activity becomes zero. Compared with optimum temperature in range 30 °C– 55 °C from the liver of the freshwater fish *Monopterus albus* [30], optimum temperature between 25-35°C from

the rat livers [31], optimum temperature 50 °C in turkey liver [17] and optimum temperature 30 °C from the liver of the Van Lake fish [23] in the literature in which result nearest to the optimum temperature of GST from the turkey liver and liver of the freshwater fish *Monopterus albus*. In continuous study, the effect of ionic-strength on glutathione S-transferase was studied as 1.2 M of Tris-HCl buffer pH = 8.0. The optimum ionic strength for GST 600 mM turkey liver [17], 100 mM in liver Van Lake fish [23], our results were found to be different compared to the literature, the quail heart GST enzyme has maximum activity at that concentration.

In addition, kinetic study was performed to determine K_M and V_{max} for glutathione S-transferase enzyme from the quail heart tissues. The results obtained are 1.642 mM and 0.502 EU/mL respectively for GSH substrate and 3.880 mM and 0.588 EU/mL respectively for CDNB substrate. The results is compare with K_M 0.085 mM for GSH and 2.0 mM for CDNB from the human placental tissues [12], K_M and V_{max} for CDNB are 0.28 mM and 15.68 EU/ml, respectively from the liver of the freshwater fish *Monopterus albus* [15], K_M values 0.786 mM for GSH and 0.205 mM for CDNB from human erythrocyte [30], K_M and V_{max} values 0.154 mM, 1.803 EU/mL for GSH and 0.380 mM, 2.125 EU/mL for CDNB substrate, respectively for GST enzyme in turkey liver [17], K_M and V_{max} determined as 0.060 mM, 0.562 EU / mL for GSH and 0.891 mM, 1.245 EU / mL for CDNB, respectively from the liver of the Van Lake fish [23], K_M and V_{max} determined as 0.53mM, 1.88 EU/mL for GSH and 1.59 mM, 5.58 EU/mL for CDND, respectively from the muscle tissue of Van Lake fish [18], in the literature. Results illustrated that the quail heart GST enzyme has low affinity for CDNB substrate than GSH substrate because the K_M value for CDNB substrate greater than K_M value for GSH substrate. The result was similar to the GST enzyme in liver and muscle tissue of the Van Lake fish, turkey liver and human placental tissues, but different from human erythrocyte. Also the results illustrated that the V_{max} for both GSH and CDNB are closest value but V_{max} for CDNB substrate was greater than V_{max} for GSH substrate it mean the CDNB substrate concentration has the more influence on the rate of reaction that catalyzed by GST enzyme than GSH substrate concentration, in which results was similar to the GST from turkey liver, muscle tissue of Van Lake fish and liver of the Van Lake fish.

Recent research has been found that various metal ions and organic compounds cause toxicity on enzyme activities purified from different sources [37-41]. The effect of metal ions on the GST enzyme were determined. (Co^{2+} , Fe^{2+} , Fe^{3+} , Ti^{+} , Zn^{2+}) increase activity that indicated those metal ions are activator But (Cd^{2+} , Cu^{2+} , Ag^{+}) decreased enzyme activity that indicated those are inhibitors. Further studied determined the types of inhibition for GST enzyme from quail heart, results obtained Cd^{2+} and Cu^{2+} ions were non-competitive inhibitors, because K_M is constant but V_{\max} changed (Figure 12) as an example for Cd^{2+} , also for Ag^{+} ion was competitive inhibitor, since K_M is constant and V_{\max} was changed (Figure 13) also K_i constant and IC_{50} values were determined for inhibitor metal ions that are the most suitable parameters for seeing inhibitor effects. Which in vitro studies revealed that the enzyme activity was inhibited by Cd^{2+} , Cu^{2+} , and Ag^{+} . IC_{50} values and K_i constants were 0.57 ± 0.236 mM for Cd^{2+} , 0.0028 mM, 0.63 ± 0.224 mM for Cu^{2+} and 0.382 mM, 0.27 ± 0.098 mM for Ag^{+} , respectively (Table 2). In recent studies, the effects of various heavy metals on different enzyme activities have been investigated. In a recent study, have been investigated the inhibitor effects of Ag^{+} , Cd^{2+} , Fe^{3+} , Cu^{2+} and Zn^{2+} ions on turkey liver mitochondrial thioredoxin reductase enzyme [42]. it was reported in different study that Fe^{2+} , Pb^{2+} , Cd^{2+} , Ag^{+} and Zn^{2+} ions inhibitory effect on rat erythrocytes G6PD enzyme activities [43].

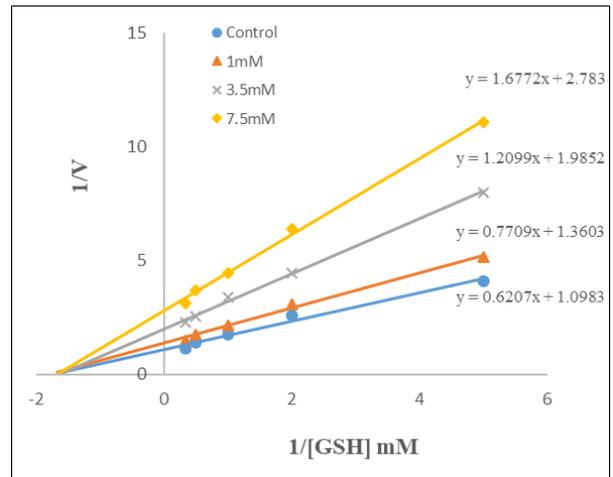


Figure 12. Lineweaver-Burk graph of five different GSH concentrations for the determination of K_i in three different concentration of Cd^{2+} .

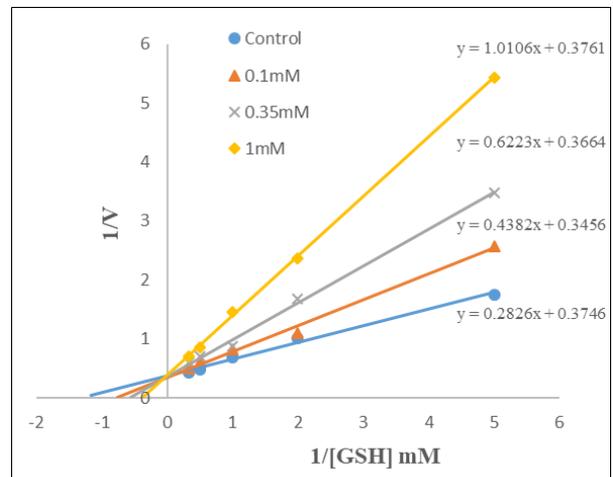


Figure 13. Lineweaver-Burk graph of five different GSH concentrations for the determination of K_i in three different concentration of Ag^{+} .

Table 1. Quail's heart tissue Glutathione S-transferase purification results table.

Samples	Volume mL	Activity EU/mL	Total activity EU	Protein content mg/mL	Total protein mg	Specific activity EU/mg	Purification Yield%	Purification fold
Homogenate	10	0.632	6.319	1.455	14.55	0.434	100	1
Ammonium sulfate precipitation	2	1.753	3.506	1.189	2.378	1.474	55.494	3.395
Pure enzyme	2	0.329	0.659	0.0097	0.0194	34.0	10.439	78.296

Table 2. K_i and IC_{50} values obtained from regression analysis graphs for quail heart GST in the presence of different metal ions concentration

Heavy metal	IC_{50} (mM)	K_i (mM)	Inhibition type
Cd^{2+}	3.127	0.57 ± 0.236	Non-competitive
Cu^{2+}	0.0028	0.63 ± 0.224	Non-competitive
Ag^{+}	0.382	0.27 ± 0.098	Competitive

5. CONCLUSION

In this study, glutathione S-transferase enzyme for the first time was purified from the Japanese quails heart tissue based on the protein purification process with three steps. After purification, characterization studies were carried out. In addition the effect of metal ions on the GST enzyme activity were determined. Our study showed that Co^{2+} , Fe^{2+} , Fe^{3+} , Ti^{+} , Zn^{2+} metal ions increased GST enzyme activity, while Cd^{2+} , Cu^{2+} and Ag^{+} ions had an inhibitory effect on enzyme activity.

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