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Characterization of Glutathione Transferase in Some Organs of Nile Tilapia (*Oreochromis niloticus*)

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Abstract: Distribution of the detoxifing glutathione transferase (GST) isoenzymes in mammalian tissues is not uniform and shows a marked diversity. The objectives of this study were to evaluate the difference in the substrate selectivity toward GST between four purified *Oreochromis niloticus* tissues, liver, kidney, ovary and testis; and to characterize the purified GST from *O. niloticus* testis illustrating its role in gonad functions and reproduction of tilapia. A simple one step reproducible procedure for the purification of GST from the four *O. niloticus* organs using GSH-Sepharose affinity column was carried out. The enzymes proved to be homogenous as judged by SDS-PAGE. The molecular weights were calculated to be between 25.1 KDa and 27.5 KDa. GST specific activities toward nine different electrophillic substrates were determined. The highest GST activity towards 1-chloro-2, 4-dinitrobenzene [CDNB] and 1, 2-epoxy-3-[4-nitrophenoxy]–propane [EPNP] was observed in testis and liver. GST of ovary and kidney showed high activity towards styrene oxide [SO], however, testis and liver have no activity toward SO. GST of *O. niloticus* testis exhibited maximum pH at pH 8.0. The Michaelis-Menten constants of the purified testis for GSH and CDNB were 0.45mM and 0.5 mM, respectively. Hematin was the most potent inhibitor of testis GST activity (IC_{50} value equal 7.94 μM).

Key words: *Oreochromis niloticus* · Gonads · Glutathione Transferase · Enzyme Kinetic · Substrate Selectivity · Enzyme Inhibitors

INTRODUCTION

Nile tilapia [*Oreochromis niloticus*] is an extensively cultured fish species in aquaculture. Because the growth rate of male Nile tilapia is significantly higher than that of females during grow-out period, it is necessary to investigate the molecular mechanism of sex determination and maintenance [1].

The release of FAO statistics in the State of World Fisheries and Aquaculture 2012, confirmed tilapias role as second only to the carps in global production. Egypt, which is the world's second largest producer of tilapia after China, reported the greatest increase in tilapia production, with a reported production of 557,049 mt in 2010 and estimates approaching 620,000 mt for 2012 [2].

The production of fish is being adversely affected due to non-sustainable activities of man that exert both, direct and indirect effects upon fish fauna. The former is expressed in the form of over fishing, while the latter through altering the physicochemical status of the aquatic environment by discharging treated, untreated industrial and domestic effluents. The polluted water destroys the suitable conditions needed for reproduction and also disrupts the metabolism of fishes leading to the large-scale mortality for a number of times [3]. The deformities change of the sperm's flagellum of *O. niloticus* of Lake Manzala may lead to decreasing the capacity of sperm motility and reducing its fertilization capacity and consequently lead to failure of reproduction. The changes in sperms structure as a result of environmental hazards giving an alarm into how the toxic heavy metals could alter the natural biology of feral fish and their ability to produce new offspring [4].

Glutathione S-transferases [GSTs; EC 2.5.1.18] comprise a large family of dimeric enzymes that catalyze the nucleophilic addition of the tripeptide glutathione [GSH] to a wide spectrum of compounds that have electrophilic functional groups. These isozymes can be found in most aerobic micro-organisms, plants and animals [5]. GSTs function primarily as phase II

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detoxification enzymes, protecting cells against both endogenous and xenobiotic alkylating agents [6]. This group of enzymes can catalyze reactions towards a large number of diverse substrates, showing the remarkable tolerance of these isozymes for both the types of electrophilic functional group and the structure of the molecules to which they are catalyzed [7]. GSTs may also function as intracellular carrier proteins. The binding of bilirubin [8], haem derivatives and thyroid hormones [9] to GSTs has been well documented. It has been proposed that GSTs may have an additional protective role by binding and immobilizing certain reactive electrophilic molecules and subsequently inactivating these compounds [9]. Besides their detoxification roles, these dimeric proteins are also involved in the reduction of organic hydroperoxides [10] and isomerization of prostaglandins [11].

Distribution of GST isoenzymes in different tissues is not uniform and mammalian organs show a marked diversity in their GST content. The testis has high levels of GSTs. Different GST isoenzymes have been identified in the male gonad. Specifically, GST alpha and GST pi have been identified in testicular somatic cells and particularly in Leydig and Sertoli cells. GST mu was shown to be expressed in somatic and germ cells [12].

The main objectives of the present study were: 1. evaluation of the difference in the substrate selectivity between four affinity purified *O. niloticus* tissues [Liver, kidney, ovary and testis]; 2. Characterization of the purified GST enzyme from *O. niloticus* testis illustrating the role of this enzyme in gonad functions and facilitates studies on the reproduction of tilapia which is important for aquaculture programs.

MATERIALS AND METHODS

Chemicals: Bovine serum albumin, 2, 4-dithiotheritol [DTT], reduced glutathione [GSH] and 1-chloro-2, 4-dinitrobenzene [CDNB] were purchased from Merck Company, epoxy activated Sepharose 6B and molecular weight standard proteins from Pharmacia Company. 1, 2-epoxy-3-[4-nitrophenoxy]–propane [EPNP], ethacrynic acid [EA], 4-nitrophenethyl bromide [4-NPB], styrene-7, 8-oxide [SO], *p* - nitrophenyl acetate [*p*-NPA], bromosufophthalein [BSP], 1,2-dichloro-4-nitrobenzene [DCNB] and cumene hydroperoxide (CuOOH) were purchased from Sigma Company. All other chemicals were of the highest purity commercially available.

Biological Material: A total of 50 mature Nile Tilapia fish, *O. niloticus* of both sexes (25 males and 25 females) were collected during March to June, 2014 from different local fish markets in Cairo, Egypt, ranging in total length and total weight from 20 to 25 cm and 108 - 249 g, respectively. The date of capture, total length to nearest cm and total weight to nearest g were recorded. The fish were dissected to determine sex and maturity stages in agreement with gonadosomatic index [GSI, percentage ratio of the gonad weight and body weight used to determine fecundity among fish].

Preparation of Crude Homogenates: Known weights of the *O. niloticus* liver, kidney, ovary and testis were homogenized using a glass homogenizer in 50% [w/v] 25 mM Tris-HCl buffer, pH 8.0 containing 1 mM EDTA and 1 mM DTT. The homogenates were then centrifuged at 10,000 *xg* for 15 min, the supernatants [cytosol] filtered through a plug of glass wool; the filtrates were termed crude homogenate and saved at -4°C for further analyses.

Preparation of GST Enzyme Using Glutathione-Sepharose Affinity Chromatography: Reduced glutathione was coupled to Epoxy-activated Sepharose 6B according to Simons and Vander Jagt [13]. Crude homogenates of the four *O. niloticus* organs were mixed individually with 15 cm of GSH-Sepharose matrix previously equilibrated with 25 mM Tris-HCl buffer, pH 8.0 containing 1 mM EDTA and 1 mM DTT and allowed to proceed for 2hrs at 4°C with shaking. The matrix with GST was collected by filtration through centered glass funnel and extensively washed with the same buffer, until no absorbance at 280 nm was observed. The matrix with bound proteins was packed to a column [15 × 1 cm i.d.] and the bound GST was eluted with 50 mM Tris-HCl buffer, pH 9.6 containing 10 mM GSH at a flow rate of 15 mL / h. Two-milliliter column fractions were collected and monitored for protein at 280 nm and for GST activity at pH 6.5 [14].

Protein Determination: Protein concentration was measured by the Bio-Rad [15] assay, using bovine serum albumin as standard. Measurements were done on Shimadzu UV Spectrophotometer at 595 nm.

Biochemical Studies

Determination of Specific Activity of GST: The specific activities of the GST were measured with CDNB, NPB, EPNP, *p*-NPA, EA and DCNB according to the method described by Habig *et al.* [16]. The activity was measured with CuOOH by coupling the glutathione reductase-dependent NADPH oxidation [17]. The activity was also measured with SO [18]. All assays were carried out at

30°C. All initial rates were corrected for the background nonenzymatic reaction. One unit of activity is defined as the amount of enzyme which catalyze the formation of 1 µmole of thioether per min at 30°C.

Electrophoresis: Purity of the purified enzyme fractions was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis [SDS-PAGE], carried out on 12% slab gels as described by Laemmli [19]. 7 % Native PAGE was performed according to the method of Davis [20] and stained for GST activity using the method of Ricci *et al.* [21]. Blue insoluble formazan appeared on the gel surface in about 3–5 min, except in the GST.

RESULTS

Gonadosomatic Index [GSI] of *O. niloticus*: *O. niloticus* reaches the maturity in the first year of life and the general manner of gonad development in *O. niloticus* conforms to that of other teleosts. The tilapia has a pair of bilateral gonads suspended from the dorsal portion of the body cavity. The gonads of both male and female are found ventral to the kidney. The values of GSI index for both sexes of *O. niloticus* began to increase from March, expressing the beginning of the breeding season. These values increased progressively to attain a majority of 1.3 for males and 2.9 for females in May as shown in Table 1.

Extraction of *O. niloticus* GST: Crude homogenates of the four *O. niloticus* organs, ovary, testis, liver and kidney collected in May were prepared as described in the materials and methods section. GST activity using CDNB as substrate was determined and represented as GST units/g of homogenized tissue [Table 2]. The highest GST activity was observed with the liver crude homogenate [22 µmole/min /g tissue] with specific activity equal 1.83 µmole/min/mg protein. High GST activity was also recorded in a decreasing order for testis, kidney and ovary [7.1, 3.58 & 1.98 µmole/min/g tissue, respectively]. However, the protein content of *O. niloticus* ovary represented the highest value compared to the other organs. Specific activity of the four organs represented the following decreasing order, liver >testis >kidney > ovary [Table 2].

Chromatographic Behavior of *O. niloticus* GSTs on GSH-Sepharose Affinity Column: Total GSTs extracted from *O. niloticus* liver, kidney, ovary and testis were subjected to a single purification step by affinity chromatography over a column of GSH linked to epoxy-activated Sepharose, yielding a single peak of enzymatic activity with CDNB as substrate. The four tilapia organs (liver, testis, ovary and kidney) exhibited the same chromatographic behavior on GSH-Sepharose affinity column (Fig.1 a, b, c, & d), respectively. The specific activity increased to 4.93 units / mg protein with 34.14 % recovery and 3.83 units / mg protein with 47.24 % recovery for both *O. niloticus* ovary and testis, respectively. While the specific activity increased to 38.4 units/mg protein with 103 % recovery for *O. niloticus* liver compared to 5.42 units / mg protein with 68.1 % recovery for *O. niloticus* kidney (Table 3). The four *O. niloticus* organs exhibited the same electrophoretic pattern on 12% SDS- PAGE. When the purified proteins of liver, kidney, ovary and testis were analyzed by SDS-PAGE, a single band was detected for each tissue after Coomassie staining having molecular weights of 27.5 KDa, 25.1 KDa, 25.1 KDa and 27.5 KDa, respectively. One band have the same electrophoretic mobility was detected for *O. niloticus* crude homogenates of ovary, testis, liver and kidney GSTs as judged by 7% PAGE after GST activity staining.

Table 1: Gonadosomatic index of *O. niloticus* gonads during three months

	Weight range (g)		Weight of range gonad (g)		GSI index	
Month/2014	Female	Male	Ovary	Testis	Ovary	Testis
February	108 - 148	108 - 148	0.4 - 2.5	0.7 - 1.7	0.37 - 0.86	0.65
March	112 - 128	60 - 123	0.84 -1.4	0.7 - 1.5	0.49 - 0.99	1.2
May	112 - 170	180 - 249	3.7 - 4.0	1.14 - 4.07	2.5 - 3.3	1.07 - 1.62

Table 2: GST activity and protein contents of different crude *O. niloticus* organs

Organ	GST activity [µmole/min /g tissue]	Protein [mg/g tissue]	Specific activity [µmole/min/mg protein]
ovary	1.98	156	0.013
kidney	3.58	52.3	0.069
liver	22.0	12.0	1.83
testis	7.10	31.3	0.23

Table 3: Purification scheme of GST from *O. niloticus* organs using GSH-Sepharose affinity chromatography

Organ name	Purification step	Activity [units]	Protein [mg]	Specific activity [unit/mg protein]	% Recovery	Fold
Liver	Crude homogenate [10g]	220	120	1.83	100	1
	Unbound fraction	36.6	38.4	0.95	16.6	0.52
	Affinity purified fraction	226	5.89	38.4	103	21
Testis	Crude homogenate [11g]	77.9	465.86	0.167	100	1
	Unbound fraction	11.5	18.5	0.62	14.8	3.7
	Affinity purified fraction	36.8	9.6	3.83	47.2	22.9
Ovary	Crude homogenate [34g]	53.4	5305	0.01	100	1
	Unbound fraction	15.0	1265	0.012	28	1.18
	Affinity purified fraction	18.2	3.7	4.93	34.4	493
Kidney	Crude homogenate [2.5g]	8.96	1310.7	0.07	100	1
	Unbound fraction	2.65	22.6	0.29	29.6	4.29
	Affinity purified fraction	6.1	1.13	5.42	68.1	79

Table 4: Specific activity (µmole/min/mg protein) of GSTs affinity isolated from *O. niloticus* organs using different electrophilic substrates

	Ovary		Testis		Liver		Kidney	
Substrate	Specific activity	% Relative activity	Specific activity	% Relative activity	Specific activity	% Relative activity	Specific activity	% Relative activity
CDNB	9.84	100	0.463	100	12.7	100	4.023	100
EPNP	17.2	175	2.16	467	16.8	132	7.7	191
EA	2.15	21.9	ND	ND	ND	ND	ND	ND
4-NPB	3.14	31.9	ND	ND	13.2	134	104	34.8
SO	24.6	250	ND	ND	ND	ND	16.8	416
p-NPA	0.68	6.9	0.01	2.21	0.053	0.42	0.77	19.2
BSP	ND	ND	0.099	21.4	1.16	9.16	ND	ND
DCNB	ND	ND	0.013	2.9	0.188	1.48	0.26	6.4
CuOOH	86.6	880	1.22	264	24	196	213	5297

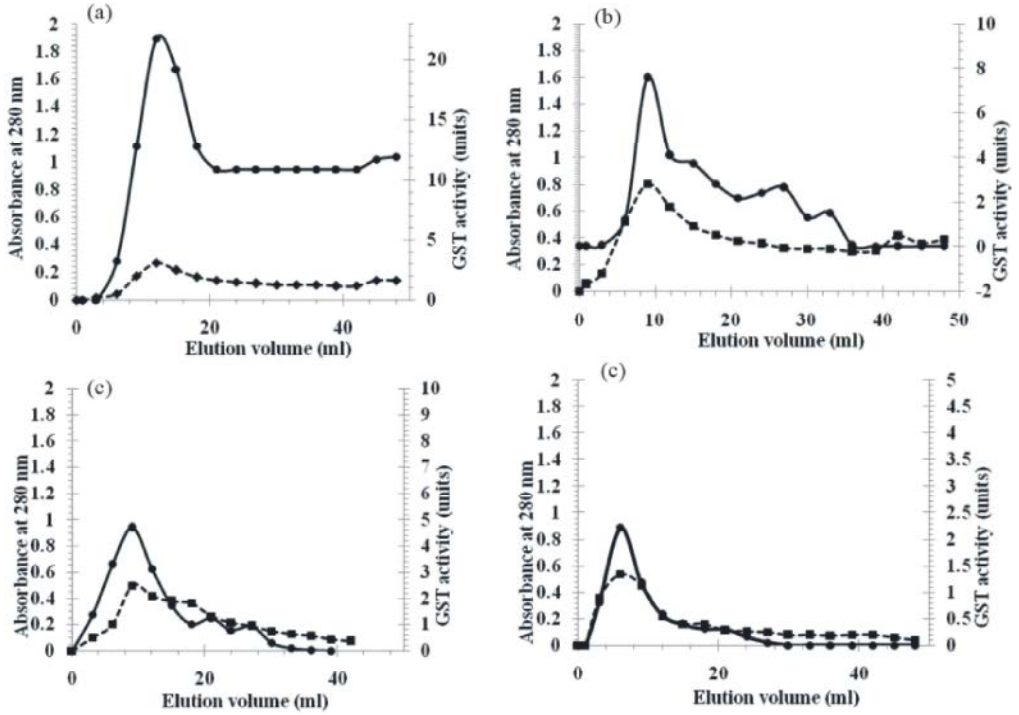

Fig. 1: Typical elution profiles for the chromatography of *O. niloticus* GST on GSH-Sepharose affinity column of [a] liver [b] testis [c] ovary and [d] kidney. Absorbance at 280 nm (– ● –) and GST activity using CDNB (…■|…).

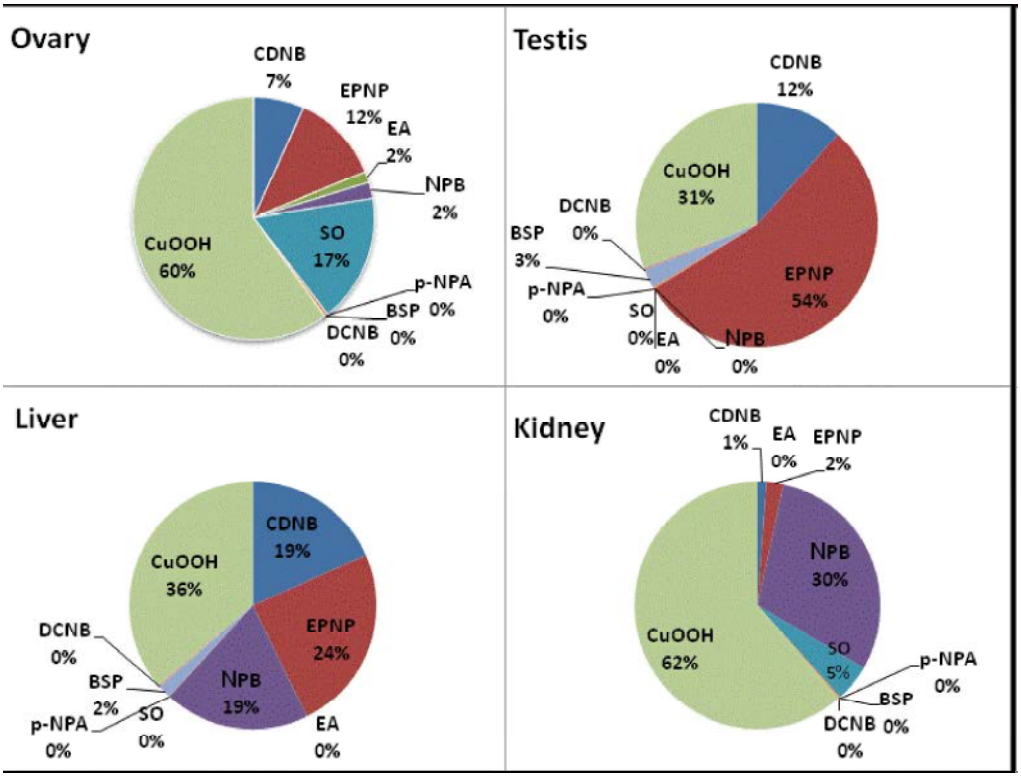

Fig. 2: Substrate selectivity profile of *O. niloticus* GST [ovary, testis, liver and kidney], Pie chart show relative activities with 9 substrates of different organs of *O. niloticus* GSTs

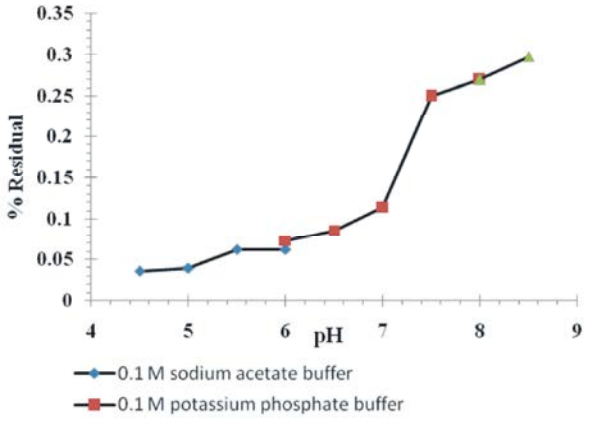

Fig. 3: The effect of pH on the enzymatic activity of the purified *O. niloticus* GST from testis

Substrate Selectivity of *O. niloticus* GST: Specific activities of the affinity-isolated enzyme fractions towards various electrophilic substrates, namely EPNP, EA, NPB, SO, *P*-NPA, BSP and DCNB were determined. The enzyme fractions were also investigated for the presence of catalytic activity towards CuOOH. The results represented in Table 4 and Fig. 2 indicated that there was a clear difference between the *O. niloticus* GST activities isolated from different tissues [Ovary, testis, liver & kidney] on the examined substrates. The highest activity was not obtained with CDNB as has been found for most transferases. The isolated enzymes from *O. niloticus* ovary and kidney were quite efficient in catalyzing the conjugation of GSH reduced glutathione with SO (24.6 µmole/min/mg protein and 16.8 µmole/min/mg protein, respectively), representing 250% and 416% of the relative activity on CDNB, respectively. Also, the highest catalytic activity was obtained with EPNP for both of testies and liver, representing 466.5 % [very reactive] and 243 %, respectively of the relative activity on CDNB. Both of the isolated ovary and kidney *O. niloticus* GSTs were reactive toward EPNP with specific activity equal 17.24 µmole/min/mg protein [175%] and 7.7 µmole/min/mg protein [191%], respectively. Liver GST was reactive toward 4-NPB representing 134 % of the relative activity on CDNB. The activities of GSTs show its highest values towards CuOOH among all the tested organs. The highest contents indicated in the kidney [213 µmole/min/mg protein] and the lowest contents showed in testis of *O. niloticus* [1.22 µmole/min/mg protein].

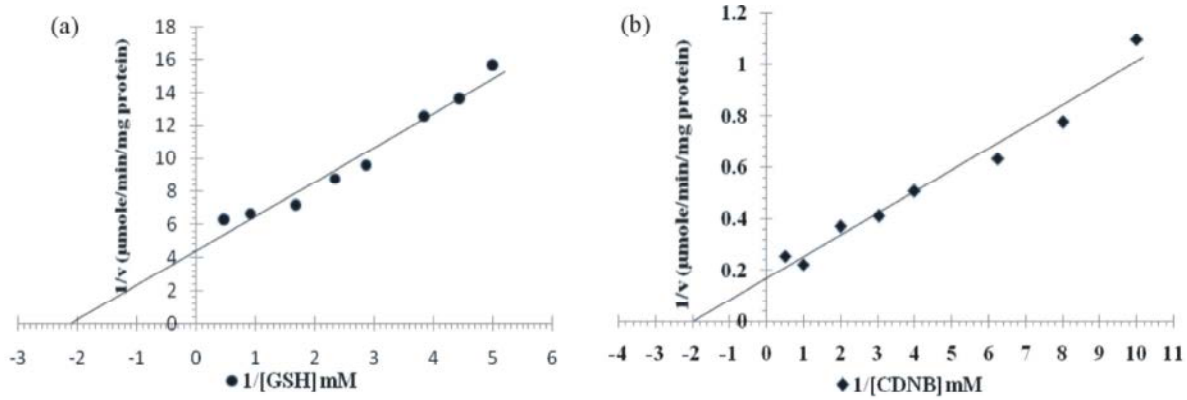

Fig. 4: Line weaver-Burk plot relating the GST activity purified from *O. niloticus* testis to (a) GSH concentration and (b) CDNB concentration.

Characterization of the Purified GST from *O. niloticus* Testis:

Effect of pH: The effect of pH on the affinity purified enzyme activity was examined between pH 4.5 and 9.0 using CDNB as a substrate. The affinity purified *O. niloticus* testis GST exhibited maximum pH at pH 8.0 and increasing the pH above 8.0 did not affect the observed maximum [Fig. 3].

Kinetic Parameters: The enzyme's steady state kinetics was studied in assays with various concentrations of GSH and CDNB. Initial velocities were determined in the presence of 2mM CDNB and varying GSH concentrations [0.2–2.2 mM]. Alternatively, 5 mM GSH was used at a fixed concentration and the CDNB concentration varied in the range of 0.1 – 2 mM. The Michaelis – Menten constants [K_m] of the purified testis for GSH and CDNB were 0.45mM and 0.5 mM, respectively [Fig. 4 a, b].

Effect of Inhibitors: The IC_{50} values [Concentration of inhibitors that will give 50% inhibition] of the activity of testis gonad GSTs were calculated to be 7.94 µM, 17.8 µM and 44.7 µM for hematin, bromosulfophtalein and ethacrynic acid, respectively. The *O. niloticus* testis GST showed different patterns of sensitivities to the small range of inhibitors tested. Hematin was the most potent inhibitor of testis GST activity [IC_{50} value equal 7.94 µM] compared to the other inhibitors tested. Testis GST was almost 2.24 times more sensitive to hematin than bromosulfophtalein [IC_{50} value equal 17.8 µM]. Sensitivity of *O. niloticus* testis GST to bromosulfophtalein was almost 2.5 times more then its sensitivity to ethacrynic acid [IC_{50} value equal 44.7 µM]. However, no effect could be detected for cibacron blue on *O. niloticus* testis GST.

DISCUSSION

GSTs are a family of intracellular enzymes with the main function in detoxification processes by catalyzing the conjugation of tripeptide GSH with some endogenous toxic metabolites and many environmental contaminants [22]. Fish GSTs were classified into seven classes depending on sequence identity named Alpha, Mu, Pi, Kappa, Theta, Omega and Rho classes [23, 24]. The GST is one of the enzymes that lack the substrate specificity because of the overlap of the substrate activities among different isoenzymes [25].

The gonadosomatic index is generally used to determine the maturity stage of fish and subsequently degree of gonadal development. The increase of gonadosomatic index is related to the percentages of ripe females and males towards the spawning season [26]. In relation to reproduction, the maximum value of the gonadosomatic index for females (2.5-3.3) and males (1.07-1.62) of *O. niloticus* was obtained in May; our results are in accordance with the results of Payne and Collinson [27], Bayoumi and Khalil [28] and El-Shazly [29]. Six stages of gonads maturation for both sexes of *O. niloticus* were identified; many authors described these maturity stages [30-33]. So, the isolation and characterization of gonadal *O. niloticus* GST glutathione transferase was measured from both sex gonads extracted in May.

A simple reproducible one step procedure for the purification of the major GST from *O. niloticus* liver, kidney, ovary and testis using affinity chromatography on GSH-Sepharose column was carried out. The four organs exhibited almost the same chromatographic behavior on GSH-Sepharose affinity column. One peak of GST bound to the column and eluted with 10 mM GSH.

After affinity purification step, a single protein was obtained suggesting this protein was the predominant GST expressed in *Monopterus albus* GST [maGST] liver [34]. The purified *O. niloticus* liver GST proved to be homogenous after affinity chromatography with a molecular weight of 25.460 kDa [14]. The same behavior was observed for *Claris lazera* kidney GST where the molecular weight was found to be 26.1 kDa [35]. Our results are in good agreement with the molecular weights reported for the major isoforms of cytosolic GST from different species of fish possessed a molecular mass ranging from 22.4 to 26.9 kDa [14, 36]. In salmon fish livers, a similar electrophoretic pattern was observed where the predominant GST expressed comprised subunits of molecular mass equal 24.8 kDa [32]. In another study on digestive gland of Icelandic scallop, also a single GST protein is detected by SDS-PAGE after GSH affinity chromatography. However, two and four GST isoenzymes are observed in Gilthead Sea bream livers and rainbow trout livers [34]. In the present study, electrophoretic pattern of the four examined *O. niloticus* organs indicated that, one enzyme binds to GSH affinity column and gave a single activity band on PAGE. *O. niloticus* organs may possess other minor enzyme forms but their amounts considered to be negligible.

Substrate Selectivity of *O. niloticus* GSTs: GST isoenzymes exhibit marked differences in their ability to conjugate GSH with various electrophiles [37]. There is a clear variation of specific activities among all the tissues of the examined *O. niloticus* GST. Both the ovary and kidney show similar peroxidatic activity with CuOOH, 60%, 62%, respectively, while the testis and liver have almost the same activity, 31%, 36%, respectively (Fig. 3). The activity towards CDNB is also varied; the highest ratio is shown in liver and testis. The activity of GST towards EPNP, characteristic substrate of mammalian GST T1-1, shows great variation. The highest activity is indicated in testis of about 54% and liver of about 24%. The GST activities of ovary and kidney show high activity towards SO, characteristic substrate of mammalian GST M1-1, meanwhile both testis and liver have no detectable activity toward SO. Setlíková and Wiegand [38] suggested that, the liver of silver carp fish has similar amount of pi and/or alpha class, which were the dominant GST classes in liver. Another study by Suzuki *et al.* [39] showed that Pi class GST genes are also strongly induced in zebrafish liver. Our This study is considered the first study dealing with changes between ovary and testis GST in fishes. The variation of the substrate selectivity of tilapia GST between ovary and testis may suggest a critical role of the GST in the reproduction process and sex differentiation.

The GST enzyme from *O. niloticus* testis was further investigated in the present study for the following reasons: Among the four tissues examined, the purified *O. niloticus* testis GST showed a quite different activity towards the nine examined substrates, where: 1- The enzyme showed the lowest conjugating activity with CDNB [0.463 µmole/min/mg protein] and showed the highest conjugating activity with EPNP [216 µmole/min/mg protein] representing 466 % of its activity toward CDNB. 2-The only examined enzyme have no activity on both 4-NPB and SO.

Characterization of *O. niloticus* Testis: This is the first study to our knowledge examining GST kinetic properties of in *O. niloticus* testis. Generally optimum pH values for GST with a variety of different substrates have values ranging from 6.0 to 9.5. When CDNB is considered; a narrow range of pH 7.0 - 9.0 is obtained, but the mode is in the vicinity of pH 8.0 [40]. The hepatic *O. niloticus* GST exhibited optimum pH between pH 8.0 and pH 9.0 [14]. Also, affinity purified GST from *O. niloticus* kidney exhibited maximum pH at pH 8.0, by increasing pH to 9.0, kidney GST still had 80 % of its maximum activity [35]. In the study of Huang et al. [34], optimum activity of freshwater fish *Monopterus albus* GST [maGST] was observed in the pH range 7.0–7.5. In the present study, the observed activity of the purified enzymes in the alkaline side may suggest a high stability in the alkaline conditions.

The kinetic studies of the purified GST from *O. niloticus* testis displayed distinct Michaelis-Menten constants with respect to GSH and CDNB as substrates. The K_m values for the purified testis GST for GSH and CDNB are similar to those of the hepatic *O. niloticus* GST [14], rainbow trout isoenzymes [41]. The present K_m^{GSH} values are in agreement with those of other GST isoenzymes [4.2-5.9 mM for GST 5-5 rat class θ, 0.5 mM for ρ] and disagreement with 2 mM for σ, 0.11 mM for P1-1, 0.08 mM for A1-1 and 0.16 mM for A2-2] [42].

Different kinetic mechanisms are employed to handle detoxification of toxins and these may be very complex and class dependent. Thus, the structural differences within the active site may influence the type of mechanism of the GST isoenzymes used to catalyze its reactions [43]. Information on purification and kinetics of fish gonads GST is not reported in the available literature. The present investigation is the first report to our knowledge on kinetic properties of *O. niloticus* testis.

The *O. niloticus* testis GST was strongly affected by hematin followed by bromosulfophtalein and EA, however, no effect could be observed with cibacron blue. In terms of inhibition, class Alpha is noted for a low IC_{50} value for hematin and a high value for cibacron blue [the ratio of the IC_{50} values for a given isoenzyme being ≈0.1]; class Mmu has a high IC_{50} for hematin and a low value for cibacron blue [the ratio of the IC_{50} values ranging from 3 to 20]; class Ppi have a high IC_{50} for hematin and a low value for cibacron blue [the ratio being similar to class Mmu] [44].

Tilapia GSTs are sensitive to hematin and EA ethacrynic acid and the result agrees with the values reported earlier for the Pi-class GST [45]. The sensitivity to hematin and EA indicated that GSTs from liver of *T. zilli* are closely related to the Pi-class than Alpha or Mu classes. Hamed *et al.* [14] indicated that GST purified from *O. niloticus* liver was strongly affected by cibacron blue and resembles the hepatic GST of the class alpha. Purified *O. niloticus* testis GST resembles GST of class Alpha in its IC_{50} for hematin. The differences in sensitivity of the isoenzymes to the different inhibitors may reflect different structural features. The variation in response to inhibitors may be due to the variation in the structure of the active sites of the enzyme.

CONCLUSION

The substrate selectivity of GST using 9 different substrates of the four organs of tilapia shows a wide variation among different organs. The variation of the substrate selectivity of testis GST and its characterization may suggest a critical role for GST in the reproduction process and sex differentiation of tilapia which is important for aquaculture programs.

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