

## Glutathione and its Related Enzymes in Fasciola Snails (*Lymnaea natalensis*): Purification and Characterization of Glutathione Transferase.

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Egypt.

**Abstract:** The effect of molluscicides – niclosamide and bromoacetamide on the glutathione (GSH) quantity and the activities of enzymes glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and gamma glutamyl transferase (GGT) were determined in *L. natalensis* snails. The LC<sub>50</sub> for niclosamide was 2.2 mg/L and 10 mg/L for bromoacetamide after 24 h of exposure. Niclosamide caused a decrease in GSH concentration and increase in the GST, GPx and GGT activity. Bromoacetamide caused a decrease in all the enzymes studied. A chromatographic procedure for purification of GST from *L. natalensis* by DEAE- Sepharose followed by GSH-Sepharose revealed two major isoenzymes GSTI and GSTII with the same pH maxima at pH 7 and temperature maxima at 40°C. GSTI and GSTII differed in K<sub>m</sub> for GSH (0.30 mM, 0.58 mM) and K<sub>m</sub> for 1-chloro-2,4- dinitrobenzene (CDNB) (1.78 mM, 0.85 mM).

**Key words:** glutathione S-transferase, purification, characterization, niclosamide, bromoacetamide.

### INTRODUCTION

The tripeptide glutathione (GSH) is the main non protein intracellular thiol. It plays a crucial role in the maintenance of the intracellular redox state and thus protects the cell against oxidative injuries. Glutathione reacts also with many toxic agents to form conjugates which can be easily excreted by means of specific transporters Meister and Anderson,<sup>[20]</sup>. Detoxification of xenobiotics or their metabolites is one of the major functions of GSH. These compounds are electrophiles and form conjugates with GSH either spontaneously or enzymatically in reactions catalyzed by glutathione S-transferase (GST) Meister,<sup>[19]</sup> DeLeve and Kaplowitz,<sup>[11]</sup> Hayes *et al.*,<sup>[17]</sup>. The conjugates formed are usually excreted from the cell. The metabolism of GSH conjugates begins with cleavage of the gamma-glutamyl moiety by gamma glutamyl transferase (GGT), leaving a cysteinyl-glycine conjugate.

As a consequence of aerobic metabolism, all aerobic organisms are subjected to a certain level of physiological oxidative stress. The intermediates that are formed, such as superoxide (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide, can lead to the further production of toxic oxygen radicals that can cause lipid peroxidation and cell injury. The endogenously produced hydrogen peroxide is reduced by GSH in the presence of selenium-dependent GSH peroxidase. As a result, GSH is oxidized to glutathione disulfide (GSSG), which in turn is reduced back to GSH by GSSG reductase at the

expense of NADPH, forming a redox cycle. Either glutathione peroxidase or GST can reduce organic peroxides. Hydrogen peroxide can also be reduced by catalase DeLeve and Kaplowitz,<sup>[11]</sup> Fernandez-Checa *et al.*,<sup>[12]</sup>.

Mollusca are a group of animals whose GSH and its related enzymes has not been extensively investigated Ajele and Afolayan,<sup>[5]</sup> Hamed *et al.*,<sup>[14]</sup> Abdalla *et al.*,<sup>[2,3]</sup>. Apart from being important horticultural and agricultural pests, molluscs, are intermediate hosts to Schistosoma and Fasciola which are the causative agent of Schistosomiasis and Fascioliasis. Fascioliasis is an increasingly recognized public health problem in Egypt for both animal and human Hassan,<sup>[16]</sup> Haridy *et al.*,<sup>[15]</sup>. However, *Lymnaea* species, as intermediate hosts of *Fasciola gigantica* or *Fasciola hepatica*, have a crucial role in completion of the life cycle and the transmission of the parasite to animals and man Shahlapour *et al.*,<sup>[25]</sup> Haridy *et al.*,<sup>[15]</sup> Shalaby *et al.*,<sup>[23]</sup>.

Chemical control using molluscicides has been proved to be the most effective method and many synthetic chemicals are tested as molluscicides (bromoacetamide, niclosamide, nicotinanilide and copper sulfate). Copper sulfate has been used for snail control for a long time but it has disadvantages, including adsorption by organic matter and toxicity to other organisms Webbe and Jordan,<sup>[30]</sup> Appleton,<sup>[7]</sup>. Niclosamide is highly toxic to developing and mature snail *Oncomelania hupensis* and its eggs Zhang,<sup>[34]</sup>.

Further, niclosamide exhibits a low toxicity to both humans and domestic animals and does not damage crops. Important constraints of this chemical is the high cost, its relatively slow killing effect on *O. hupensis* Zhang, [34]. Yuan *et al.*, [32]. Bromoacetamide was first synthesized in the 1980s Zhu, [37]. It is highly soluble in water and exhibits high toxicity to snails and has lower toxicity to fish and shrimps, but this chemical is expensive Yuan *et al.*, [32].

The aim of this work is to obtain information on GSH and its metabolizing enzymes in snail *L. natalensis* exposed to niclosamide and bromoacetamide and to purify and characterize GST which plays an important role in detoxification.

## MATERIALS AND METHODS

**Chemicals:** The reduced glutathione, glutathione reductase (Type III from Baker's yeast 200 IU/mg protein), nicotinamide adenine dinucleotide phosphate reduced form (NADPH) Epoxy-activated Sepharose 6B were purchased from Sigma Company, DEAE-Sepharose and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Amersham Pharmacia Biotech (CDNB), glutathione disulfide (GSSG) and H<sub>2</sub>O<sub>2</sub> from Fluka Company. Other general chemicals were of the highest purity commercially available.

**Snails:** *L. natalensis* snails (14-20 mm) used to study the effect of molluscicides, were maintained in the laboratory under standard conditions of aeration and temperature (25-30° C). They were fed fresh lettuce leaves and placed in dechlorinated water (aerated in a container for several days prior to being used in the experiments). The snails are exposed to different concentrations of niclosamide (0.02-50 mg/L) and bromoacetamide (10-50 mg/L) for 24 h. Six batches (3 snails each) were collected, snails tissues were removed and weighed.

**Enzymatic activity:** 1. The activity of GST was determined spectrophotometrically by following the formation of GSH conjugate with 1-chloro-2,4-dinitrobenzen (CDNB) at 340 nm using extinction coefficient of 9.6 mM<sup>-1</sup>cm<sup>-1</sup> [13]. The reaction mixture contained in 1 ml volume: 0.1 M potassium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM CDNB in ethanol and the enzyme solution. One unit of transferase activity is defined as the amount of enzyme which catalyse the formation of 1 μmole of thioether per min. 2. The activity of GR was determined spectrophotometrically at 25°C following the decrease in absorbance at 340 nm according to the method described by Zanetti [35]. The reaction mixture contained

in 1 ml volume: 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 0.1 mM NADPH, 0.5 mM GSSG and the enzyme solution. 3. The activity of GPx was determined spectrophotometrically at 25°C according to the method described by Weinhold *et al.* [31]. in which the GSSG produced due to peroxidase activity is grouped to the reaction catalyzed by GR. The reaction mixture contained in 1 ml volume: 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 0.75 mM H<sub>2</sub>O<sub>2</sub>, 1 mM GSH, 0.2 mM NADPH, 1.6 IU/ml GR and enzyme solution. One unit of GR or GPx activity is defined as the amount of enzyme which oxidize 1 μmole of NADPH/min under the assay conditions. The controls containing buffer instead of the substrate CDNB for GST, NADPH or GSSG for GR and NADPH or GSH for GPx were routinely included and treated under the same conditions of the enzyme assay. 4. GGT activity was measured by kinetic colorimetric method using commercial kits according to the method of Szasz [25]. The total GSH was measured colorimetrically according to the method of Saville [22]. Tissues were homogenized in 0.1M potassium phosphate buffer, pH 6.5. The tissue extract was mixed with an equal volume of 13% trichloroacetic acid (TCA). The precipitated protein was removed by centrifugation at 2000 g for 10 min and the supernatant was used for GSH analyses. The known weights of the whole snails were homogenized using glass homogenizer in 20% (w/v) of either 0.1 M potassium phosphate buffer, pH 7.0 containing 1 mM EDTA for GR and GPx enzymes. The homogenates were then centrifuged at 10,000 g for 1 h. The supernatants were filtered through a plug of glass wool to remove floating lipids. The filterates were termed 'crude extract' and saved at -20°C for further analyses.

**Purification:** The known weights of the whole untreated snails were homogenized in 25 mM Tris-HCl buffer, pH 8.0 containing 2 mM β-mercaptoethanol. The homogenate was then centrifuged at 15,000 g for 1 h. The supernatant was filtered through a plug of glass wool to remove floating lipids. The filterate was termed 'enzyme homogenate' and saved at -20°C for further purification. The enzyme homogenate was applied to 1- a DEAE-Sepharose ion exchange column (20 x 1.6 cm i.d.) previously equilibrated with 25 mM Tris-HCl buffer, pH 8.0 containing 2 mM β-mercaptoethanol. Protein was eluted with stepwise increase in the molarity of NaCl ranging from 0.02 to 0.20 M in the equilibration buffer at a flow rate of 60 ml/h. Fractions were collected in 3 ml and monitored at 280 nm and assayed for GST activity. The active fractions were pooled, dialyzed against 0.02 M Tris-HCl buffer and applied to 2- a GSH-Sepharose affinity

column (10 x 1cm i.d.). The column was washed with 0.02 M Tris-HCl buffer, pH 7.0. The bound GST was eluted with 0.1 M Tris-HCl buffer, pH 9.6 containing 10 mM GSH. Fractions containing GST activity were pooled and stored at -20°C. Protein was determined by Coomassie brilliant blue- G250 using bovine serum albumin as standard Bradford, [8]. The response of the purified isoenzymes to the variation of CDNB and GSH concentrations were studied at 25°C. GST activity was assayed taking different concentrations of GSH (0.05-2.0 mM) and holding CDNB concentrations at 2 mM, and different concentrations of CDNB (0.05-2.0 mM) and holding GSH at 2 mM. The  $K_m$  was calculated from double reciprocal plot of  $1/v$  versus  $1/[S]$ . The effect of temperature on CDNB conjugation reaction catalyzed by *L. natalensis* GSTI and GSTII was examined under the standard conditions. The reaction was carried out at different temperature ranging from 20°C to 60°C and the activity of the enzyme was measured.

**Statistical analysis:** The mean and standard error were calculated for each test and the significance of the difference between the means assessed by the student test.

## RESULTS AND DISCUSSION

The effect of different concentrations of molluscicides, niclosamide (0.02-50 mg/L) and bromoacetamide (10-50 mg/L) was examined after 24 h (Figs.1a, b). The mortality increased with increasing the concentrations of the two molluscicides.  $LC_{50}$  for niclosamide was found to be 2.2 mg/L and 10 mg/L for bromoacetamide after 24 h of exposure. The  $LC_{90}$  for niclosamide and bromoacetamide was 15.8 and 31.6 respectively.

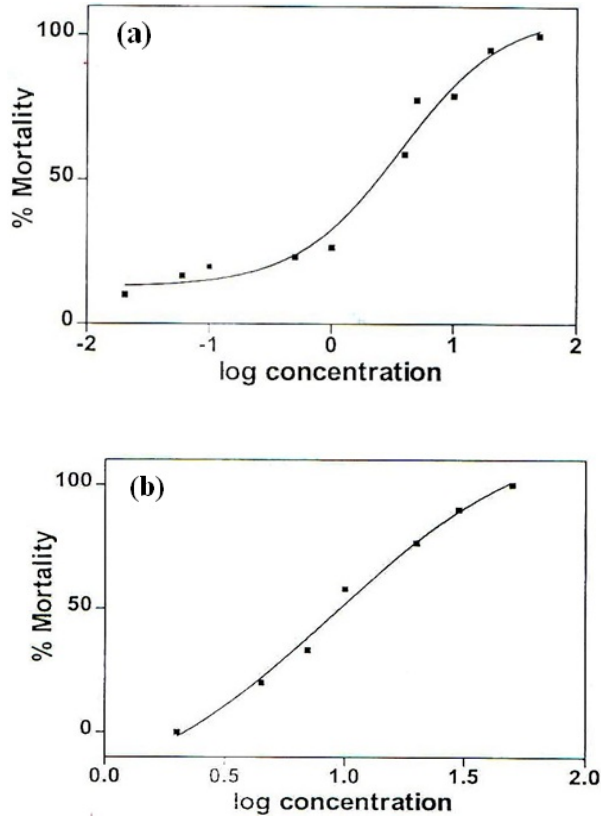
The effect of increasing concentrations of niclosamide ranging from (0.02-10 mg/L) and bromoacetamide (1-10 mg/L) on *L. natalensis* GSH concentration and GST, GGT, GR and GPx activity was examined after exposure of the snails for 24 h. Glutathione concentration decreased with increasing the concentration of niclosamide and bromoacetamide (Tables 1 and 2). GST and GGT are slightly increased with increasing the concentrations of niclosamide however, these increases are statistically insignificant (Table 1). The increase in GPx is statistically significant at concentration 0.06 mg/L ( $P<0.05$ ) (Table 1). GR decreased at the concentration 0.02 mg/L (statistically highly significant,  $P<01$ ) (Table 1). The treatment with bromoacetamide showed a highly significant decrease in the specific activity of GST concentrations of 3, 5 and 7 mg/L ( $p<01$ ,  $P<001$ ) while

the changes in GGT is statistically insignificant. However, the decreases in GPx are statistically highly significant ( $P<001$ ,  $P<01$ ) at all the concentrations tested while the decrease in GR was observed only at highly concentrations (7.0 mg/L, 10 mg/L) (Table 2).

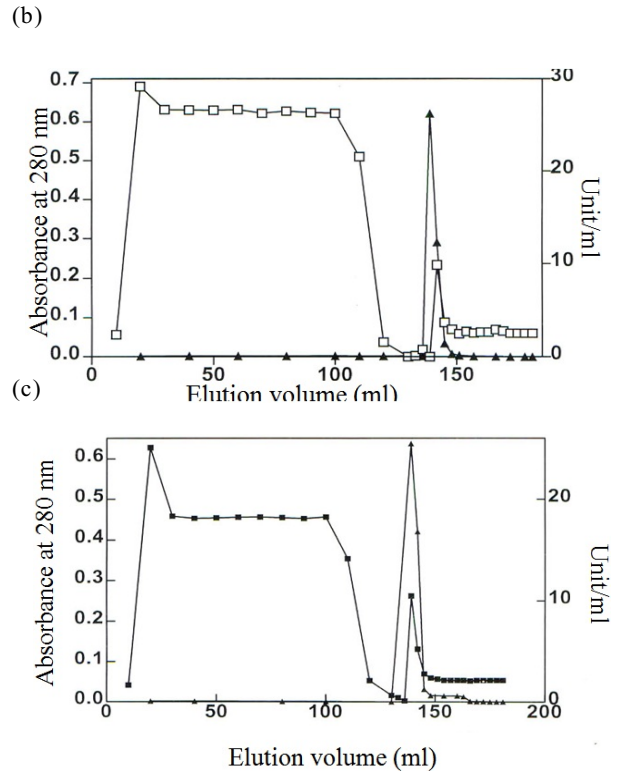
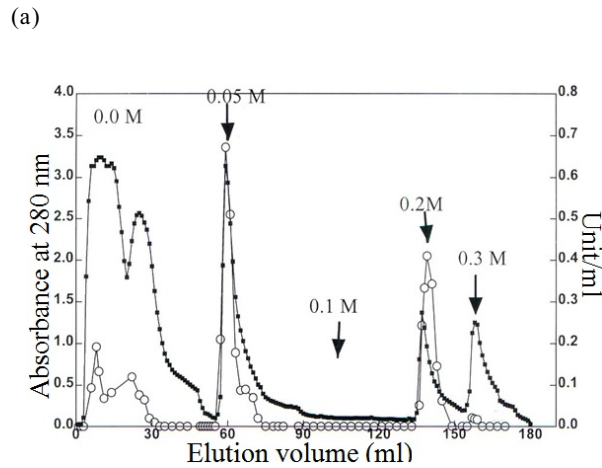
Chromatographic procedures for the purification of GSTI and GSTII were established. The procedure included chromatography on DEAE-Sepharose followed by chromatography on GSH-Sepharose. The crude extract (48.9 units and 217 mg protein, with specific activity of 0.23 unit/mg protein) was applied onto DEAE- Sepharose column (10 x 1 cm i.d.) previously equilibrated with 25 mM Tris-HCl buffer, pH 8.0 containing 2 mM  $\beta$ -mercaptoethanol. The applied protein was eluted by stepwise NaCl concentrations of NaCl dissolved in the same buffer. A typical example for the chromatography of the crude extract of *L. natalensis* GST isoenzymes on DEAE- Sepharose is shown in Fig. (2a). The elution profile shows the presence of two major isoenzymes peaks, designated GSTI and GSTII (Table 3). The enzyme activity of these peaks represented 37.1%, 30.5% of the crude extract activity, respectively. The fractions exhibited GST activity under each peak were pooled and saved at -20°C.

GSTI (18.1 units) and GSTII (14.9 units) were passed separately through the GSH-Sepharose column (9 x 1 cm i.d.) previously equilibrated with 25 mM Tris-HCl buffer, pH 8.0 at a flow rate of 20 ml/h. The column was washed with 150 mM NaCl dissolved in the equilibration buffer, at a flow rate of 30 ml/h until the absorbance of the effluent at 280 nm falls to zero. The column was then developed with 50 mM Tris-HCl buffer, pH 9.6 containing 10 mM GSH at a flow rate of 15/ml/h. Activity appeared as a single peak containing 11.7units and 1.75 mg protein with specific activity of 6.71units/mg protein and 23.9% recovery for GSTI and 8.2 units and 1.4 mg protein with specific activity of 5.8 units/mg protein and 167.8% recovery for GSTII. The purification fold was increased to 29.8 and 26.1for GSTI and GSTII, respectively (Figs. 2b, c and Table 3). The response of the purified isoenzymes to the variation of GSH and CDNB concentrations were studied at 25°C. The effect of GSH and CDNB concentrations on GSTI and GSTII activity was examined between 0.05 and 2.0 mM at pH 6.5, under the standard assay condition of GST (Figs. 3a, b and 4a, b). The two isoenzymes exhibited typical Michaelian behavior in these ranges of substrate concentrations. The  $K_m$  values were calculated and represented in Table 4. The effect of pH on GSTI and GSTII isoenzymes activity was examined using 0.1 M citrate buffer for pH 4.5- 6.0, 0.1 M phosphate buffer for pH 6.5-8.0 and 0.1 M Tris-HCl buffer for pH 8.5,

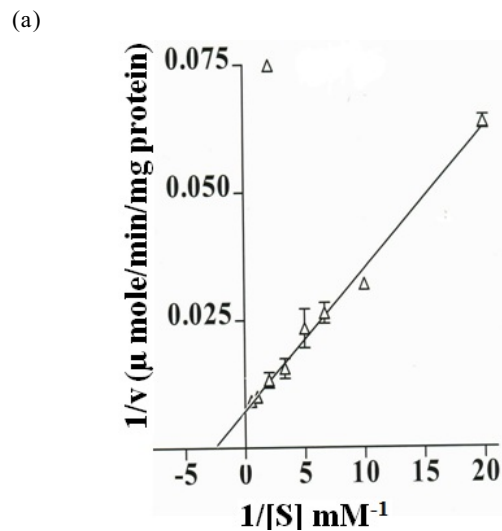
9.0. GSTI and GSTII exhibited optimum pH at pH 7 (Figs. 5a, b). At temperature ranging from 20°C to 60°C, the enzymatic activity of GSTI and GSTII increased by increasing the temperature up to 40°C, then the activity starts to decrease with increasing the temperature (Figs. 6a, b).



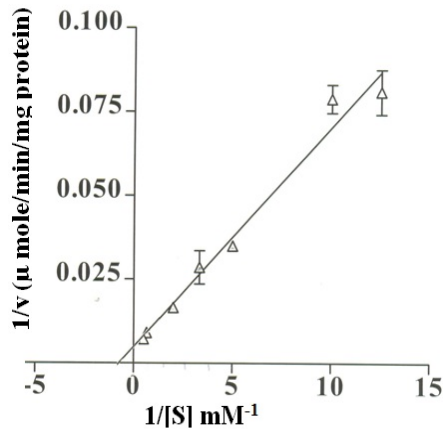
**Fig. 1:** Effect of niclosamide concentrations (a) and bromoacetamide concentrations (b) on mortality of *L. natalensis* snails.



**Fig. 2:** Typical elution profile for chromatography of GST of *L. natalensis* on DEAE-Sepharose column (a). Typical elution profile for chromatography of GSTI (0.05M NaCl) on GSH-Sepharose affinity column (b). Typical elution profile for chromatography of GSTII (0.2M NaCl) on GSH-Sepharose affinity column (c). Absorbance at 280 nm (-○-, -□-, -■-). Activity in unit/ml (-●-, -▲-).

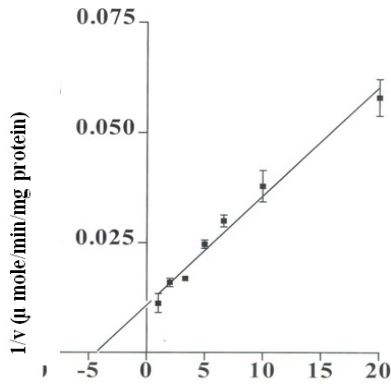


(b)

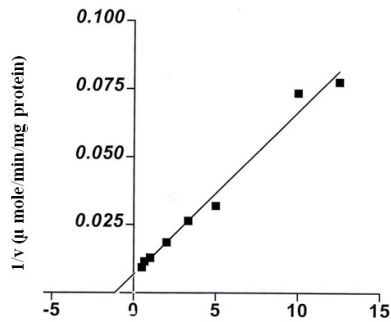


**Fig. 3:** Lineweaver-Burk plot relating the purified isoenzyme GSTI activity to GSH (a) and CDNB (b) as substrates at pH.

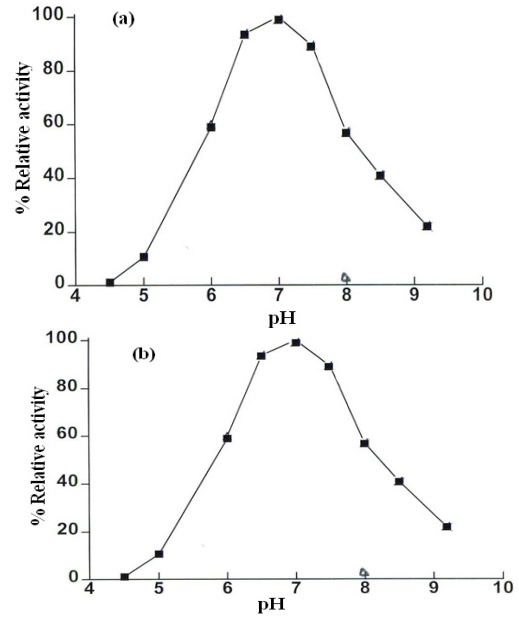
(a)



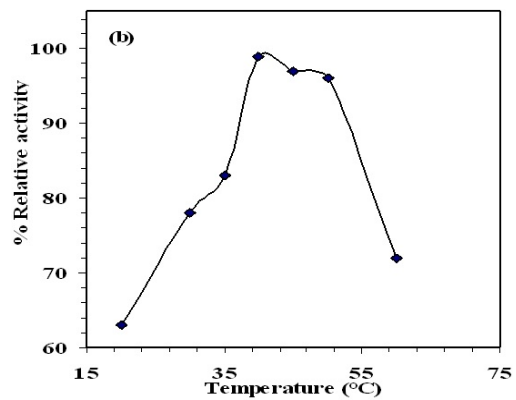
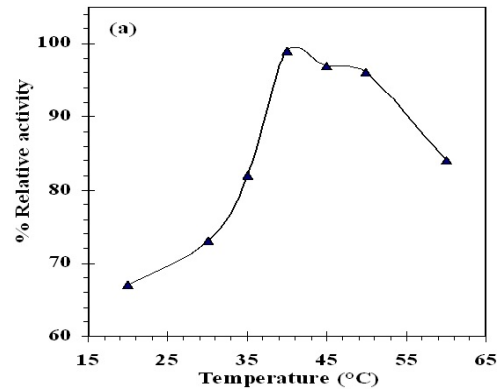
(b)



**Fig. 4:** Lineweaver-Burk plot relating the purified isoenzyme GSTII activity to GSH (a) and CDNB (b) as substrates at pH 6.5.



**Fig. 5:** Effect of pH on enzymatic activity of GSTI (a) and GSTII (b). The buffers used were: 0.1 M citrate for pH 4.5- 6.0, 0.1 M phosphate for pH 6.5-8.0 and 0.1 M Tris-HCl for pH 8.5, 9.0.



**Fig. 6:** Effect of temperature on enzymatic activity of (a) purified GSTI and (b) purified GSTII.

**Discussion:** Niclosamide is a potent molluscicide. The concentration of niclosamide that cause 100% mortality reported so far ranged between 0.2 and 1 mg/L for different species of snails. Tchounwou *et al.* [28], found that a 0.2 mg/L of niclosamide caused 100% mortality of adults *Helisoma trivolvis* and *Biomphalaria havanensis* snails after 24 h exposure. Abdel Aziz [4], found that niclosamide at a concentration of 0.54 mg/L caused the death of 50% of the *B. alexandrina* after 24 h of exposure of the snails. This concentration is higher than that reported by Andrews *et al.* [6]. They reported that 100% mortality was achieved at concentration of 0.3 mg/L. This may be due to a difference in the purity of the molluscicide used or to a difference in the strain of *B. alexandrina*. In the present investigation 2.2 mg/L of niclosamide caused the death of 50% of the studied snails. This value is higher than that reported by the previously mentioned authors. Bromoacetamide has proved to be safe to fish and effective molluscicide against schistosoma snails (*Oncomelania hupensis* snails) Zhu *et al.*, [36]. The LC<sub>90</sub> of bromoacetamide is 1.0 mg/L against *O. hupensis* snails for 24 h immersion under 25°C and has a low toxicity to fish Zhou *et al.*, [35]. However the LC<sub>90</sub> of bromoacetamide against the studied snails is 31.6 mg/L. Wang *et al.* [29], studied the mode of action of bromoacetamide, it caused a decrease in action of enzymes located in mitochondria (ornithine carbamyl-transferase, citric acid synthase). In our study bromoacetamide also caused a decrease in GGT, GST, GR and GPx activity.

Glutathione and its related enzymes are efficient protective mechanisms against chemical reactive species. The biochemical analysis revealed numerous differences related to glutathione and its metabolizing enzymes GST, GPx and GR in the fresh water snails *B. alexandrina*, *Cleopatra bulimoids*, *Melanoid tuberculata*, *Bellamya unicolor*, *Bulinus truncatus*, *Lymnaea trunculata* and *Physia acuta* Abdalla, [1]. Hamed *et al.*, [14]. In mussels *Mytilus edulis* and *Mytilus galloprovincialis* Viarengo *et al.*, [28] Regoli and principato, [23]. glutathione has been reported as a biomarker in various tissues. Da Silva *et al.* [10]. found that GST activity in oyster *Crassostrea rhizophore* increased in a concentration-dependent manner after exposure to diesel oil. Li *et al.* [18]. studied the sensitivity of GSH and its related enzymes (GST, GPx and GR) of edible freshwater snail *Bellamya purificata* to the contaminated aquatic environment by landfill leachate effluent and bisphenol A. Their results showed that activities in both gills and digestive glands increased with increasing pollutant concentrations. The

total GSH levels had a significant depletion in both the gills and digestive glands. The same results had been reported by Canesi *et al.* [9]. In our study a similar results were observed using niclosamide. The decrease in GSH is mainly due to the increase of all the tested parameters, showing its roll in detoxification of niclosamide and survival of the snails. A highly significant decrease in GSH and its related enzymes (GGT, GST, GR and GPx activity) was observed in snails exposed to bromoacetamide. In this case all the factors tested affecting GSH production (Redox cycle, GR/GPx) or withdrawal of GSH (GGT and GST) are decreased. This decrease may indicate its roll in another system. In case of niclosamide the decrease in the ratio of GSH to the tested enzymes is mainly due to the increase in enzymes activities. While the increase in the ratio of GSH to the tested enzymes using bromoacetamide is due to the decrease in enzymes activities which is comparably higher than the decreased in GSH. Hamed *et al.* [14]. studied glutathione and its related enzymes (GST, GPx and GR activity) in four Egyptian freshwater snails (*B. alexandrina*, *C. bulimoids*, *M. tuberculata* and *B. unicolor*). *B. alexandrina* and *M. tuberculata* the results show the highest concentrations of GSH and lowest specific activity of GST and GPx. The high content of GSH may give non enzymatic protection of the organism from xenobiotics.

The kinetic studies of the purified GSTI and GSTII isoenzymes from *L. natalensis* display very distinct Michaelis-Menten constants with respect to GSH and CDNB as substrates. K<sub>m</sub> values of GSH were 0.3 μM and 0.58 μM for GSTI and GSTII respectively. Optimum pH values for GST of invertebrate with a variety of different substrates have values ranging from 6.5 to 9.5. Different substrates of molluscs, *Achatina fulica*, *Indophanorbis exustus* and *Cerithidea obtuse* exhibited pH optimum values of 8.3, 7.0 and 9.0, respectively. Abdalla *et al.*, [2]. showed that the two purified isoenzymes exhibited pH optimum at 6.5. In the present study, the two purified isoenzymes showed pH optimum at 7.0.

In conclusion, although both molluscicides are potent for *L. natalensis* and glutathione decreased by increasing the molluscicide concentrations, the glutathione related enzymes exhibited different behavior suggesting different mechanism. Two main GST isoenzymes may have different role in detoxification as they are similar in some property as optimum pH and temperature but their affinity toward the substrates GSH and CDNB are different.

**Table 1:** Effect of niclosamide concentrations on specific activities of related enzymes of *L. natalensis* snails.

Niclosamide (mg/L)	GSH (nmole)	Specific activity (mmole/ min/ g/mg protein)			
		GST	GR	GPx	GGT
0.00	28.1± 1.20	0.176±0.009	0.022±0.002	0.014±0.004	0.039±0.007
0.02	20.7± 0.63	0.185±0.017	0.010±0.01**	0.020±0.004	0.049±0.007
0.06	22.1± 1.47	0.199±0.009	0.024±0.003	0.044±0.01*	0.047±0.003
0.10	21.5± 1.07	0.021±0.211	0.024±0.003	0.016±0.006	0.061±0.015
0.50	19.9± 3.04	0.199±0.022	0.013±0.002	0.0150±0.003	0.018±0.003*
1.00	21.1± 1.40	0.163±0.036	0.011±0.003	0.013±0.001	0.046±0.014
10.0	20.9± 3.30	0.182±0.017	0.013±0.002	0.0202±0.004	0.055±0.005

Each figure represents the mean±SE.; \*Significant P<0.05; \*\*Highly significant P<0.01, 0.001.

**Table 2:** Effect of bromoacetamide concentrations on specific activities of related enzymes of *L. natalensis* snails.

Bromoacetamide (mg/L)	GSH (nmole)	Specific activity (mmole/ min/ g/mg protein)			
		GST	GR	GPx	GGT
0.0	28.1±1.20	0.141±0.01	0.047±0.006	0.048±0.003	0.040±0.009
1.0	26.4±1.93	0.128±0.01	0.064±0.010	0.023±0.01**	0.039±0.009
2.0	22.1±0.26	0.076±0.01**	0.017±0.007	0.013±0.01**	0.026±0.002
3.0	22.9±0.25	0.078±0.01**	0.017±0.004	0.018±0.01**	0.030±0.004
4.5	19.7±0.73	0.139±0.011	0.018±0.002	0.011±0.01**	0.041±0.005
5.0	19.3±1.10	0.124±0.02**	0.024±0.001	0.012±0.001*	0.022±0.004
7.0	17.5±0.49	0.066±0.01**	0.020±0.01**	0.022±0.01**	0.046±0.001
10.0	15.9±0.28	0.049±0.01	0.016±0.01**	0.020±0.01**	0.028±0.010

Each figure represents the mean±SE.; \*Significant P<0.05; \*\*Highly significant P<0.01, 0.001.

**Table 3:** Purification scheme of *L. natalensis* GST.

Fraction	Protein (mg)	Activity (unit)	Specific activity	Recovery %	Fold Purification
Cude extract DEAE- Cellulose	217	48.9	0.225	100	1
0.05 M	26.8	18.2	0.65	37.1	2.99
0.2 M	26.2	14.9	0.57	30.5	2.54
0.3 M	9.5	0.766	0.08	1.56	0.36
GSH- Sepharose GSTI	1.75	1117	6.71	23.9	29.8
GSTII	1.40	82.2	5.89	16.7	26.1

**Table 4:** K<sub>m</sub>, maximum pH and temperature for GSTI and GSTII of *L. natalensis*.

Parameters	GSTI	GSTII
K <sub>m</sub> GSH	0.304±0.031	0.584±0.053
K <sub>m</sub> CDNB	1.78±0.272	0.848±0.112
Maximum pH	7.0	7.0
Maximum temperature	40°C	40°C

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