

Lipid peroxide, glutathione and glutathione-dependent enzyme (GST) in mixed zooplankton from the North-West Coast of India: implication for the use of environmental monitoring

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Abstract:

This paper deals with an experiment of mixed zooplanktonic organisms were collected from shore water off, Diu coast, analysed and measured for lipid peroxidation (LPX), as a marker of oxidative stress and glutathione-S-transferase (GST) activity, as marker of organic pollution. Both LPX and GST activities were highest at stations close to the shore and marginally decreased along with transect. The reduced glutathione (GSH) levels were highly variable. Results are discussed in relation to biomarker application of mixed zooplankton, lipid peroxidation (LPX), glutathione-S-transferase (GST) and reduced glutathione (GSH). The present results indicated that these three potential markers (LPX, GST & GSH), could be used as a measure for bio-monitoring the coastal ecosystem using mixed zooplankton as suitable organisms.

Key notes: Zooplankton, lipid peroxidation, glutathione-S-transferase and reduced glutathione.

Abbreviations: **LPX;** lipid peroxidation, **GST;** glutathione-S-transferase, **GSH;** reduced glutathione, **ROS;** reactive oxygen species, **SOD;** superoxide dismutase, **GPx's;** Peroxidations, **SH;** cystin, **TBA;** thiobarbituric acid, **SDS;** sodium dodecyl sulfate, **TBARS;** thiobarbituric acid reactive substances, **BHT;** Butylated hydroxyl toluene, **PAH;** petroleum aromatic hydrocarbon, **DPPH;** 2,2-Diphenyl-1-picrylhydrazyl, **CDNB;** 1-chloro-2, 4-dinitrobenzene.

Introduction

Marine environments are subjected to several forms of disturbance related to anthropogenic activities such as shipbuilding, transport, oil refinery spill, industrial and urban effluents, dredging and dumping of sediments, often extending their influence through diffusion of pollutants. The water born pollutants which generate reactive oxygen species (ROS) are the source of toxicity for aquatic organisms living in the polluted environment and practically responsible for disruption in physiological functions (Livingstone DR, 2001).

ROS such as superoxide radical (O_2^\bullet), hydroxyl radical (OH^\bullet), and hydrogen peroxide (H_2O_2) etc., are highly reactive compounds, which if not neutralized efficiently, will attack almost all bio-molecules of cells, including proteins and DNA. Membrane lipids are more susceptible to their attack generating lipid peroxidation (Elstner EL, 1991). To protect themselves from these highly reactive compounds, cells have their own interdependent antioxidative defense system, composed of protective proteins removing the ROS/RNS. These chemical radicals are normally metabolized by the enzymatic antioxidants, such superoxidants and superoxide dismutase (SOD), that converts superoxide into anion ($O_2^{\bullet-}$) to H_2O_2 in all aerobic organisms. Catalase degrades H_2O_2 to water and oxygen, although GPx detoxifies both H_2O_2 and hydro peroxides (ROOH) using reduced glutathione (GSH) as a cofactor. Intracellular homeostasis cycling is regulated by antioxidants, mainly by thiol-containing molecules such as GSH. Glutathione reductase (GR) has important role in maintaining intracellular status of GSH. Glutathione S-transferase (GST) catalyzes conjugation reaction with GSH. Apart from this, cells possess some other antioxidant defense mechanism to neutralize the effect of toxic compounds. Metallothionein (MT), a high content of cystein (SH) group protein, which detoxifies heavy metals (Halliwell B et al, 1986a. Romero-Isart N et al, 2002b).

Zooplanktons are small heterotrophic animals inhabiting almost every type of aquatic environment. Numerous studies have demonstrated the suitability of zooplankton for biomarker study (Fossi MC Minutoli R et al, 2001a. Petres LD et al, 2001b). Marine zooplanktons have relatively high lipid reserves and can accumulate hydrophobic compounds directly from the sea or by ingestion of contaminated prey. Several studies have focused on the distribution of the conservative contaminants in zooplanktons (Harding GC, 1986a. Shailaja MS et al, 1991b). Kureishy et al (1978) and Kanan & Sen Gupta (1987) also reported the values of total DDT in zooplankton from the Arabian Sea as 0.3-3.2 $\mu\text{g}/$

g, but very little has been concluded about their toxic effects in marine organisms, especially on zooplankton. Zooplanktons are close to the base of the marine food web and therefore can play a vital role in transfer of pollutants through several trophic levels. Ecotoxicological risks to zooplankton can be used as an early warning signal of risk to the health of the marine ecosystem. In the present study, we investigate the potential use of certain biomarkers in mixed zooplankton. Attempts have been made to measure level of LPX, GST activity and GSH level (Table 1) in mixed zooplankton.

Material and methods

Zooplankton samples were collected from 12 different stations along and off Diu coast on 5th and 7th October 2009 covering the geographical limits of latitude 20° 39' 53.3 to 20° 42' 02.3 N and longitude 71° 00' 26.0 to 71° 04' 59.7 E. (Fig-1). For this purpose horizontal hauls were taken using Heron Traton net of mesh size 200 µm. A trawler was used to operate the net (Tow speed at 1.5 knot, haul time 5 min). Total water filtered was computed with the help of the current speed, mouth area of the sampling net and towing time. Fifty percent (50%) of the collected sample was preserved in 5% neutralized formaldehyde for identification and quantitative analysis and the remaining 50% samples were filtered, washed in distilled water and cryopreserved in liquid nitrogen cans for biochemical estimation.

TBARS Assay:

The whole animals were processed and were assayed according to the method of Ohkawa et al (1979) with minor modifications. In brief, the reaction mixture containing approximately 1 mg of protein, 1.5 mL of 0.8% aqueous solution of thiobarbituric acid (TBA), 1.5 mL of 20% acetic acid (CH₃COOH) (pH 3.5) with NaOH, 0.2 mL of (8.1%) sodium dodecyl sulfate (SDS), 0.2 mL of double distilled water and 0.1 mL of (0.76%) Butylated hydroxyl toluene (BHT) was heated at 95 ° C for 60 min, then cooled to room temperature and centrifuged at 2000 X g for 10 min. The absorbance of the supernatant was read at 532 nm. The amount of TBARS formed was calculated by using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$, and expressed as nano mol TBARS performing protein.

Measurement of GST activity

The mixed zooplankton was carefully surface dried with filter paper, thoroughly washed with phosphate buffer (pH 7.4) and homogenized with 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM DTT, 0.15 M KCL, 0.01% PMSF. Homogenization was carried out at 4° C using 12-15 strokes of a motor driven Teflon Potter homogenizer and centrifuge at 10,000 X g. The supernatant was taken for enzyme assay. The enzyme GST was measured at 340 nm according to Habig et al (1974) using 0.3 mM CDNB as substrate.

Measurement of Reduced glutathione (GSH)

A crude homogenate 20 % was prepared with 5% trichloro-acetic acid (TCA) and was centrifuged at 2000 X g for 10 min, the deproteinised supernatant was used for assay of GSH using 0.6 mM DTNB in 0.1 M Phosphate buffer (pH 8.0) and the formation of thiol anion was measured at 412 nm ¹⁵.

Estimation of Proteins

Protein content was estimated by the Folin-Phenol reaction as described by Lowry *et al*, using bovine serum albumin (BSA) as a standard.

RESULTS

In the present investigation 17 different zooplanktonic groups were observed during study period at 12 stations, where Copepoda contributed almost 97% of the total zooplankton density followed by Doliolids (0.95%), Brachyuran zoea (0.83%), Eupliausiid (0.25%), and Chaetognatha (0.23%). Hydromedusae (0.16%), Amphipoda (0.15%) and salpa (0.13%) could contribute a little higher share in the total population while other groups like Lucifer (0.01%), Bivalve (0.00%), Fish eggs (0.02%) and Polychaeta (0.00%) were very rare in their occurrence. A total number of 4 orders were recorded belonging to the group Copepoda: Calanoida, Cyclopoida, Harpacticoida and Mostrilloida. Calanida was the dominant order among the copepods accounting for 96.65% of the total Copepod density followed by Cyclopoida (3.28%), Harpacticoida (0.07%) and Mostrilloida (0.004%)

The Lipid peroxidation (LPX) was determined in sub-cellular fraction of this mixed zooplankton. The highest LPX level was observed in stations 2, 3, 5, 7, 8, 9, 10, 11 & 12 (Fig-2A). Glutathione-S-

transferase (GST) and reduced glutathione (GSH) level was reported in greater variation. The GST activities were indicated to be in stations 3, 10, 11 & 12 (Fig-2B). The non-enzymatic antioxidant reduced glutathione contents high in stations 2, 3, 5, 7, 8, 10, 11 & 12 (Fig-2C).

DISCUSSION

The aim of these studies was to evaluate the potential use of certain biomarkers (LPX, GST and GSH) in mixed zooplanktonic organisms. LPX, GST and GSH level were measured in the sub-cellular fraction of mixed zooplankton from the Arabian Sea. Attack by molecular oxygen produces lipid peroxynulical that can abstract a hydrogen atom from an adjacent lipid to form a lipid hydroperoxide. The LPX process affects membrane fluidity and integrity of biomolecules associated with membrane, and can be quantified by measuring TBARS assay. The formation of TBARS is commonly used as a marker of biomembrane lipid peroxidation, which is an indicator of oxidative stress (Oakes KD et al, 2003). Therefore, Lipid peroxidation (LPX) has been taken as an index of oxidative stress. The highest LPX level was observed at stations 2, 3, 5, 7, 8, 9 10, 11 & 12 (Fig-2A), closer to the outflow of the river Ghogla (Fig-1). Since TBARS is primarily an outcome of generation of free radicals, it is suggested that this organism is facing an oxidative challenge. This result agrees with results obtained with mussels, when exposed to copper (Cu), aluminum (Al), lead (Pb), cadmium (Cd) and in the field study (Viarengo A et al, 1999a. Torres MA et al, 2002a). Our laboratory early findings suggested that exposure of mercury (Hg), WSF of petrol and diesel, naphthalene and pyrene to *P. viridis* generated LPX.

GSH is the most abundant intracellular thiol, and through its antioxidant properties, it is able to protect cells from ROS formed during normal mitochondrial respiration and metabolism of foreign chemicals (Hammond CL et al, 2004). Glutathione exerts its antioxidant function by reaction with superoxide radical (O_2^\bullet), peroxy radical and singlet oxygen (1O_2) followed by the formation of oxidized glutathione and other sulfides. A decrease in tissue concentration (Fig-2C) of available GSH may be due to increased consumption of GSH via GST or may directly react with ROS, in turn leading to alteration in the redox balance. Decreased GSH level is found in mussel exposed to copper (Cu). Di Giulio et al (1995) suggested that trace metals can bind to GSH and thereby limit its availability to counteract lipid peroxidation and DNA damage. Further when GSH values were plotted vs LPX it showed a correlation ($r = -0.596$, $p < 0.05$, Fig-3A). This suggests that GSH might play an important role in protecting the organisms from ROS.

Potential source of contaminant mediated ROS production by marine organisms include interactions with metals, PAH (petroleum aromatic hydrocarbon) and other organic chemicals such as nitroaromatics (Lemaire P et al, 1994). The present study also focused on the phase II GST enzymes at different stations. GSTs are a multigen family of enzymes (isoforms) grouped into seven classes: alpha, mu, pi, theta, sigma, zeta and omega (Lenartova V et al, 1997a, Mennervick B, 1985b). The enzyme catalyzes the conjugation of a large variety of compounds bearing an electrophilic site, with reduced glutathione (Edwards R et al, 2000). GST activity varies in different stations (Fig-2B). Interestingly a positive correlation is obtained with LPX level (Fig-3B). Edwards et al (2000), reported that glutathione transferase play an important role in the detoxification of ROS in the cells protecting lipids from peroxidation. The Induction of GST activity of mussels in field study, by toxicant has been done by several authors in the organisms (Prakash NT, 1995. Torres MA, 2002. Canesi L, 1999. Bainy AC, 2000. Strmac M, 2002. Lau PS, 2003). The increase in GST activity may be a strategy to prepare for oxidative stress in an effort to defend cells against oxidative damage ($r= 0.56$, $p < 0.05$, Fig-3 B). From the result it suggests that these zooplanktons more effectively use the glutathione metabolism pathway as an adaptive response against ROS generated pollutants.

Conclusion

In the present study, we report variations of LPX, GSH and GST activity in the mixed zooplankton. We have shown an increase in LPX and GST activity in mixed zooplankton collected in the near shore area and marginally decrease along the transect until it levels off. Present studies permit a better characterization of the impact of pollution on marine organisms taken together. The present results indicate that these three potential markers (LPX, GST and GSH), could be used as measure for bio-monitoring the coastal ecosystem using mixed zooplankton as suitable organism.

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Table 1. Measurement of LPX, GST and GSH per gram proteins by TBARS and CNDB methods

Stations	1	2	3	4	5	6	7	8	9	10	11	12
TBARS/mg proteins	5	10	17	4	7.5	5	10	11	6.25	9	10	13.5
nMole CNDB conjugated/ proteins	249	258	600	239	253	255	184	175	177	480	320	409
GSH μ M/gm wt tissues	0.7	0.2	0.25	2.5	1.74	1.77	1.1	1.2	1.45	0.75	0.67	0.49

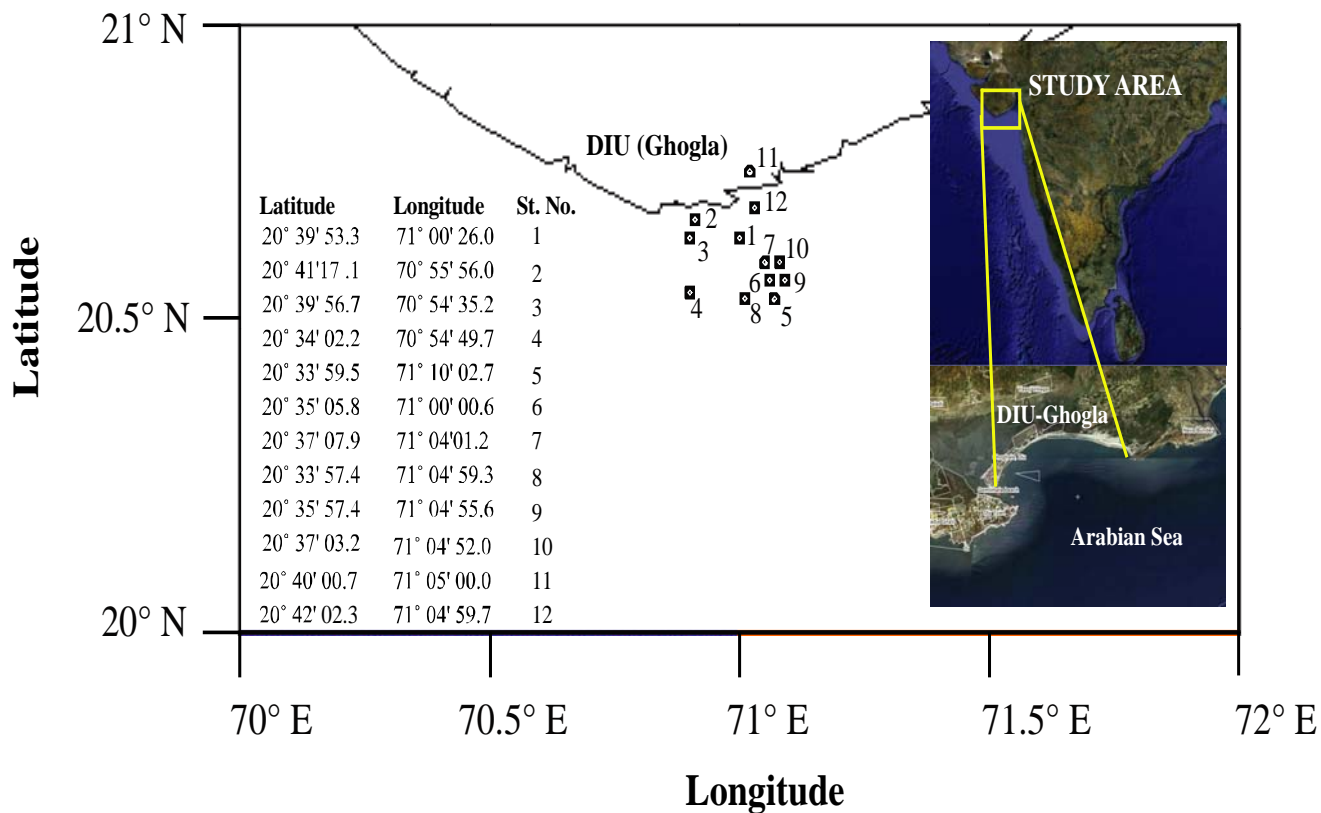


Fig. 1. Sampling site of Zooplankton collection near shore water of Diu Island.

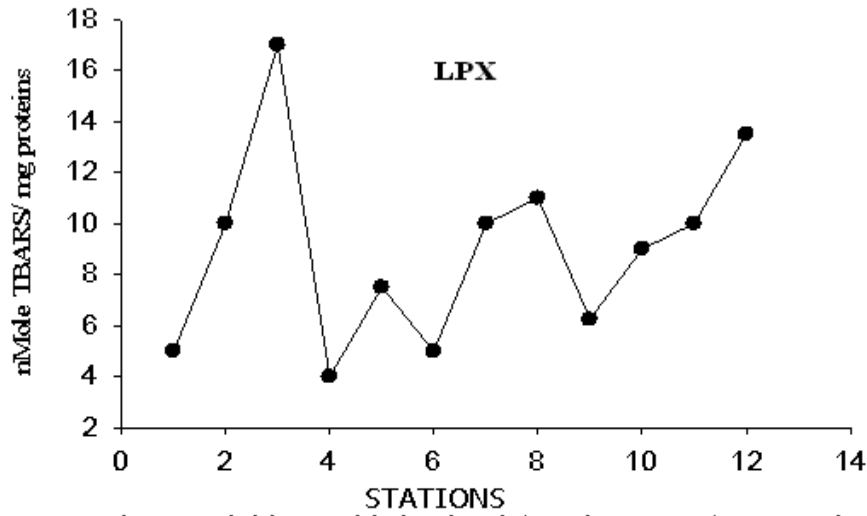


Fig-2A. Lipid peroxidation level (nMol. TBARS/ mg protein)

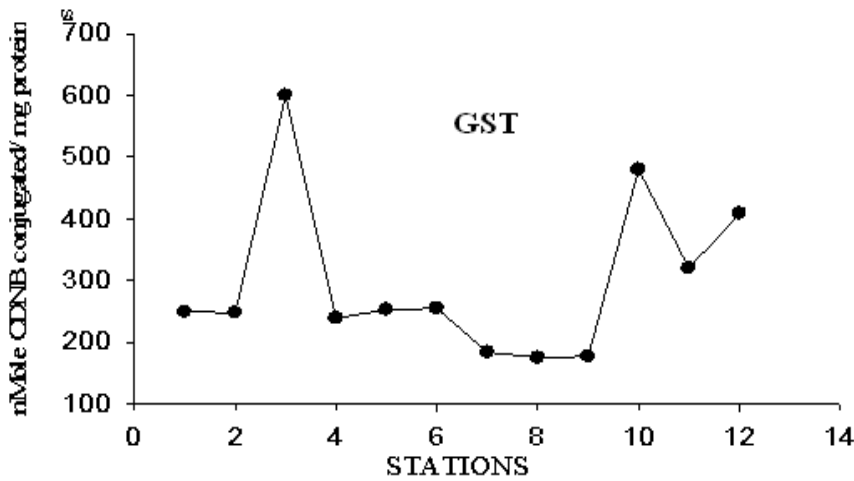


Fig-2B. Glutathione S-transferase (nMole CDNB formed/ mg proteins)

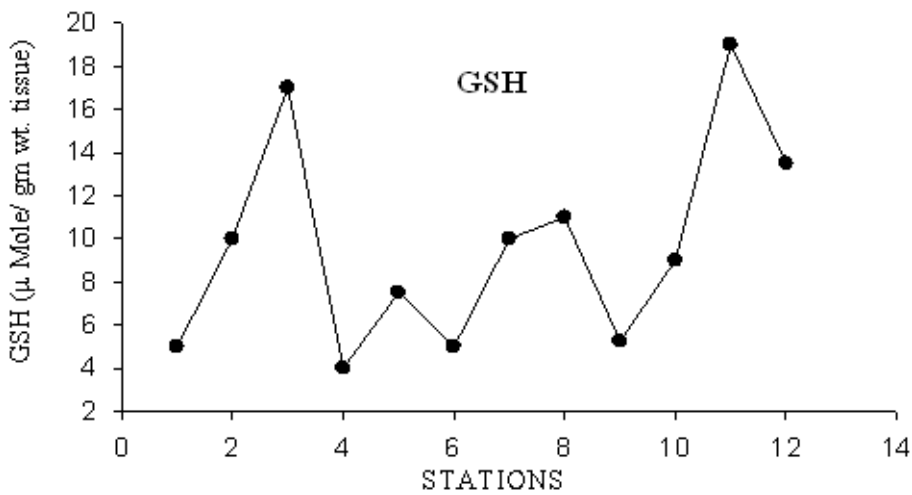


Fig-2C. Reduce glutathione level (µ mole/ mg weight tissue)

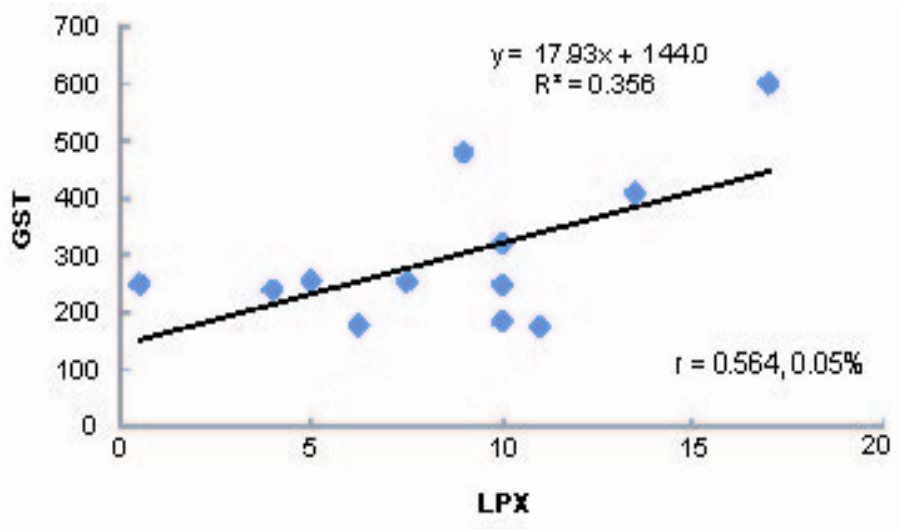


Fig. 3B. Correlation between GST (nMole CDNB conjugated formed/min/mg proteins) and LPX (nMole TBARS/mg protein) of mixed Zooplankton

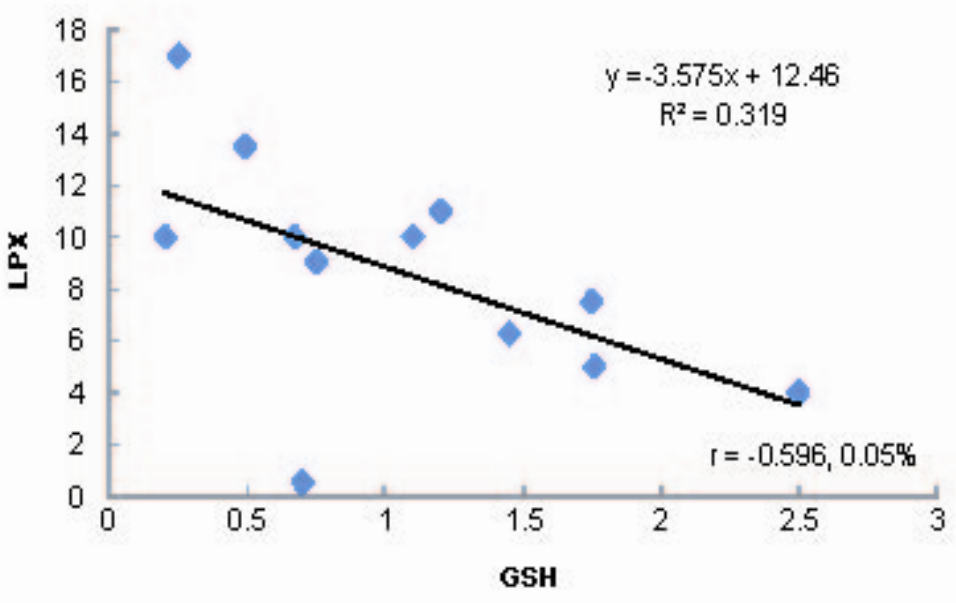


Fig. 3A. Correlation between GSH (m Mole /gm wt. tissue) and LPX (nMole TBARS/mg proteins)