Pyruvate suppresses cysteine-ascorbate deprived medium induced cytotoxicity in Giardia lamblia
6.1 Introduction:

*Giardia lamblia* also known as *Giardia intestinalis* or *Giardia duodenalis* is a causative agent of Giardiasis. It is mostly responsible for food-borne and waterborne diarrhoea worldwide (Adam, 2001). It imparts to an estimated 280 million symptomatic human infections (called Giardiasis) every year (Lane & Lloyd, 2002) and has been included as part of WHO Neglected Disease Initiative since 2004 (Bhatnagar et al., 2014). The symptoms of Giardiasis appear after 6-15 days of infection and it is characterized by watery diarrhoea, epigastric pain, nausea, and vomiting, irritable bowel syndrome and weight loss (Farthing, 1997). The clinical impact of the disease is stronger in the case of children and in undernourished or immunodeficient individuals. Although it cannot invade and secrete any known toxin but recent data suggest that *Giardia* increases intestinal permeability by induction of apoptosis of intestinal epithelial cells. There are many drugs available, but metronidazole is the most commonly used drug for the treatment of Giardiasis. *Giardia lamblia* does not usually tolerate elevated oxygen pressure and in the upper intestine where this organism generally inhabits, the oxygen (O$_2$) concentration there has been measured at 60μM (Atkinson, 1980). In addition to this, the amitochondriate parasite lacks some of the conventional enzymes that detoxify reactive oxygen species (ROS), such as superoxide dismutase (SOD), catalase, peroxidase, glutathione reductase, (Brown et al., 1995). Cysteine is not synthesized de novo and is not synthesized from cystine. It appears to be imported into the cell by passive diffusion, although active transport may account for some of the acquisition of cysteine (Lujan et al. 1994). The importance of free thiol (-SH) groups on the surfaces of trophozoites was demonstrated by the toxicity of thiol-blocking agents that are unable to penetrate intact cells (Gillin et al. 1984). This toxicity suggests that these agents are reacting with thiol (-SH) groups on the trophozoite 64 surfaces, killing the trophozoites. Cysteine appears to be the major thiol (-SH) group present in *Giardia* (Brown et al., 1993). The trophozoites of *Giardia* are also protected by ascorbic acid under high partial pressure of oxygen (Tekwani et al. 1999). We have previously observed that cysteine-ascorbate deprivation generates reactive oxygen species (ROS) in *Giardia lamblia* that had a tendency towards apoptotic death (Ghosh et al., 2009). Some of the genes which are differentially regulated during cysteine-ascorbate deprived medium stress are involved in different pathways in *Giardia lamblia* (Raj et al., 2014).

Pyruvate formed by glycolysis is a key intermediate of energy metabolism and is the precursor of most metabolic end products which are dependent on the ambient values for
oxygen tension (Paget et al., 1993). In *Giardia lamblia* the conversion of acetate from pyruvate, a very important metabolic sequence of energy metabolism occurs in the cytoplasm. The energy of thioester bond of acetyl-CoA is always conserved by substrate level phosphorylation. Thus acetate formation plays a crucial role in *Giardia*’s energy metabolism. It has been hypothesized that change in redox state of NAD(P)H pools affect the relative rates of end product formation (Mead, 1976). The detailed mechanism is unknown till today by which the parasite could relieve the detoxification of ROS produced during an oxidative stress. In this study, we have demonstrated the effect of pyruvate on oxidative stress management in *Giardia lamblia* under cysteine-ascorbate deprived medium stress.

6.2 Materials and methods:

Chemicals and solutions

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pyruvate (sodium salt), menadione, acetate, malate, α-ketoglutarate, mannitol, oxaloacetate etc. solutions were prepared freshly on the day of the experiment. For experimentation, same set of cells were taken from individual experiment.

6.2.1 Culture methods

*Giardia lamblia* Portland1 strain trophozoites were maintained in TYIS-33 medium, supplemented with Penicillin (100 U/ml), Streptomycin (100 mg/ml) and 10% adult bovine serum. All experiments were conducted with trophozoites that had been harvested during the mid-exponential phase of growth by centrifugation at 2000 rpm for 10 min and resuspended in PBS buffer (pH-7.2) containing 150 mM NaCl, 5 mM K₂HPO₄ and 1.8 mM KH₂PO₄. *Giardia* trophozoites that were exposed to cysteine-ascorbate deprived medium lost their viability at a greater rate than the trophozoites that were incubated with TYIS-33 medium (Raj *et al.*, 2014).

6.2.2 Confocal microscopy

Reactive oxygen species in *Giardia* were assessed by using dichlorodihydrofluoresceine diacetate (H₂DCFDA) fluorescent probe, according to Schuessel *et al.*, (2006) with few modifications. Cysteine-ascorbate deprived medium treated trophozoites were previously incubated with different metabolites (pyruvate, acetate, ascorbate etc.) at a concentration of 2mM. H₂DCFDA is a membrane permeable dye and therefore we have not used any mild
detergent for permeabilization and the cells were fixed with 2% paraformaldehyde after washing them with PBS twice. Consequently, observations were made with a confocal microscope (LSM510, Meta; Carl Zeiss, Thornwood, NY, USA).

6.2.3 Determination of total ROS generation by spectrofluorometer

*Giardia* trophozoites (10⁷ cells/ml) were incubated in 10⁹ the presence and absence of different concentration of pyruvate (0-5 mM) under cysteine-ascorbate deprivation. After 2 hours trophozoites were washed and resuspended in phosphate buffer saline. ROS Levels were determined by spectrofluorometer (QuantaMaster30, Photon Technology International) at 530 nm after excitation at 488 nm.

6.2.4 Viability assessment by Flowcytometric analysis

Treated and untreated trophozoites were previously incubated with or without pyruvate. Measurement of PI fluorescence with a Becton-Dickinson FACSARIA-III flowcytometer (BD Biosciences, San Jose, USA) was performed. Menadione (2-methyl-1, 4-napthoquinone) induced ROS generation was also determined in *Giardia* trophozoites previously incubated with or without pyruvate. After 2 hours incubation, trophozoites were washed with PBS and added H₂DCFDA (1.5 μM). The H₂DCFDA loaded trophozoites were incubated in the dark at 37 °C for 15 min. Then, the trophozoites were washed twice with PBS and resuspended in PBS for flowcytometric analysis. All the data were analyzed by WinMDI 2.9 software.

6.2.5 TBA assay

The TBA assay was used to assess the MDA concentration as described by the Bar-Or *et al.*, (2001) with a slight modification. Malondialdehyde (MDA) is produced by the process of lipid peroxidation. This is a later product in the sequence of lipid peroxidation reactions (Evans *et al.*, 1999; Rael *et al.*, 2004). It produces a chromophore when reacts with thioburbituric acid. The absorbance of the chromophore was measured at 535 nm. The MDA concentration is presented as nmoles of MDA produced/mg protein using a molar extinction coefficient of 1.56 x105 M-1cm⁻¹.

6.2.6 Determination of intracellular pyruvate concentration

Intracellular pyruvate concentration was determined under cysteine-ascorbate deprivation at several time points by using the Pyruvate Assay Kit (ab65342). Trophozoites (10⁷ cells/ml) were incubated under cysteine-ascorbate deprived medium stress for 0 to 8 hours.
Trophozoites were harvested and homogenization procedure was performed. 6% perchloric acid (PA) was added to inactivate the enzyme. Supernatant was collected and used for pyruvate assay according to the manufacturer’s protocol.

6.2.7 DNA fragmentation assay
Untreated and cysteine-ascorbate deprived medium treated (for 12 hours at 35.5°C) trophozoites were taken. The assay was performed according to the Nandi et al., 2010 with little modification. After phenol–chloroform treatment, salt precipitation and 70% ethanol wash, the pellet was air dried and resuspended in autoclaved triple distilled water and checked in a 1.5% agarose gel stained with ethidium bromide.

6.2.8 Cell death study with different metabolites
Demonstration of cell death was studied by the use of Annexin-V FITC assay kit (IM3546). As cysteine-ascorbate deprived medium have been reported to induce DNA damage (Ghosh et al., 2009), we have estimated the effects of pyruvate during incubation under cysteine-ascorbate deprived medium on DNA damage in Giardia lamblia. Trophozoites were incubated for 8 h cysteine-ascorbate deprived medium (previously incubated for 2 h) with different substrates at same concentration (2 mm). Trophozoites were harvested and washed with ice-cold PBS and collected. After that, the assay was performed according to the manufacturer’s protocol and analyzed by flowcytometry.

6.2.9 Real-Time PCR for gene expression of pyruvate metabolism pathway
Treated and untreated trophozoites were harvested as per experimental procedure and the total RNA was extracted by TRIZOL (Invitrogen) method and gene expression studies were performed according to the Raj et al., 2014.

6.2.10 Statistical analysis
All the experiments were performed at least thrice in triplicates and the results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was evaluated by t-test or one way ANOVA followed by Kruskal-Wallis test (wherever applicable), using Graph Pad Prism software, version 4 (GraphPad Software, Inc, San Diego, CA); P<0.05 was considered as statistically significant.
6.3 Results:

6.3.1 Effect of pyruvate on ROS generation in trophozoites of *Giardia* under CAD stress

The H2DCFDA, a non-fluorescent molecule, is able to enter the cells. After getting entry into the cytosol of the cell, esterase activity renders the indicator, non-permeable by forming fluorescent product dichlorofluoresceine and the fluorescence intensity of the dye is proportional to the rate of oxidation by reactive oxygen species. Observation of cellular fluorescence in the trophozoites was examined by confocal microscopy under cysteine-ascorbate deprived medium stress conditions. Our results confirmed that exogenously added physiological concentrations of pyruvate attenuate fluorescence, produced by reactive oxygen species (Fig. 6.1).

![Figure 6.1: H2DCFDA-loaded cells under confocal microscope after cysteine-ascorbate stress.](image)

Increases in fluorescence are representative of increase in the rate of oxidative species generated. Fluorescence was monitored from the suspension of live cells treated with cysteine-ascorbate deprived medium in the absence and presence of pyruvate (2 mM) and acetate (2 mM). We used mannitol (2 mM) and ascorbate (2 mM) as a positive control and without metabolite as a negative control.

6.3.2 Pyruvate attenuate total ROS production in *Giardia* trophozoites upon cysteine-ascorbate deprivation

Pyruvate decreases total ROS production in *Giardia* during cysteine-ascorbate deprivation stress. Trophozoites were previously incubated with increasing concentrations of pyruvate from 0.0001 to 5 mM and then exposed in cysteine-ascorbate deprived medium. The level of
ROS was measured in *Giardia* trophozoites with or without pyruvate. The ROS level increased significantly (P < 0.01) by cysteine-ascorbate deprivation than H2DCFDA-loaded untreated trophozoites. The fluorescence intensity was lowered significantly (P < 0.05) in the presence of pyruvate for the range of concentrations from 2 to 5 mM (Fig. 6.2).

**Figure 6.2:** Pyruvate decreases the level of ROS in the medium of *Giardia* lamblia trophozoites after treated with cysteine-ascorbate deprived medium. *Giardia* trophozoites were exposed to cysteine-ascorbate deprived medium with increasing doses of pyruvate (from 0-2mM). Levels of ROS were estimated by spectrofluorometry using 2’, 7’- dichlorodihydrofluoresceine diacetate. The data are from six representative experiments.

6.3.3 Pyruvate protects *Giardia* trophozoites from the toxicity induced cysteine-ascorbate deprived (CAD) medium

Flowcytometry was performed to confirm the antioxidant activity of pyruvate in *Giardia* trophozoites. Trophozoites were incubated for 2.5 h with increasing concentration of sodium pyruvate under CAD medium for 6 h. Cysteine-ascorbate deprivation reduces trophozoites viability to 48.33%, which was significantly lower than the untreated trophozoites (93.6%, P < 0.001) (Fig. 6.3). The trophozoites previously incubated with Pyruvate (2 mM) and (5 mM) protects trophozoites significantly at 87.1% and 89.3% from cysteine-ascorbate deprived medium toxicity. When treated under CAD medium, trophozoites were increasingly protected by increasing concentrations of pyruvate. Acetate, produced from pyruvate, did not vary the
viability of *Giardia* either in control conditions or treated with cysteine-ascorbate deprived medium.

**Figure 6.3:** Pyruvate protects *Giardia* trophozoites from cysteine-ascorbate deprived medium induced toxicity. Cultured *Giardia* trophozoites were incubated at 35.5 °C under cysteine-ascorbate deprivation for 4 h with increasing concentration of pyruvate. Data are the mean ± SEM of three independent experiments, each performed in triplicate.

### 6.3.4 Pyruvate suppresses menadione induced cytotoxicity caused by cysteine-ascorbate deprivation in *Giardia* trophozoites:

A progressive increase in the production of reactive oxygen species (as indicated by the enhancement in fluorescence intensity) with the incubation under CA-deprived medium increased the rate of reactive oxygen species generation (**Fig.6.4**). Prior incubation of H2DCFDA loaded cells with increasing concentration of pyruvate (0.01 mM, 0.5 mM, and 2 mM) for 2 h attenuated the both stressed induced fluorescence. Exposure of *Giardia* trophozoites in increasing concentrations of menadione for 1 h induced ROS generation that was significantly reduced by 2 mM sodium pyruvate.
Figure 6.4: (B) Flowcytometric histograms of intracellular fluorescence intensity (488nm excitation, 530nm emission) in *Giardia lamblia* trophozoites under cysteine-ascorbate deprivation. The various cell populations reflect different treatments: (a) fluorescence arising from H$_2$DCFDA-loaded without cysteine-ascorbate deprived medium treated cells, (b) H$_2$DCFDA-loaded cells under cysteine-ascorbate deprived medium previously incubated with pyruvate (2 mM). (c) H$_2$DCFDA-loaded cells under cysteine-ascorbate deprived medium previously incubated with pyruvate (0.5 mM), (d) H$_2$DCFDA-loaded cells under cysteine-ascorbate deprived medium previously incubated with pyruvate (2 mM) in the presence of menadione (10 µM), (e) H$_2$DCFDA-loaded cells under cysteine-ascorbate deprived medium previously incubated with pyruvate (2 mM) in the presence of menadione (30 µM), (f) H$_2$DCFDA-loaded cells under cysteine-ascorbate deprived medium, (g) H$_2$DCFDA-loaded cells under cysteine-ascorbate deprived medium previously incubated with menadione (10 µM), (h) H$_2$DCFDA-loaded cells under cysteine-ascorbate deprived medium previously incubated with menadione (30 µM).

6.3.5 Determination of antioxidant activity of different metabolite pre-incubated with trophozoites under CAD stress

We have also observed that the protective effects of pyruvate and other α-ketoacids against cysteine-ascorbate stress. *Giardia* trophozoites were incubated under cysteine-ascorbate deprived medium for 6 hours after 2 hours initial incubation in the presence of α-ketoglutarate and oxaloacetate, mannitol, malate, β-ketoglutarate. All the compounds were added at a concentration of 2 mM. In particular, acetate was found to be ineffective, whereas oxaloacetate strongly prevented CAD medium cytotoxicity by 60.6% . Viability of trophozoites under CAD medium treated samples incubated with pyruvate and malate was restored to 88% (P < 0.01) and 78%, respectively; however, the viability of trophozoites were 53% and 40.6%
when it was previously supplemented with mannitol and acetate, respectively, and was found to be significantly lower than the control (P < 0.001) (Fig.6.5).

Figure 6.5: ROS scavenging capacities and antioxidant properties of various α-ketoacids.

Cultured *Giardia* trophozoites were preincubated for 60 min with a 2 mM concentration of each compound (oxaloacetate, malate, pyruvate, α-ketoglutarate, mannitol, acetate) and further incubated under cysteine-ascorbate deprived medium for 4 h in their presence or absence. Data are the mean ± SEM of three independent experiments, each performed in triplicate.

6.3.6 Measurement of degree of lipid peroxidation in trophozoites under cysteine-ascorbate deprivation

The degree of lipid peroxidation has been determined on the basis of malondialdehyde (MDA) formation. We have measured lipid peroxidation status in *Giardia lamblia* under cysteine-ascorbate deprived medium stress with or without pyruvate supplementation. Lipid peroxidation was found to be increased significantly by 43% (P < 0.01) in trophozoites under cysteine-ascorbate compared to the untreated trophozoites. Supplementation of pyruvate (5mM) significantly decreased the lipid peroxidation to 48% (P < 0.05) in the trophozoites under CA-deprived medium stress compared to the stressed trophozoites without pyruvate incubation (Fig.6.6).
Figure 6.6: Measurement of MDA concentration in cultured *Giardia* trophozoites under cysteine-ascorbate deprivation: MDA concentration in *Giardia* trophozoites treated with cysteine-ascorbate deprived medium after 8 h incubation. Values are mean ± SEM of three independent experiments.

### 6.3.7 Measurement of pyruvate concentration in *Giardia* trophozoites under cysteine-ascorbate deprivation

The intracellular pyruvate concentration in *Giardia lamblia* trophozoites was measured during cysteine-ascorbate deprived medium induced stress conditions. Pyruvate content was quantified in trophozoites under cysteine-ascorbate deprived medium stress. Intracellular pyruvate concentration was observed to be increasing significantly from 0.5 μmol/mg proteins to 2.1 μmol/mg proteins (P < 0.001) after 2 h under cysteine-ascorbate deprivation in *Giardia* trophozoites (Fig.6.7). However, with time pyruvate concentration was decreased to 1.35 μmol/mg proteins from 2.1 μmol/mg proteins between 2nd and 4th hour and it remained almost same 1.3 μmol/mg proteins at 4th and 6th hour. Interestingly, after 8 hours of stress under cysteine-ascorbate deprivation, the concentration of pyruvate was significantly lowered to 0.97 μmol/mg proteins (P < 0.01). However, the trophozoites seemed to adjust between production and consumption of pyruvate in order to maintain the intracellular pyruvate level.
at and around 0.5 μmol/mg proteins (the normal pyruvate concentration in the trophozoites) cysteine-ascorbate deprivation.

![Graph showing intracellular pyruvate concentration](image)

**Figure 6.7: Intracellular pyruvate concentration in *Giardia* trophozoites during oxidative stress.** Intracellular pyruvate content in *Giardia lamblia* under cysteine-ascorbate deprived medium. The level of intracellular pyruvate was quantified every two hours interval. Values are means ± SEM of three independent experiments.

### 6.3.8 DNA fragmentation assay

The hallmark of apoptosis in mammalian cell is the degradation of genomic DNA. Therefore, we examined the DNA fragmentation pattern for untreated and stressed-induced trophozoites and also in cysteine-ascorbate stressed-induced trophozoites previously supplemented with pyruvate. The stressed-induced trophozoites showed a DNA fragmentation pattern after 8 h exposure to cysteine-ascorbate deprived medium. The ladder pattern was not clear as a metazoan DNA ladder and showed some degree of smearing with fragmented DNA in the low molecular weight region, identified by electrophoresis on a 1.5% agarose gel (Fig.6.8).
**Figure 6.8: Effect of pyruvate on DNA fragmentation.** Electrophoretic analysis of DNA fragmentation on a 1.5% agarose for *Giardia* trophozoites treated with H$_2$O$_2$ and also treated with cysteine-ascorbate deprived medium in the absence and presence of pyruvate.

**6.3.9 Quantification of viable *Giardia* trophozoites recovery from stress after incubation with pyruvate**

Externalization of phosphatidyl serine upon exposure under cysteine-ascorbate deprived medium. We observed that the rate of recovery of the stressed *Giardia* trophozoites increased with the increase in pyruvate concentration. In these rescue studies the major goal for administering pyruvate was to provide the substrate distal to glycolysis, thereby boosting ATP generation and acetate production. We tried to rescue trophozoites of *Giardia lamblia* by adding a pyruvate. Survival rate was estimated 24 h later. We have observed that with time, the rate of recovery has increased for pyruvate after cysteine-ascorbate deprivation stress (Fig. 6.9).
Figure 6.9: **Quantification of viable cells:** Counts of viable cells are given as percentages of the total number of cells. *Giardia* trophozoites were incubated under cysteine-ascorbate deprived medium for 6 h and after that pyruvate was added and then further incubated for 30-120 min. Results are from three independent experiments. Pyr = pyruvate.

6.3.10 Transcriptional regulation of gene expression in *Giardia* trophozoites under cysteine-ascorbate deprivation

To understand the effect of cysteine-ascorbate deprivation stress in transcriptional regulation of gene expression in *Giardia lamblia*, we performed a time course analysis of gene expression of pyruvate metabolism pathway under cysteine-ascorbate deprived medium stress using a quantitative RT-PCR. We have chosen six genes, related to the pyruvate metabolism of *Giardia lamblia* modulated by at least 2 fold at one or more time points in response to cysteine-ascorbate deprivation. In our study, we have shown that arginine deiminase (ARGD)-encoding gene was up-regulated in *Giardia* trophozoites during cysteine-ascorbate deprived medium stress. In *Giardia lamblia*, pyruvate can be produced by three different pathways. Malate dehydrogenase (MDH) gene was up regulated at one or more time points upon cysteine-ascorbate deprivation. The gene showed a maximum induction at 6th hour of cysteine-ascorbate deprivation. In response to cysteine-ascorbate deprivation pyruvate kinase remained down-regulated after 6th hour time points, but up-regulated after 8 hour time points (Fig.6.10). In our study, PFOR-encoding gene was up-regulated during the first couple of hours under cysteine-ascorbate deprived medium stress. The enzyme acetyl-CoA synthase
transcript was up-regulated during cysteine-ascorbate deprived medium stress. However, cysteine-ascorbate deprivation has been observed to significantly modulate the metabolic flux across the pyruvate metabolism in *Giardia lamblia*.

**Figure 6.10: Effect of CA deprived medium on the expression of genes involved in pyruvate metabolism.** Modulation of transcripts encoding enzymes involved in pyruvate metabolism. A. Gene expression (fold change) under cysteine-ascorbate deprivation. Data are shown as fold change in relative expression compared with Actin on the basis of Comparative Ct ($2^{-\Delta\Delta Ct}$) method. Values are shown as mean ± SEM of three independent experiments, each performed in triplicate. (Gene abbreviation used: **Metabolic enzymes:** PFOR: Pyruvate-ferredoxin oxidoreductase, MALDH: Malate dehydrogenase, ARGD: Arginine deiminase, PK: Pyruvate kinase, PDK: Pyruvate dikinase, ACS: Acetyl coA synthase).

**6.4. Discussion:**

The present study demonstrates that pyruvate administration protects *Giardia* trophozoites against the cytotoxicity induced by cysteine-ascorbate deprived medium. It was previously stated that cysteine-ascorbate deprived medium produces reactive oxygen species (ROS) in *Giardia*. To establish its pathogenesis *Giardia* has to develop an antioxidant protective mechanism. The antioxidant protective effect of α-ketoacids has already been investigated both in vitro in several cell types (Andrae *et al.*, 1985) and in vivo in whole organs such as heart (Crestanello *et al.*, 1995). It is well-known that pyruvate nonenzymatically reacts with hydrogen peroxide produces acetate, CO$_2$, H$_2$O (Holleman, 1904).

With the help of the H$_2$DCFDA-based assays, intracellular generation of reactive oxygen species by *Giardia lamblia* was monitored (Biagini *et al.*, 2001). Cysteine-ascorbate deprived medium produces intracellular ROS in *Giardia* and the fluorescence was also augmented by
the addition of synthetic quinine menadione. Our results proved that intracellular generation of ROS was reducing by the supplementation of pyruvate and also attenuate the menadione induced ROS generation. It was also shown to reduce the generation of total fluorescence arising from the oxidation of the H$_2$DCFDA by spectrofluorometer and may also have played a direct antioxidant role in *Giardia*. The protective effect of pyruvate was reproduced by several α-ketoacids, which share with pyruvate the ability to react with intracellular ROS; these compounds include mannitol, which is not an energy substrate. We have observed that pyruvate showed that higher capacity to scavenge ROS.

It has been observed that elevated ROS generation is very much related to a decline in the production of ATP as well as protein synthesis and increase in DNA breakage and lipid peroxidation (Herbener, 1976; Vorbeck *et al*., 1982). In trophozoites of *Giardia* hydroxyl and super oxide radicals are generated during cysteine-ascorbate stress. These radicals then initiate membrane lipid peroxidation. Thus, pyruvate, by eliminating hydroxyl and superoxide radical, prevented lipid peroxidation. It was exciting that acetate showed inhibition of lipid peroxidation but the key mechanism is still unknown.

In our present study it was shown that intracellular pyruvate concentration was found to be increasing and decreased after several hours. It can be explained by the inactivation of pyruvate:ferredoxin oxidoreductase, an enzyme which is sensitive to oxidative stress. However, it might also result from a metabolic need. Pyruvate concentration can also be increased by the activities of pyruvate dikinase, pyruvate kinase and also malate dehydrogenase that *Giardia* can produce more pyruvate. This is indicating an enhancement of glycolysis activity in the presence of pyruvate. Increased glycolysis might thus maintain the ATP supply during oxidative stress. Such an increase in the intracellular pyruvate in response to elevated O$_2$ concentration has been reported to have protective effects against oxidative stress (Herbener, 1976).

### 6.4 Conclusion:

This study has revealed the dynamics of the transcriptional and metabolic regulatory networks during cysteine-ascorbate stress. However, the transcriptomic results have shown that the oxidative regulation is not only controlled by some other genes, but also some metabolic genes have taken a significant role in ROS detoxification (Raj *et al*., 2014). As *Giardia* does not contain any known machinery for oxidative stress management by modulating intracellular pyruvate concentration it can combat oxidative stress.