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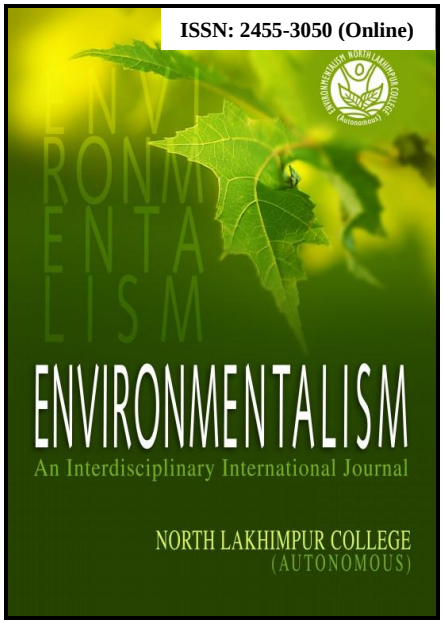

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ASTRAGALIN INDUCED ALTERATION IN THE GLYCOLYTIC ENZYMES OF HELMINTH PARASITE, *FASCIOLOPSIS BUSKI*

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Abstract

The shoot extract of *Alpinia nigra* (Family - Zingiberaceae) has been used as an ethnomedicine against parasitic helminth infections in the Northeast region of India. One of the major bioactive compounds found in the shoots of the plant is astragalin. Therefore, the present study was conducted to evaluate the anthelmintic efficacy of astragalin in *Fasciolopsis buski* using mortality observation, biochemical analysis and kinetic studies of four major glycolytic enzymes vis. PEPCK, PK, MDH and LDH. *In-vitro* study showed complete inactivation and flaccid paralysis of the parasites followed by death. Biochemical analysis showed significant inhibition of all the enzymes in the treated parasites. Kinetic studies revealed the changes in kinetic parameters on exposure to astragalin and praziquantel. The present study therefore suggests that astragalin may be useful as a therapeutic agent to treat trematode parasite.

Keywords: Astragalin, glycolytic enzymes, *Fasciolopsis buski*, kinetic study

1 Introduction

Extractions of energies from glucose through various metabolic pathways are important to organism's survival and therefore act as potential targets for chemotherapeutic drugs (Timson 2015). Helminth parasites are a group of cryptic organisms with poorly known reasons which enable them to adapt inside the hosts by switching on to different metabolic pathways causing considerable damages to the host body (Kita *et al.* 2010; Wolff *et al.* 2012). They can fortify their parasitic mode of life by changing the metabolic pathway from aerobic (in miracidia and cercaria stage) to anaerobic (in adult stage) mode of respiration (Von brand 1979; Mansour 2012). Besides, the high degree of similarity between the host and parasite enzymes there are some enzymes with functions opposite to each other. In helminth parasites, phosphoenolpyruvate (PEP) an intermediate of glucose metabolism can be metabolized either to pyruvate via pyruvate kinase (PK) and hence to lactate in presence of lactate dehydrogenase (LDH) or via phosphoenolpyruvate carboxykinase (PEPCK) to oxaloacetate which is reduced to malate in presence of malate dehydrogenase (MDH) (Mansour 2012; Barrett 2009). PEPCK, therefore, acts as a distinguishing enzyme between

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parasites and its mammalian hosts. This difference in energy transducing systems between helminths and hosts indicates the presence of unique pathways in helminth parasites which can be exploited selectively as target for chemotherapy ((Mansour 2012).

A large number of existing drugs to treat helminth infections were found to interfere with the metabolic pathways of helminth parasites. Effects of praziquantel (PZQ), levamisole (LEV), mebendazole (MBZ), fenbendazole (FBZ), diethylcarbamazine (DEC), tetramisole and albendazole (ABZ) on activity of different enzymes like LDH, HK, PEPCK, glucose-6-phosphatase etc. were well documented. Similarly, trivalent antimonials which target phosphofructokinase, clorsulon which targets phosphoglycerate mutase and phosphoglycerate kinase etc. are also used as effective anthelmintic drugs (Oztop *et al.* 1999; James *et al.* 2009). The effectiveness of commercial drugs is, however, interfered by the development of resistance capacities against the existing drugs. Researches has endorsed to such kind of resistance capacity to frequent and unlimited use of the same drug (James *et al.* 2009; Kaplan and Vidyashankar2012). Furthermore, its limited availability and high costs has encouraged the use of traditional medicinal plants as an alternative to the synthetic drugs to treat helminthiasis (Mali and Mehta 2008; Dasgupta *et al.* 2010; Tandon *et al.* 2011).

Astragalin (synonym: kaempferol-3-O-glucoside) is a newly found bioactive compound from the shoots of *Alpinia nigra* (Qiao *et al.* 2007). Recently, this compound has also been reported from many other plants such as leaves of *Cassia alata*, *Rosa agrestis* and green tea seeds (Bitis *et al.* 2010; Lee *et al.* 2011; Saito *et al.* 2012). Biologically, the compound is found to be active as antioxidant, anti-inflammatory, antitumor and inhibitor of endotoxin-induced oxidative stress (Bitis *et al.* 2010; Burmistrova *et al.* 2011; Ma *et al.* 2015). Further, this compound inhibits the dermatitis development and IgE elevation in models of passive cutaneous anaphylaxis and atopic dermatitis NC/Nga mice and autophagy-associated airway epithelial fibrosis (Kotani *et al.* 2012). Recent studies in our laboratory have confirmed the effectiveness of *Alpinia nigra* and its bioactive compound astragalin as potential anthelmintic agent (Roy *et al.* 2012; Swargiary and Roy 2015; Swargiary 2015). The tegumental structure and its enzymes like acid – and alkaline phosphatase of *Fasciolopsis buski* were found to be altered significantly when exposed to astragalin. Because of its pharmacological significance, the present study was designed to explore the *in-vitro* effect of astragalin on the glycolytic enzymes activities of *F. buski*.

2 Materials and Methods

2.1 Drugs and chemicals

Astragalin (AST) was obtained from Sigma (St. Louis, USA), whereas praziquantel (PZQ) was obtained from Chandra Bhagat Pharma Pvt. Ltd, Mumbai, India. All other enzymes and co-enzymes were obtained either from Himedia (Mumbai, India) or SRL (Mumbai, India). All the chemicals used were of analytical grade. Milli-Q water was used for all the experiments.

2.2 *In-vitro* mortality study

Fresh, adult *F. buski* were collected from the intestine of freshly slaughtered pig from local pig slaughterhouse at Shillong. After acclimatization in laboratory, the parasites were processed for mortality study following the protocol as described earlier (Swargiary *et al.* 2013a). The various concentrations of AST and PZQ taken were 25 and 50 µg/mL phosphate buffered saline (pH 7.4), respectively. As soon as the parasites were paralyzed they were collected and kept at -20ºC for enzymatic studies.

2.3 Biochemical Studies for PEPCK, PK, LDH and MDH

2.3.1 Tissue processing

A 10% tissue homogenate (w/v) was prepared in ice –cold Tris-HCl buffer (150 mM, pH 7.5) containing 250 mM sucrose. The homogenate was centrifuged at 10,000 rpm for 20 min at Hermle Z 233MK-2 and the resultant supernatant was used for all enzyme assays. All the steps were carried out at 4°C.

2.3.2 Enzyme assays

PEPCK and PK were assayed following McManus and Smyth (1982) and McManus and Smyth (1982). Assay mixture of PEPCK consisted of 23.3 mM Tris-HCl (pH 7.4), 4 mM PEP, 40 mM $MgSO_4$, 7 mM $NaHCO_3$, 40 mM KCl, 0.1 mM GDP, 0.2 mM NADH, MDH 8 units and 50 µL tissue supernatant. PK assay mixture consists of 43.3 mM Tris-HCl (pH 7.4), 4 mM PEP, 53.3 mM KCl, 0.2 mM ADP, 5.6 mM $MgSO_4$, LDH 6 units, 0.2 mM NADH and 50 µL tissue supernatant.

Activities of LDH and MDH were measured following Bergmeyer (1974) and Bergmeyer and Bernt (1981) with little modification. For LDH, the assay composition consisted of 47 mM phosphate buffer (pH 7.4), 0.4 mM pyruvic acid, 0.2 mM NADH and 50µl of tissue supernatant. Similarly, for MDH the assay mixture was composed of 26.63 mM phosphate buffer (pH 7.4), 0.5 mM Oxaloacetic acid, 0.2 mM NADH and 50µl tissue supernatant.

2.3.3 Partial Characterization of PEPCK, PK, LDH and MDH

Partial characterizations of all the four glycolytic enzymes were performed in crude tissue homogenate of fresh untreated parasites. The effects of pH, temperature (T), substrate concentration (S) were studied to provide maximum activity of all the enzymes studied. Inhibition properties of AST on enzymes have also been studied by direct mixing of astragalin to the assay mixture. The concentrations taken were 10 and 25 µM for both the drugs. Partial characterization of the enzymes were done following the method as described earlier in our publication (Swargiary *et al.* 2013b) keeping the content of assay mixture as mentioned above.

Enzyme activities (V) were measured using double beam UV-visible spectrophotometer (Beckman Model-26) at 340 nm wavelength keeping 3 mL as the final volume of the reaction mixture. The enzyme activity was calculated using 6.2×10^3 $m^{-1}cm^{-1}$ as the molar extinction coefficient value for NADH. One unit of enzyme activity is defined as the amount of enzyme which catalyzes the oxidation of 1 µM of NADH per minute. For kinetic studies, enzyme activities were represented only as specific activity and the values of PEPCK, PK and LDH were multiplied by 100 and MDH by 10 because of decimal values of the same.

The protein content of all the tissues was estimated following the method of Lowry *et al*. (1951) using bovine serum albumin as a standard protein.

3 Statistical calculations

Data collected from three replicates (n = 3) were statistically analyzed and were presented as means ± standard error of means (SEM). Comparisons of the paired mean values between the experimental data and respective controls were made using Student's t-test with $p<0.05$ taken as the threshold of significance.

4 Results

4.1 In-vitro mortality and enzyme study

The efficacy of *A. nigra* bioactive compound astragalin and PZQ were presented in the table 1. On exposure to different concentrations of AST and PZQ, the parasite showed dose-dependent mortality. At maximum concentration (0.10 mg/mL) of astragalin the time taken for paralysis and death were 9.76±1.05 h and 10.78±1.47 h, respectively which is almost similar to PZQ at concentration 25 µg/mL. Commercial drug, PZQ showed better activity compared to astragalin. The mortality study however, indicates the potency of astragalin as anthelmintic agent.

Table 1: Effects of different concentrations of astragalin and PZQ on the mortality of *F. buski*.

Incubation	**Doses** (µg/mL)	**Mortality (in hour)**	
		Paralysis	**Death**
Control	PBS	-	21.05±0.25
AST	25	15.79±0.71	16.70±0.14
	50	13.56±1.28	15.44±0.51
	100	9.76±1.05	10.78±1.47
PZQ	25	9.62±1.18	11.26±1.33
	50	8.77±0.81	9.57±2.03
	100	6.76±1.44	8.37±1.27

Values are represented as mean ± SEM (n = 3). All the values are significant at $p<0.05$ probability level.

4.2 Biochemical analysis and Kinetic studies

Biochemical enzymatic study of the four major glycolytic enzymes namely PEPCK, PK, LDH and MDH both in treated and control parasites revealed significant ($p<0.05$) difference between the two. All the enzymes showed

higher activity in control untreated parasites (Table 2). MDH showed highest activity (28.72±0.57 Units/mg protein) among all the four enzymes followed by LDH (2.30±0.32 U/mg protein), PK (2.13±0.32 U/mg protein) and PEPCK (1.30±0.15 U/mg protein), respectively. The tissue protein of control *F. buski* was found to be 10.99±0.16 mg/g of wet tissue. On exposure to AST treatment, the parasites showed reduction in all the four glycolytic enzyme activities. Highest reduction in enzyme activity was observed in LDH (36%) followed by PEPCK (31%), PK (19) and MDH (17%), respectively. The reference drug PZQ also showed more or less similar type of activity against the enzymes studied. Like AST, PZQ also showed strong activity against PEPCK and LDH, while AST showed better activity against PK and MDH compared to reference drug PZQ (table 2).

Table 2: Effects of different plant extracts on vital glycolytic enzymes of *F. buski*.

	Enzyme activities (Total/Specific*)							
	PEPCK	**%In**	**PK**	**%In**	**LDH**	**%In**	**MDH**	**%In**
Control	1.30±0.15/ 0.12±0.01*		2.13±0.32/ 0.19±0.01*		2.30±0.32/ 0.21±0.01*		28.72±0.57/ 2.89±0.06*	
AST	0.89±0.11/ 0.08±0.02*	31.54/ 33.33*	1.71±0.05/ 0.14±0.04*	19.72/ 15.79*	1.47±0.44/ 0.14±0.05*	36.09 33.32*	23.66±0.56/ 2.15+0.16*	17.62 25.60*
PZQ	0.78±0.46/ 0.09±0.01*	40.00/ 25.00*	1.97±0.36/ 0.18±0.16*	7.50/ 5.23*	1.40±0.59/ 0.18±0.31*	39.13 14.29*	26.12±0.03/ 2.66±0.01*	9.05 7.96*

Values are given as mean ± SEM from three replicates (n = 3). Total activity - formation of 1 µmole of product per min per gram of wet tissue weight. *Specific activity - total activity/mg tissue protein. %In – percentage inhibition

The influence of pH, reaction temperature, substrate concentrations and Lineweaver-Burk plot of substrate *vs*. PEPCK activity *in-vitro* exposure to AST and PZQ has been shown in figure 1. In normal untreated conditions, PEPCK showed optimum enzyme activity (11.67±0.09 U/mg protein) in the pH range 7.0 to 8.0 when the reaction was conducted at temperature 30ºC (Fig. 1a & b). A good correlation could be observed between the pH and enzyme activity. Above or below the optimum pH range, the enzyme showed reduction in activity. Similarly, when the reaction was conducted at the lowest temperature of 5ºC PEPCK showed very less activity (Fig. 1b) followed by increase in activity with the increase of reaction temperature showing maximum at 30ºC (12.38±19 U/mg protein). Further increase in temperature decreased the enzyme activity. Phosphoenolpyruvate, the substrate for PEPCK enzyme when increased from 0.5 mM to 5.0 mM showed good correlation (R^2 = 0.9717) between enzyme activity and substrate concentration. Figure 1c showed the increase in PEPCK activity in relation to the increase concentration of PEP showing hyperbolic curve. When the substrate concentrations were plotted against the enzyme activities in a Lineweaver-Burk Plot the K*m* and V*max* values of control parasite were found to be 2.54 mM and 17.18 U/mg tissue proteins, respectively (Table 3). Assay mixture when incubated with different concentrations (25 and 50 µM) of AST and PZQ, changes were observed in the enzyme activities as well as kinetic parameters of PEPCK. The V*max* values were found to be reduced on exposure to different treatments compared to control *F. buski* while the K*m* value were observed to have been increased in both the astragalin and PZQ treated parasites.

Alterations in the kinetic parameters on exposure to different treatments suggest a mixed type of enzyme inhibition in PEPCK under experimental conditions (Table 3).

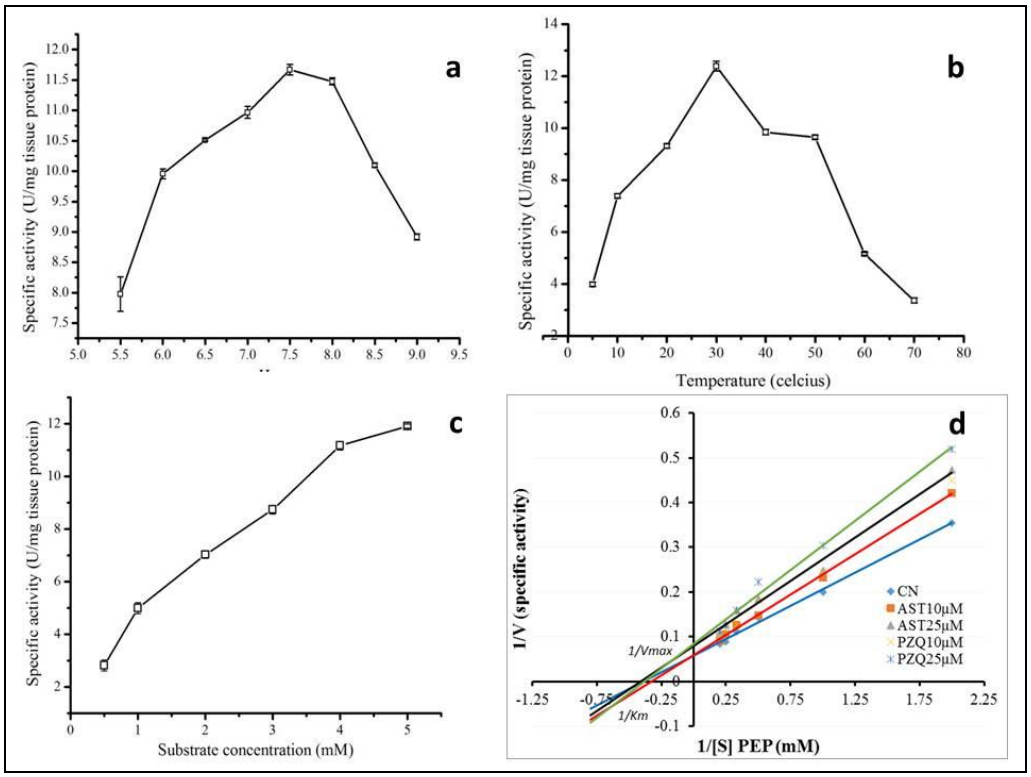

Figure 1. Influence of (a) pH, (b) temperature and (c) substrate concentration and (d) Lineweaver-Burk plot of substrate vs. phosphoenolpyruvate carboxykinase activities in control *F. buski*. Values were shown in *specific activity x 100* because of decimal value of enzyme activity.

Similarly, the influence of pH, temperature and substrate concentration on the PK enzyme activity were shown in figure 2. At the *in-vitro* experimental conditions, the PK enzyme showed its maximum enzyme activity at the pH 7.5 when the reaction temperature was set at the range of 30 to 40ºC. Further increase of pH from 7.5 reduced the enzyme activity (fig. 2a). Similarly, the influence of temperature on the PK enzyme activity could be seen where increasing the temperature beyond 40ºC have negative effect that decreases the functioning the activity to a lowest of 1.08 ± 0.34 U/mg tissue protein at 70ºC (Fig. 2b). A plot of [V] against increasing concentrations of [S] from 0.25 to 5 mM (Fig. 2c) showed a linear increase (R^2 = 0.9683) in the enzyme activities in accordance with the normal pattern of enzymatic reactions. The maximum activity (V*max*) and K*m* was found to be 28.33 U/mg tissue protein and 3.89 mM, respectively (Table 3). On incubation of assay mixture with different concentrations of astragalin and PZQ, changes have been observed in enzyme activity of PK. When the assay mixture was treated with 25 µM of AST, the V*max* and K*m* values of PK have been reduced to 15.23 U/mg protein and 2.16 mM, respectively. Figure 2d and table 3 showed the alterations of kinetic parameters of PK enzyme on exposure to *in-vitro* treatment with astragalin indicating a mixed type of inhibition while PZQ showed uncompetitive type of inhibition in pyruvate

kinase activity.

The influence of pH, temperature and substrate concentrations on *in-vitro* LDH activity is presented in the figure 3. LDH showed optimum activity at pH 7 to 7.5 (Fig. 3a). Like other enzymes, LDH also showed increase in enzyme activity with the increase of pH up to 7.5 which decreased with the further increase of pH values. Similarly, when the reaction temperatures were set at 30 to 40ºC, LDH showed maximum activity. In accordance with the normal enzyme activity, increase in enzyme activity was observed in LDH with the increase of substrate concentration from 0.05 to 1.0 mM (Fig. 3c). The K*m* and V*max* values obtained from the Lineweaver-Burk plot of control *F. buski* were 0.15 mM and 34.96 U/mg tissue proteins (Table 3). When the assay mixture was incubated with AST and PZQ, alterations in the kinetic parameters were noticed indicating mixed and un-competitive type of inhibition, respectively.

Table 3. Alteration in kinetic parameters (K*m* and V*max*) of PEPCK, PK, LDH and MDH enzymes on control and treated *F. buski*.

Kinetic parameters		Control	Astragalin		Praziquantel	
			10 µM	25 µM	10 µM	25 µM
PEPCK	V*max*	17.18	17.03	12.67	14.64	11.99
	K*m*	2.54	3.07	2.45	2.762	2.64
Inferences			Mixed type of inhibition			
PK	V*max*	28.33	23.47	15.23	18.53	18.54
	K*m*	3.89	2.93	2.16	1.93	2.23
Inferences			Mixed type inhibition		Uncompetitive inhibition	
LDH	V*max*	34.96	28.41	23.31	22.32	22.52
	K*m*	0.15	0.13	0.12	0.10	0.13
Inferences			Mixed type inhibition		Un-competitive inhibition	
MDH	V*max*	45.45	43.29	29.33	40.5	42.14
	K*m*	0.77	1.04	0.64	1.03	0.96
Inferences			Un-competitive type inhibition		Mixed type inhibition	

V*max* – maximum enzyme activity or oxidation of µM of NADH per minute per mg tissue protein, K*m* – substrate concentration at which PEPCK activity is ½ of V*max*.

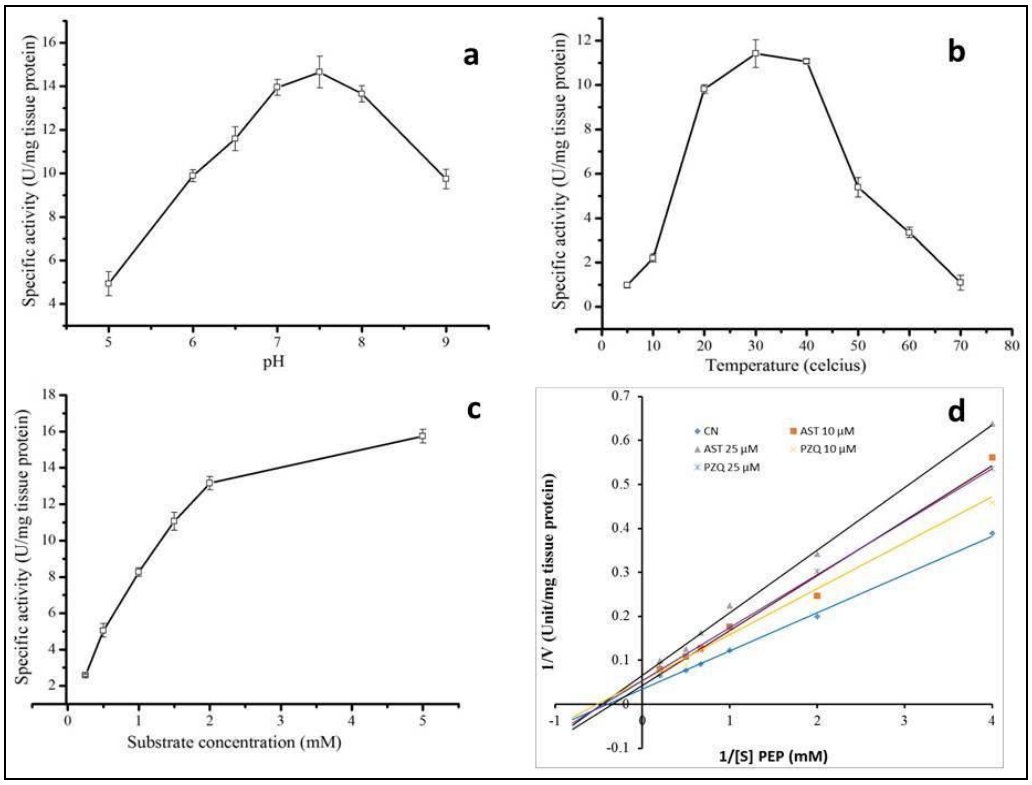

Figure 2. Influence of (a) pH, (b) temperature and (c) substrate concentration and (d) Lineweaver Burk plot showing alterations of kinetic parameters of Pyruvate kinase activities of *Fasciolopsis buski*.

Similarly, the suitable pH level and reaction temperature for optimum MDH activity was studied taking into account the range of pH 5.5 to 9.0 along with temperature in the range of 5 to 70ºC. Figure 4 depicts the influence of pH and reaction temperature on enzyme activity showing optimum activity at pH 7.5 and 40ºC temperature, respectively. Present studies have shown that lower and higher values of pH and reaction temperature have negative effect on the MDH enzyme activity (Fig. 4a & b). A good linearity (R^2 = 0.9986) relationship between the oxaloacetate concentrations vs. enzyme activity have been observed in the present experimental conditions (Fig. 4a). The kinetic parameters K*m* and V*max* values of control tissue showed 0.77 mM and 45.25 U/mg tissue proteins, respectively (Table 3). The possible mode of action of astragalin and PZQ on MDH activity revealed alterations in the enzyme activity. At tested concentrations 10 and 25 µM, decreased value were observed both in K*m* and V*max*, whereas the K*m* value showed little increase in PZQ treated parasite with decreased MDH (V*max*) activity. The study therefore, indicates un-competitive nature of enzyme inhibition by astragalin against MDH. However, the PZQ showed a mixed type of enzyme inhibition (Table 3).

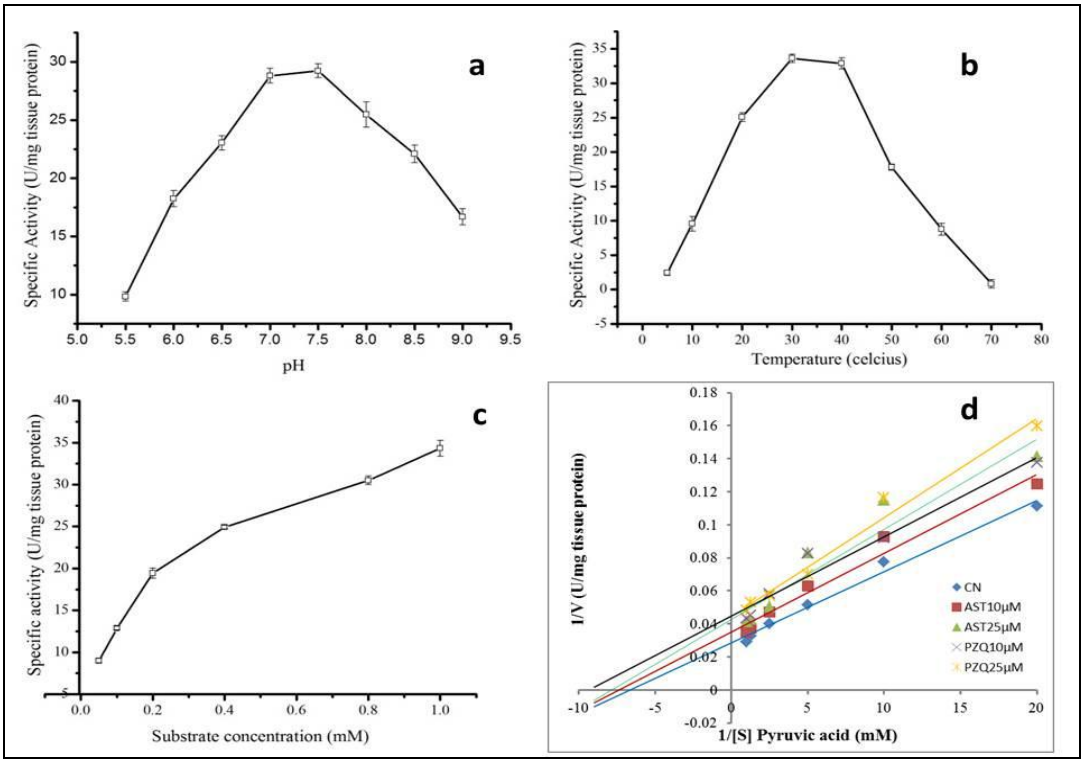

Figure 3. Influence of (a) pH, (b) temperature and (c) substrate concentration and (d) Lineweaver-Burk plot of substrate vs. lactate dehydrogenase activities in control *Fasciolopsis buski*.

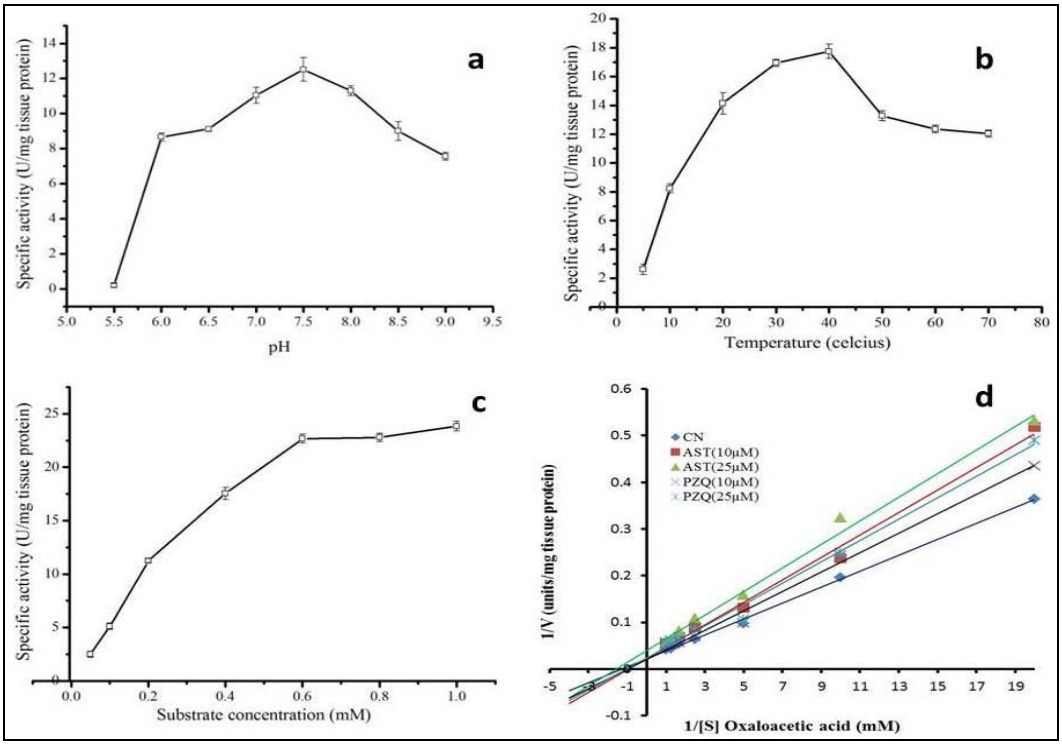

Figure 4. Influence of (a) pH, (b) temperature and (c) substrate concentration on the malate dehydrogenase activities in *Fasciolopsis buski*.

5 Discussions

All living organisms including parasitic helminths have hundreds of metabolic pathways that are vital for normal functioning of their body cells. Glycolysis is the major energy-yielding pathway in the parasites since the Krebs cycle and hexose monophosphate pathways are less functional. In most helminth parasites, glucose and other simple carbohydrates are metabolized following type 2 glucose fermentation, which is characterized by a CO_2-fixation step (by phosphoenolpyruvate carboxykinase) and malate dismutation (Omar *et al.* 1996; Lakhdar-Ghazal *et al.* 2002). In the present study, the bioactive compound astragalin showed better inhibitory activity in PEPCK and LDH activity compared to PK and MDH. Reference anthelmintic drug, praziquantel also showed more or less similar extend of enzyme inhibition. Similarly, commercial drugs like sumarmin, centperazine, diethylcarbamazine and LEV were showed marked inhibition of several glycolytic enzymes (Hussain *et al.* 1990; Xiao *et al.* 1998). Pampori and Srivastava (1987) recorded a significant inhibition of different glycolytic enzymes like PEPCK, HK, G6PDH and MDH in *Cotugnia digonophora* when treated with different anthelmintics like MBZ, niclosamide and PZQ. The main role of PEPCK in helminth parasites is the degradation of glucose rather than its synthesis through the gluconeogenic pathway (Smyth and McManus 1989). However in *S. mansoni*, experiments with inhibitors of PEPCK gave no indication that this enzyme is involved in the degradation of glucose, and it was confirmed that lactate is formed from PEP *via* the actions of PK and LDH (Tielens *et al.* 1991). In helminth parasites the energy metabolisms shift from aerobic to anaerobic respiration with the nearly complete disappearance of PK (Tielens *et al.* 1992). The value of PK/PEPCK ratio of helminth parasites lies in the region of 2-10 for aerobic and 0.10-0.05 for anaerobic respiration (Barrett 1981). However, transition of PK/PEPCK value occurs during the developmental stages of *Hymenolepis diminuta* (Fioravanti *et al.* 1998). The PK/PEPCK ratio of *Shistosoma mansoni* and *Fasciola hepatica* were found to be in the range of 5-10 and 0.25-0.4, respectively (Barrett 1981). In the present study, PK was found to be slightly higher than PEPCK in untreated *F. buski* with low PEPCK/PK ratio indicating slight inclination towards aerobic type of respiration. Similarly, in a recent study by Bera and Manna (2007), the PK/PEPCK ratio was found to be 0.104 followed by gradual decrease during *in-vitro* starvation. However, the *in-vitro* treatment of parasites with astragalin showed maximum inhibitory activity against LDH followed by PEPCK, MDH and LDH. In a similar type of study all the four important glycolytic enzymes namely PEPCK, PK, MDH and LDH showed reduced enzyme activity on exposure to different doses of *Syzygium aromaticum* in *Cotylophoron cotylophorum* (Dhanraj and Veerakumari 2015). Recent studies by Roy and Giri (2016) established the inhibitory activities of α-viniferin and resveratrol against PEPCK, MDH and LDH.

Partial characterization of all the four enzymes showed good correlation between the different kinetic parameters and enzyme activities. Almost all the enzymes under study showed maximum activities at reaction temperature of 30 to 40ºC. Similarly most of the enzymes studied were found to show optimum activity within pH range of 7.0 to 8.0 like other mammalian enzymes. On exposure to *in-vitro* treatment with AST and PZQ at doses of 10 and 25 µM, the enzymes activities were found to be affected. In PEPCK, at low dose of 10 M the V*max* remained almost similar while K*m* increases indicating competitive type of enzyme inhibition. While increasing the AST concentration the V*max* and K*m* values were decreased which may be an indication of AST's affinity towards substrate or enzyme-substrate complex. Similar is the case with PZQ showing mixed-type of inhibition. Of all the

enzymes studied LDH was found to be affected most reducing the V*max* value while keeping the K*m* values more or less equal indicating slightly more affinity of AST and PZQ towards enzyme-substrate complex thereby showing mixed and uncompetitive inhibition, respectively. When the assay mixture was treated with AST and PZQ, the maximum activity MDH remained almost similar while increasing the K*m* values. However, it has been revealed from the study that the reduction in enzyme activity of all the glycolytic enzymes in whole parasite treatment and assay mixture treatment is not similar. The in-vitro treatment of whole parasite showed reduced enzyme activities in PEPCK and LDH whereas the in-vitro assay mixture treatment with AST and PZQ showed higher reduction in PK and LDH activity. It therefore suggests that the activity of an enzyme is regulated not only by its substrate concentration but also by other regulatory molecules present inside the helminth body and help the parasite in extracting the energy by one way or the other.

6 Conclusions

The present study revealed the first report of the anthelmintic effect of astragalin on the helminth parasite, *Fasciolopsis buski*. Astragalin showed inhibitory activity against the energy metabolism of the parasites by inhibiting the enzymes PK, PEPCK, LDH and MDH. Kinetic study also showed potential anthelmintic activity against the *F. buski* by interfering the activities of glycolytic enzymes. It may therefore be concluded that astragalin may proof to be an alternative for anthelmintic chemotherapeutic agents. However, in depth study regarding the actual mode of action and its molecular mechanisms need to be established for successful therapeutic applications.

Conflict of Interest

Author declares there is no conflict of interest.

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