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Effect of *Allium sativum* on the phosphoenolpyruvate carboxykinase and pyruvate kinase activity of *Haemonchus contortus in vitro*

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ABSTRACT

Effect of *Allium sativum* bulb extract (*As*BE) on the phosphoenolpyruvate carboxykinase (PEP) and pyruvate kinase (PK) activity of *Haemonchus contortus* was studied.Maximum level of inhibition in both PEPCK and PK activities were observed in worms treated at 10 mg ml^{-1} concentration of *As*BE after 8 h incubation. This inhibition in the enzyme activity affects the energy generating process, which ultimately proves fatal to the parasite.

Keywords: *Haemonchus contortus; Allium sativum;* phosphoenolpyruvate carboxykinase; pyruvate kinase.

INTRODUCTION

Carbohydrate metabolism in parasitic helminths is characterised by the production of reduced end products, and this situation persists even under aerobic conditions. The difference in carbohydrate catabolism between parasitic helminths and their mammalian hosts are highlighted by the fact that acetyl-CoA does not play the same central role in helminths as it does in mammals, this function being taken over in helminths by phosphoenolpyruvate (PEP) (1). In parasitic helminths majority of PEP is diverted to oxaloacetate (OAA) by the action of phosphoenolpyruvate carboxykinase (PEPCK). A smaller amount is converted to pyruvate via pyruvate kinase, which is further reduced to lactate by lactate dehydrogenase (LDH). OAA is then reduced to malate, which undergoes dismutation, one part being converted to fumarate, succinate and propionate, and the other to pyruvate. The PEP-succinate pathway is considered the major source of ATP anaerobically in parasitic helminths (2). Any anthelmintic drug which could cause adverse changes in the respiratory metabolism of a parasite but not in the host is said to be a potential anthelmintic drug. In view of the functional significance of PEPCK in the energy metabolism of parasitic helminths, any change in PEPCK activity following exposure to an anthelmintic drug is important in explaining the mode of action of the anthelmintic agent.

In the face of escalating resistance to chemical anthelmintic drugs, plant products, which are a complex mixture of active phytochemicals offer an effective alternative that may be both sustainable and environmentally acceptable. Apart from its worldwide usage as a seasoning in food, *Allium sativum* (garlic) has a wide range of therapeutic applications, including antifungal, antibacterial, antiviral, anticancerous and anthelmintic properties (3–7). Further, *A. sativum* has also been demonstrated to posses hypoglycaemic effect and hypolipidemic activity (8-9). The present investigation

was carried out to elucidate the effect of *A. sativum* bulb extract (*As*BE) on the PEPCK and in addition the PK activity of *Haemonchus contortus*.

MATERIALS AND METHODS

Adult live *H. contortus* were collected from the abomasum of sheep slaughtered at the Madras slaughterhouse, Chennai. The worms were washed in physiological saline and maintained *in vitro* in Hedon-Fleig solution (10).

Preparation of plant extract

One gm of bulbils of garlic were ground to a thin paste and added to 100 ml of Hedon-Fleig solution to give 10 mg ml^{-1}concentration. This stock solution was serially diluted to 0.1, 0.5, 1 and 5 mg ml^{-1} concentrations. The worms were incubated in five sub-lethal concentrations viz., 0.1, 0.5, 1, 5 and 10 mg ml^{-1} of *A. sativum* aqueous extract for 2, 4 and 8 h. Worms maintained in Hedon-Fleig served as control.

Preparation of enzyme sample

The control and plant extract-treated worms were rinsed in distilled water and weighed wet and a 10% (w/v) homogenate was prepared by homogenizing the worms in an ice-cold 0.25M sucrose solution containing 0.15M Tris-HCl (pH 7.5), using a homogenizer in an ice bath. This homogenate was centrifuged at 1000 × g for 10 min and the sediment containing the cellular particles viz., nucleus and other heavy organelles was discarded. The clear supernatant was used as an enzyme source. The soluble fraction of the sample which served as the enzyme source (11). Protein in the sample was determined (12).

Enzyme assay

The assay system for PEPCK (EC 4.1.1.32) consisted of 1 ml of 300 mM imidazole buffer (pH 6.2), 0.4 ml of 300 mM magnesium sulphate ($MgSO_4$), 0.3 ml of 400 mM potassium chloride (KCl), 0.3 ml 70 mM sodium bicarbonate, 0.3 ml of 20 mM adenosine diphosphate (ADP), 0.3 ml of 40 mM PEP, 0.3 ml of 2 mM nicotinamide adenine dinucleotide reduced (NADH), 0.05 ml of malate dehydrogenase and 0.05 ml of enzyme sample (13). The reaction was started by the addition of the enzyme sample and the decrease in absorbance was read at 340 nm for 3 min at an interval of 15 sec. The enzyme activity was calculated from the millimolar coefficient of 6.22 for NADH and was expressed as n moles NADH oxidized / min/ mg protein.

The assay system for PK (E.C.2.7.1.4) consisted of 1 ml of 300 mM Tris-HCl buffer (pH 7.8), 0.5 ml of 42 mM $MgSO_4$, 0.5 ml of 450 mM KCl, 0.3 ml of 50 mM ADP, 0.3 ml of 50 mM PEP, 0.3 ml of 2 mM NADH, 0.025 ml of 48 mM fructose biphosphate, 0.025 ml of LDH and

Table 1 *In vitro* effect of *As* BE on the PEPCK activity of *Haemonchus contortus*

	% inhibition (mean ± S.D n = 5) at various periods of incubation **		
Concentration mg ml^{-1} *	2h	4h	8h
0.1	21.87 ± 1.31	41.51 ± 0.83	55.32 ± 0.94
0.5	27.75 ± 1.03	43.31 ± 1.14	63.76 ± 1.14
1	33.24 ± 0.52	45.44 ± 1.06	67.12 ± 1.03
5	41.83 ± 1.01	50.20 ± 0.87	68.66 ± 1.28
10	46.76 ± 0.62	57.36 ± 1.27	76.82 ± 1.10

*Kruskal-Wallis Test - $P < 0.0001$;
**Friedman Test - $P < 0.01$
*As*BE – *Allium sativum* bulb extract
PEPCK- Phosphoenol pyruvate carboxykinase

Table 2 *In vitro* effect of AsBE on the PK activity of *Haemonchus contortus*

	% inhibition (mean ± S.D n = 5) at various periods of incubation **		
Concentration mg ml^{-1} *	2h	4h	8h
0.1	19.65 ± 0.54	32.28 ± 0.76	54.12 ± 0.41
0.5	24.44 ± 0.42	37.00 ± 0.47	62.25 ± 0.40
1	34.33 ± 0.27	45.13 ± 0.49	68.67 ± 0.84
5	39.69 ± 0.42	49.49 ± 0.72	73.84 ± 0.45
10	46.16 ± 0.32	57.45 ± 0.42	80.21 ± 0.53

*Kruskal-Wallis Test - $P < 0.0001$;
**Friedman Test - $P < 0.01$
*As*BE – *Allium sativum* bulb extract
PK- Pyruvate kinase

0.05 ml of enzyme sample (13). The reaction was started by the addition of the enzyme sample and the decrease in absorbance at 340 nm was recorded for 3 min at an interval of 15 sec. The enzyme activity was calculated from the millimolar coefficient of 6.22 for NADH and was expressed as n moles NADH oxidized / min/ mg protein.

Statistical analyses

The data were analysed using the statistical software SPSS version 10.0. Since the data were not normally distributed, non-parametric tests of significance- Kruskal-Wallis test and Friedman test were applied to find out the significance of drug induced inhibition in the enzyme activities of *H. contortus* exposed to different concentrations and time.

RESULTS

The activity of both PEPCK and PK showed varying degrees of inhibition following incubation in *As*BE at different concentrations and period of exposure (Tables 1 and 2). *As*BE effectively inhibited PEPCK and PK actively at 10 mg ml^{-1} concentration after 8 h exposure. The inhibition in both the PEPCK and PK activities was concentration and time dependent. The inhibition of PEPCK and PK activity in the treated worms was significant in all the groups ($P<0.0001$).

DISCUSSION

In anaerobic helminths, fermentation in the direction of either succinate or lactate is controlled by the competing activities of PEPCK and PK. They direct the flow of carbon from PEP into the end products of anaerobic metabolism. The enzymes compete for the substrate PEP, and their relative activities account for the PEP-lactate or acetate/PEP-succinate or propionate pathways (14). It has been pointed out that in the anaerobic habitat of intestinal helminths, succinate formation has a distinct advantage for the parasite over the LDH reaction. The terminal step leading to the production of succinate is brought about by a mitochondrial system which catalyses the transfer of electrons from NADH via a flavoprotein to fumarate. Fumarate serves a terminal electron acceptor. This reaction is coupled with the anaerobic phosphorylation of ADP (15).

*As*BE significantly inhibited both the PEPCK and PK activities in *H. contortus*. Inhibition of PEPCK results in reduced production of malate, which serves as the main substrate for mitochondrial phosphorylation. Likewise, inhibition of PK activity results in reduced production of pyruvate. Similar inhibitory effect on the PEPCK and PK activities has been observed in other helminths treated with anthelmintics (1, 16–17). Inhibition of both PEPCK and PK activities arrests the PEP-succinate/PEP-lactate pathways. Consequently, the energy yielding process is impaired and deprives the parasite of its ATP production. Decreased generation of ATP proves fatal to the parasites (18). The results of the present work suggest that *As*BE could be used as a potential phytotherapeutic drug against *H. contortus* infection of livestock.

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