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In vitro Studies on the Effect of Ethanol Extract of *Trachyspermum Ammi* on Lactate Dehydrogenase of *Haemonchus Contortus*

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ABSTRACT

Gastro intestinal helminth infections is a major threat to small ruminant production. *Haemonchous contortus* is a nematode that feeds on blood of small ruminants and causes anemia, anorexia and eventually leads to death. Anthelmintic drugs are used to treat helminth infection for control of the parasitic diseases. The problem of the development of resistance against the common chemical anthelmintics has renewed the interest in the study of medicinal plants for the development of herbal anthelmintics. Lactate Dehydrogenase (LDH) a glycolytic enzyme plays a key role in the carbohydrate metabolism of the parasites. It catalyzes the reduction of pyruvate to lactate and oxidation of lactate to pyruvate. In the present investigation anthelmintic efficacy of ethanol extract of *Trachyspermum ammi* (*Ta*EE) on LDH of the nematode *H. contortus* was studied *in vitro*. The worms were exposed to five different sub-lethal concentrations (0.01, 0.05, 0.1, 0.5 and 1%) of ethanol extract of *T. ammi* (*Ta*EE), which significantly inhibited the LDH activity catalyzing both oxidation of lactate and reduction of pyruvate. Inhibition of LDH activity increased with an increase in the drug concentration and the period of exposure. Hindrance in LDH activity reduce the level of ATP production which affects the physiological functions of the parasite and ultimately leads to death of the worms.

Keywords: *Haemonchous Contortus*, *Trachyspermum Ammi*, Lactate Dehydrogenase.

I. INTRODUCTION

Small ruminants play a vital role in maintaining family stability by providing meat, milk, skin and wool, earn cash income for the farmers and landlords. India has the largest livestock population in the world, which contributes nearly 7 % towards its national income, and it has the second largest sheep population in the world, accounting for 6.4 % of the global population (FAOSTAT, 2014). *Haemonchus contortus* is a predominant and highly pathogenic nematode of sheep and goats (Hamad *et al*., 2013). Both the larva and the adults feed on blood and cause low productivity, decrease haemoglobin and blood loss that can lead to death in animals (Zaman *et al*., 2012). Keyyu *et al*. (2002) stated, the usage of pharmaceutically derived chemical anthelmintics against helminth infections in small ruminants as a major option in all areas, but continuous use of the same anthelmintic drug in a given area leads to development of resistance. Anthelmintic resistance has been reported among the gastro-intestinal nematodes of sheep and goats from different parts of the world (Bentounsi *et al*., 2006). Therefore it is imperative to search for new effective, economical and eco-friendly drugs like phytotherapeutic drugs. Phytotherapeutic drugs are safe, non-toxic, biodegradable and do not leave residues in animal products (Hammond *et al*., 1997; Veerakumari and Navaneetha Lakshmi, 2006). Hence the present study was undertaken to elucidate the anthelmintic efficacy of *Trachyspermum ammi* against *H. contortus.*

Trachyspermum ammi belongs to the family Apiaceae. *T. ammi* possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, antihypertensive, antispasmodic, broncho-dilating actions, antilithiasis, anti-diuretic, antitussive, anti-septic, anthelmintic and antifiliarial activity (Gupta,

2002; Bairwa *et al*. 2012; Dwivedi *et al*., 2012; Chahal *et al*., 2017).

Glycogen is the main source of energy in helminth parasites. Carbohydrate metabolism of the helminth parasites resembles their host animals, until the formation of phosphoenol pyruvate (PEP). PEP obtained from glycolysis is converted to pyruvate by the action of pyruvate kinase (PK), which is further reduced to lactate, by lactate dehydrogenase (LDH). Many gut dwelling parasites are capable of fixing CO_2 to PEP by phosphoenol pyruvate carboxykinase (PEPCK), which results in the formation of oxaloacetate (OAA). OAA is then reduced to malate, by malate dehydrogenase (MDH). Malate undergoes dismutation, one part being converted to fumarate, succinate and propionate, and the other to pyruvate (Kaur and Sood, 1983). LDH plays a key role in the carbohydrate metabolism of the parasite, as high activity catalyst. In doing so, they assure maintenance of the cytoplasmic redox state and continued utilization of glycogen for energy production (Moon *et al*., 1976). The present study is designed to elucidate the anthelmintic effect of *T. ammi* against digenetic nematode *H. contortus* based on its effect on LDH the key enzyme of carbohydrate metabolism.

II. METHODS AND MATERIAL

Adult live worms were collected from the abomasum of freshly euthanized sheep, slaughtered at Perambur slaughter house, Chennai. Worms were washed in physiological saline and maintained *in vitro* in Hedon-Fleig solution (pH 7.0) (Veerakumari, 1996).

Preparation of Plant Extract

Seeds of *T. ammi* were coarsely powdered and soaked in serious of solvents, hexane, chloroform, ethyl acetate and ethanol in increasing polarity for 48 hours. Aqueous extract was also prepared. Extracts were filtered using Whatman filter paper no. 1 and concentrated using, rotary evaporator (EQUITIRON). *T. ammi* ethanol extract is dried using freeze drier (Lyodel Freeze Dryer) to remove the last traces of solvent.

Sample Preparation

The parasites were incubated for 2h, 4h and 8h in various sub-lethal concentrations of *T. ammi*. Worms were maintained in Hedon-Fleig solution without plant extract served as control. The drug-treated worms were rinsed in distilled water and weighed wet and 10% (w/v) homogenate was prepared by homogenizing the worms in an ice cold 0.25 M sucrose solution containing 0.15 M Tris-HCl (pH 7.5), using a homogenizer in an ice bath. The homogenate was centrifuged at 1000 x g for 10min 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 particulate and soluble fraction of the sample was prepared following the method of Fry *et al*. (1983).

Enzyme Assay

The activity of lactate dehydrogenase (LDH) (EC 1.1.1.27) was assayed according to the method of Yoshida and Freese (1975). LDH catalyses the oxidation of lactate and reduction of pyruvate. The conversion of pyruvate to lactate occurs in anaerobic tissues and conversion of lactate to pyruvate occurs in aerobic tissues. Therefore, LDH activity can be measured spectrophotometrically either by the reduction of nicotinamide adenine dinucleotide (NAD) in the presence of lactate or by the oxidation of NADH in the presence of pyruvate. In the present study the activities in both the directions were assayed. For oxidation of lithium lactate, 0.8ml of 60 mM phosphate buffer (pH 7.5) (Veerakumari, 1996), 0.1 ml of 0.5 M lithium lactate, 0.05 ml enzyme sample and 0.05 ml of 20 mM NAD were placed in 1 ml cuvette. The increase of absorbance at 340 nm was recorded for 3 min at an interval of 15 sec. For the reduction of pyruvate, 0.05 ml of enzyme sample was added to 0.8 ml of 60 mM phosphate buffer (pH 6.5) (Veerakumari, 1996), 0.01 ml of 1 mM NADH, 0.01 ml of 10mM sodium pyruvate and final volume was adjusted to 1 ml by the addition of distilled water in 1 ml cuvette. The decrease in absorbance at 340 nm was measured for 3 min at an interval of 15 sec. The protein content in the sample was estimated following the procedure of Lowry *et al*. (1951). The enzyme activity was calculated from the millimolar coefficient of 6.22 for NAD and NADH and was expressed in n moles NAD reduced or NADH oxidised/min/mg protein.

Statistical analyses were performed with the Statistical program for the social sciences SPSS version 16.0. The significance of drug induced inhibition in LDH activity of the parasites was assessed using analysis of variance

(ANOVA) for different concentrations of ethanol extracts of *T. ammi*. The term significant had been used to indicate differences for which $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Investigations on the effect of the ethanol extract of *T. ammi* on *H. contortus* revealed a significant inhibition of the key regulatory enzyme LDH. The LDH activity catalyzing oxidation of lactate was found to be inhibited by 70.28, 85.33 and 94.35 % in worms treated with 1 mg/ml of *Ta*EE after 2, 4 and 8h of exposure, respectively (Fig. 1). Whereas the inhibition of LDH activity catalyzing pyruvate reduction was found to be 59.48, 71.71 and 97.93 % after 2, 4 and 8h respectively in 1 mg/ml of *Ta*EE–treated worms (Fig. 2). Percentage of inhibition is dose and time dependent. Inhibition of LDH is highly significant ($P < 0.05$) when compared between the period of exposure and different concentrations of *Ta*EE.

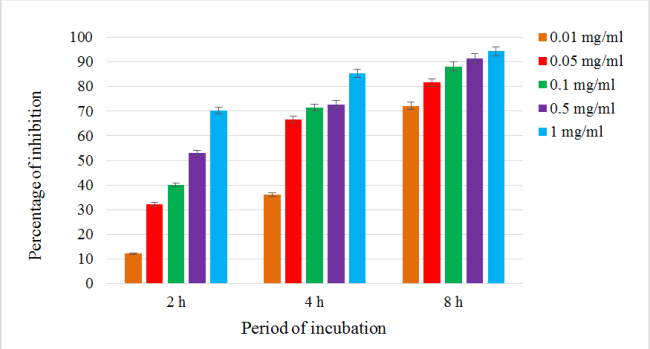

Fig. 1 Effect of *Ta*EE on the LDH activity (oxidation) of *H. contortus*

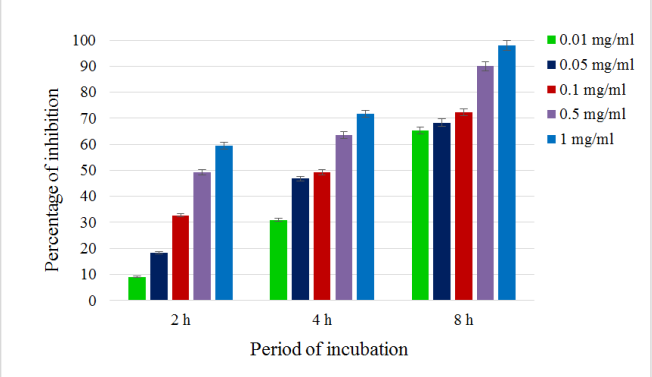

Fig. 2 Effect of *Ta*EE on the LDH activity (reduction) of *H. contortus*

Carbohydrates form the chief energy source in parasitic nematodes. Energy generation in parasitic helminths is based mainly on the anaerobic glucose metabolism (Von Brand, 1974). In view of the importance of carbohydrates in helminths, any difference in their carbohydrate metabolism and that of their hosts might be usefully exploited in helminth control. In the present study, *Ta*EE inhibited the LDH activity, catalysing both oxidation and reduction reaction. Similar inhibitory effect of *Andrographis paniculata* and *Allium sativum*, on LDH activity catalyzing oxidation of lactate in *H. contortus* were documented by Veerakumari and Navaneetha Lakshmi, (2004; 2006). A remarkable inhibition of LDH activity in *Potentilla. fulgens*, *Carex. baccans* and praziquantel treated *Fasciolopsis buski* was reported by Swargiary *et al*. (2013). Veerakumari and Munuswamy (2000) Opined that accumulation of lactate due to LDH inhibition may affect the mitochondrial energy generating process which ultimately proves to be fatal to the parasite. In the present study, inhibition of LDH catalyzing lactate reduction was comparatively more than the LDH catalyzing pyruvate oxidation. Inhibition of lactate dehydrogenase might arrest the carbon flux in glycolytic pathway and the generation of necessary energy through oxidative phosphorylation, results in the debilitation of pyruvate and the accumulation of lactate. Consequently, decarboxylation of pyruvate to acetate, an energy yielding processes is impaired. Decreased generation of the ATP proves fatal to the parasite (Srivastava *et al*., 1989; Jasra *et al*., 1990). The current study thus suggests that *Ta*EE could be used as a potential phytotherapeutic drug against *H. contortus*.

IV. CONCLUSION

The present study elucidated the anthelmintic potential of *T. ammi* on LDH of *H. contortus*. Significant inhibition in the activities of LDH, the enzyme involved in energy metabolism was observed in all the drug-treated worms. Energy deprevation results in the death of the parasites. Depletion of energy reserve might uncouple oxidation phosphorylation, hinder ATP production and cause cessation of protein synthesis, which might prove fatal to the worms. This study suggests a promising application of *T. ammi* against the significantly ambulant, invasive, *H. contortus.*

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