In Vitro Studies on the Effect of Ethanol Extract of *Syzygium Aromaticum* on the Carbohydrate Metabolism of *Cotylophoron Cotylophorum*

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**Abstract** Paramphistomosis is one of the major pathogenic diseases in domestic animals and responsible for heavy economic loss in terms of reduced milk, meat and wool production. *Cotylophoron cotylophorum* is more prevalent in Tamilnadu. In the present investigation, the effect of *Syzygium aromaticum* ethanol extract on the enzymes of carbohydrate metabolism viz. pyruvate kinase, phosphoenolpyruvate carboxykinase, lactate dehydrogenase, malate dehydrogenase, fumarate reductase and succinate dehydrogenase of *Cotylophoron cotylophorum* was studied *in vitro*. The parasites were incubated in five different sub-lethal concentrations of ethanol extract of *Syzygium aromaticum* viz. 0.005, 0.01, 0.05, 0.1 and 0.5mg/ml for 2, 4 and 8 h. The activity of the enzymes was assayed using standard procedures. The enzyme activity was expressed in terms of protein. The data obtained were analyzed statistically. Ethanol extract of *Syzygium aromaticum* significantly inhibited the enzymes of carbohydrate metabolism and the percentage of inhibition was dose and time dependent. Inhibition of these enzymes leads to decreased ATP production which may be fatal to the parasites. The present study validates the anthelmintic property of ethanol extract of *Syzygium aromaticum* against *C. cotylophorum*.

**Keywords** *Syzygium Aromaticum; Cotylophoron Cotylophorum; Pyruvate Kinase; Phosphoenolpyruvate Carboxykinase; Lactate Dehydrogenase; Malate Dehydrogenase; Fumarate Reductase; Succinate Dehydrogenase*

1. Introduction

The most important and reliable source of animal proteins in India is meat from goat and sheep. Sheep production has remained an integral part of cultural life and farming system of the rural population. Helminthiasis affects the production potential through mortality, weight loss, reduced milk yield and wool production [1]. Paramphistomosis is a major disease caused by amphistomes. The paramphistome *Cotylophoron cotylophorum* lives in the rumen and reticulum of sheep, goats, cattle and other domestic ruminants [2, 3].
Vast array of synthetic anthelmintics are used to combat paramphistomosis. However, problems have emerged with the use of synthetic anthelmintics, notably the development of resistance in helminths to various anthelmintic compounds and classes, as well as chemical residues and toxicity problems [4, 5]. In addition, recognition of the antigenic complexity of parasites has slowed vaccine development. These disadvantages have stimulated a search for alteration control methods such as the use of traditional medicinal plants. Plants are known to provide a rich source of potent botanical anthelmintics. The use of medicinal plants for the prevention and treatment of gastrointestinal parasitism has its origin in ethno veterinary medicine [6, 7].

*Syzygium aromaticum* commonly called clove belongs to the family myrtaceae. Clove bud oil has biological activities, such as antibacterial, antifungal, antiviral, antimicrobial, anticancer, antiseptic, anesthetic, insecticidal, analgesic, antispasmodic, ant carcminative and antioxidant properties [8]. Clove oil is also active against plant-parasitic nematodes [9]. The major constituents in bud oil are eugenol and β-carophyllene, vanillin, categolic acid, gallotannic acid, methyl salicylate (pain-killer) eugenin, kaempferol, rhamnetin, eugenitin, oleanolic acid, stigmasterol, campesterol and several sesquiterpenes [10, 11] Kumar and Singh, Manoj Dhanraj and Veerakumari reported the anthelmintic activity of *Syzygium aromaticum* against *Fasciola gigantica* and *Cotylophoron cotylophorum* [12, 13].

Carbohydrate is an essential energy source in all adult parasitic helminths and its metabolism is often predominantly anaerobic, even in the presence of oxygen. They depend on carbohydrate either in the form of glycogen or glucose. The inhibition of energy metabolism is the most important mode of anthelmintic action of various groups of drugs since the parasitic trematodes depend on carbohydrates for their energy metabolism and glucose is the only direct source of energy [14, 15]. Glucose is absorbed from the host via the glucose transporters located in the tegument and intestinal epithelium of trematodes [16, 17]. The main storage of carbohydrate in parasitic helminths is glycogen [18].

Carbohydrate metabolism of the helminth parasite resembles Embden-Meyerhof glycolytic pathway of their host animals, until the formation of phosphoenol pyruvate (PEP). PEP obtained from glycolysis can either be carboxylated to oxaloacetate (OAA) by phosphoenolpyruvate carboxykinase (PEPCK), or dephosphorylated to pyruvate by pyruvate kinase (PK). Pyruvate so formed is further reduced to lactate by lactate dehydrogenase (LDH) and OAA is reduced to malate by malate dehydrogenase (MDH). Malate permeates into the mitochondrion where it undergoes dismutation in which one-half of malate is oxidized to pyruvate by malic enzyme (ME) and the other half is dehydrated to fumarate by fumarase (FM), which is further reduced to succinate by fumarate reductase (FR). Succinate oxidized to fumarate by succinate dehydrogenase (SDH). Decarboxylation of pyruvate and succinate results in the final end products of acetate and propionate respectively [18, 19, 20]. Keeping this in view, an attempt has been made to the assess the anthelmintic efficacy of ethanol extract of *Syzygium aromaticum* against *Cotylophoron cotylophorum* based on its effect on the enzyme involved in carbohydrate metabolism.

2. Materials and Methods

2.1. In Vitro Maintenance of *Cotylophoron cotylophorum*

*Cotylophoron cotylophorum* were collected from the rumen of infected sheep, slaughtered at Perambur abattoir, Chennai. Adult live worms were collected, washed thoroughly in physiological saline and maintained in Hedon-Fleig solution, which is the best medium for *in vitro* maintenance [21]. It is prepared by dissolving 7gm of sodium chloride, 0.3gm of potassium chloride, 0.1gm of calcium chloride, 1.5gm of sodium bicarbonate, 0.5gm of disodium hydrogen phosphate, 0.3gm of magnesium sulphate and 1gm of glucose in 1000ml of distilled water.
2.2. Preparation of Plant Extracts

The buds of *Syzygium aromaticum* were collected from a local shop at Chennai, and were authenticated in the Department of Botany, Pachaiyappa's college; Chennai and vouched specimens are deposited in the herbarium of Pachaiyappa's College, Chennai-30. The extraction of plant materials was done following the method of Harborne [22].

2.3. Sample Preparation

Adult *C. cotylophorum* were incubated in various concentration of *Syzygium aromaticum* ethanol extract (SaEE) (0.005, 0.01, 0.05, 0.1 and 0.5mg/ml) for 2, 4 and 8h. Simultaneously, control was also maintained in Hedon-Fleig solution without the plant extract. After incubation, the parasites were rinsed in distilled water. The parasites were weighed wet and a 10% (W/V) homogenate was prepared by homogenising the flukes in ice-cold 0.25 M sucrose solution containing 0.15 M Tris-HCl (pH-7.5). This homogenate was centrifuged at 1000 rpm for 10 min. The supernatant was used as the enzyme source. The cytosolic and mitochondrial fractions of *C. cotylophorum* were prepared following the method of Fry et al. [23].

2.4. Enzyme Assay

2.4.1. Pyruvate Kinase (PK)

Pyruvate kinase (PK, EC 2.7.1.4) activity in the cytosolic fraction was assayed following the method of McManus and Smyth [24]. The reaction mixture contained 1 ml of 300 mM Tris-HCl buffer (pH 7.8) [25], 0.5 ml of 42 mM magnesium sulphate (MgSO4), 0.5 ml of 450 mM potassium chloride (KCl), 0.3 ml of 50 mM adenosine diphosphate (ADP), 0.3 ml of 50 mM PEP, 0.3 ml of 2 mM nicotinamide adenine dinucleotide reduced (NADH), 0.025 ml of 48 mM fructose biphosphate (FBP), 0.025 ml of 15 units of LDH and 0.05 ml of enzyme sample. 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 in n moles NADH oxidised / min / mg protein.

2.4.2. Phosphoenolpyruvate Carboxykinase (PEPCK)

The activity of phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) was assayed according to the method of McManus and Smyth [24]. PEPCK catalyses the formation of oxaloacetate (OAA) from PEP. The assay mixture contained 1ml of 300 mM imidazole buffer (pH 6.2) [25], 0.4 ml of 300 mM MgSO4, 0.3 ml of 400 mM KCl, 0.3 ml of 70 mM sodium bicarbonate (NaHCO3), 0.3 ml of 20 mM ADP, 0.3 ml of 40 mM PEP, 0.3 ml of 2 mM NADH, 0.05 ml of 15 units of MDH and 0.05 ml of enzyme sample. 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 in n moles NADH oxidised / min / mg protein.

2.4.3. Lactate Dehydrogenase (LDH)

The activity of lactate dehydrogenase (LDH, EC 1.1.1.27) was assayed according to the method of Yoshida and Freese [26]. LDH catalyses the oxidation of lactate and reduction of pyruvate. For oxidation of lithium lactate, 0.8 ml of 60 mM phosphate buffer (pH 7.5) [27], 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) [27].
0.01 ml of 1 mM NADH, 0.01 ml of 10 mM 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 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.

2.4.4. Malate Dehydrogenase (MDH)

Malate dehydrogenase (MDH, EC 1.1.1.37) catalyses the oxidation of malate and reduction of OAA. The activity of this enzyme catalysing the malate oxidation and OAA reduction was assayed in both cytosolic and mitochondrial fractions following the procedure of Yoshida [28]. For the oxidation of malate, the reaction mixture contained 1 ml of 150 mM Tris-HCl buffer (pH 8.4 for cMDH and pH 7.2 for mMDH) [21] 0.1 ml of 100 mM sodium malate, 0.1 ml of 10 mM NAD, 1.7 ml of distilled water and 0.1 ml of enzyme sample. For MDH catalysing the reduction of OAA, the reaction mixture contained 2.5 ml of 100 mM Tris-HCl (pH 7.4 for both cMDH and mMDH) [21], 0.05 ml of 100 mM oxaloacetate, 0.05 ml of 10 mM NADH, 0.3 ml of distilled water and 0.1 ml of the enzyme sample. The activity of the enzyme catalysing oxidation and reduction reaction was measured at 340 nm for 3 min at an interval of 15 sec each. 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.

2.4.5. Fumarate Reductase (FR)

Fumarate reductase (FR, EC 1.3.1.6) catalyses the reduction of fumarate to succinate. The enzyme was assayed as detailed by Sanadi and Fluharty [29]. The reaction mixture contained 1 ml of 150 mM Tris-HCl buffer (pH 8.6) [21], 0.3 ml of 10 mM KCN (neutralised with 0.01 N HCl), 0.3 ml of 1 mM ethylene diamine tetra acetic acid (EDTA), 0.3 ml of 50 mM fumarate, 0.7 ml of distilled water, 0.1 ml of enzyme sample and 0.3 ml of 1.6 mM NADH in a 3 ml cuvette. After the addition of NADH, decrease in absorbance at 340 nm was measured for 3 min at an interval of 15 sec. The enzyme activity was calculated by using the millimolar coefficient of 6.22 and expressed in n moles of NADH oxidised / min / mg protein.

2.4.6. Succinate Dehydrogenase (SDH)

The activity of succinate dehydrogenase (SDH, EC 1.3.99.1) was assayed according to the method of Singer [30]. The reaction mixture included 0.5 ml of 300 mM phosphate buffer (pH 7.5) [21], 0.3 ml of 0.1 M succinate, 0.1 ml of enzyme, 0.3 ml of 10 mM KCN (neutralised with 0.01 N HCl), 0.1 ml of 0.75 mM calcium chloride and 1.3 ml of water. The enzyme was incubated for 5 - 7 min to permit full activation. After incubation, 0.1 ml DCPIP (0.05 %) (W/V) and 0.3 ml of PMS (0.33 %) were added to initiate the reaction and decrease in absorbance was recorded at 600 nm. The enzyme activity was calculated using millimolar extinction coefficient of 19.1 and expressed in n moles of dye reduced / min / mg protein.

Protein in the sample was determined by the method of Lowry et al. [31].

2.5. Statistical Analysis

All the data obtained in the present study were statistically analysed using the statistical software SPSS version 16.0. One-way Anova using Bonferroni test was applied to find out the significant difference between the different concentrations of plant extracts and periods of incubation.
3. Results and Discussion

Helminth parasites derive energy for their survival mainly through the degradation of carbohydrate. Several scientists [25, 32, 33, 34, 35] have studied the influence of anthelmintics on the carbohydrate metabolism of helminth parasites. A good understanding on different carbohydrate metabolic reactions forms solid basis for choosing appropriate targets for new chemotherapeutic agents. Investigations on the effect of ethanolic extracts of *S. aromaticum* on the cytosolic and mitochondrial fraction of *C. cotylophorum* revealed a significant inhibition of the key regulatory enzymes involved in carbohydrate metabolic pathway.

PK and PEPCK activity was found to be inhibited in SaEE treated flukes (Table 1a). 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 [36]. The inhibition of PK and PEPCK activities treated with anthelmintics has been observed in other helminths [37, 38, 39]. Also, Navaneetha Lakshmi and Veerakumari [40] reported the inhibitory effect on the PK and PEPCK activities in *Haemonchus contortus* treated with *Allium sativum*.

The action of PK on PEP results in the production of pyruvate. Pyruvate so formed comes under the influence of LDH, which catalyses, the reduction of pyruvate to lactate and the oxidation of lactate to pyruvate. It is evident from the present investigation that SaEE inhibited the LDH catalysing both the lactate oxidation and pyruvate reduction (Table 1b). It is interesting to note that LDH exhibits a peculiar type of chemotherapeutic response. Inhibition of LDH activity catalyzing pyruvate reduction was found to be higher compared to LDH inhibition catalysing the oxidation of lactate. Similar findings were also reported by various workers [41, 42, 43]. Inhibitory effect of albendazole on LDH activity of *Fasciola hepatica* reported by Ozcelik [44]. Veerakumari and Munuswamy elucidated the inhibitory effect of PZQ and LEV on LDH activity of *C. cotylophorum* [27]. Similar inhibitory effect of *A. sativum* on the LDH activity catalysing both the oxidation and reduction reactions in *H. contortus* has been reported by Veerakumari and Lakshmi [45]. The inhibition of lactate dehydrogenase might arrest the carbon influx in the glycolytic pathway and the generation of the necessary energy through oxidative phosphorylation [36]. Consequently, production of malate, which serves as main substrate for mitochondrial phosphorylation is reduced, which leads to reduced production of ATP [39].

Malate dehydrogenase (MDH) has been a rate–limiting enzyme in the phosphoenolpyruvate metabolism. SaEE significantly inhibited the cytoplasmic MDH (cMDH) and mitochondrial MDH (mMDH) catalysing both the oxidation and reduction reactions in *C. cotylophorum* (Table 1c & 1d). Inhibition of MDH activity of *Ascaris suum*, *F. hepatica* and *Moniezia expansa* by mebendazole, albendazole and parbendazole was reported by Tejada et al. [46]. Also, Oztop et al. [47] reported the alteration of MDH and LDH activities of *Trichuris saginata* by albendazole and niclosamide. Similar inhibitory effect of *Acacia concinna* on the cMDH and mMDH activity of *C. cotylophorum* was reported by Priya and Veerakumari [25]. Anthelmintics may disturb the transmembrane proton gradient severely, leading to drop in cellular ATP levels [48]. Reduction in the MDH activity of the flukes exposed to SaEE suggest that, plants act transtegumentally to target vital tegumental enzymes and interfere with the energy generating pathways depriving the parasite in acquiring ATP, thereby leading to paralysis and death [49]. The inhibition of both cMDH and mMDH observed in the present study suggests the declined production of oxaloacetate (OAA) and malate. The inhibition of MDH might subsequently result in the inhibition of FR, as OAA is essential for production of fumarate [50, 51].

Fumarate is reduced to succinate using NADH as reducing equivalent and succinate formation is the final step of the glycolytic pathway [52]. In the present study, SaEE inhibited the FR and SDH activity of *C. cotylophorum* (Table 1e). Priya and Veerakumari [25] reported similar inhibition of FR in *A.
Acacia concinna treated C. cotylophorum. The FR activity of H. contortus was also inhibited by other drugs such as tetramisole, thiabendazole, cambendazole, mebendazole, morantel tartrate and disophenol [53, 54, 55]. Barrowman et al. [56] demonstrated the inhibitory effect of benzimidazole and albendazole sulphone on the FR activity of Ascaris suum. Antiparasitic drugs, inhibit fumarate binding to FR, slowdown the synthesis of body constituents, curtail the energy production in the parasites [57], uncouple oxidative phosphorylation, hamper ATP production [58] and present an excellent biochemical target in the treatment of helminthic infections [59]. The SDH activity of Heterakis, Trichuris, Ascardia, Chabertia, Bunostomum and Nematodirus was inhibited by tetramisole [60]. SDH has the ability to transfer electrons to the respiratory chain by catalysing the formation of fumarate and succinate [61]. SDH inhibition by anthelmintics could prevent the utilization of the chemical energy derived from electron transport for the net phosphorylation of ADP to ATP and deprive the parasite of its normal source of energy [62]. In addition, anthelmintics, affect tubulins bound in mitochondrial membrane of the parasites by influencing SDH–FR complex negatively inhibit succinate metabolism and diminish ATP–synthesis [63, 64]. Hence, SDH could potentially be an important target for anthelmintics against the gastrointestinal parasites of livestock [65].

The inhibition of enzymes of carbohydrate metabolism of C. cotylophorum by Acacia concinna was also reported by Priya and Veerakumari [25]. Impairment of carbohydrate metabolism in parasitic helminths may be disastrous since they depend almost entirely on it for their energy supply [66]. Present study manifested that PK, PEPCK, LDH, MDH, FR and SDH provide biochemical target for SaEE which disrupt energy generation process in C. cotylophorum, resulting in decreased production of ATP. Consequently, the energy deprived parasite unable to sustain themselves in situ may be expelled from the host. The results of the present study holds a potential promise in the future use of active principles of S. aromaticum as effective anthelmintics and may help in designing assimilated solutions for the control of paramphistomosis.

### 4. Conclusion

The present study elucidated the anthelmintic effect of SaEE on C. cotylophorum. SaEE blocked the energy metabolism of the parasites by inhibiting the enzymes PK, PEPCK, LDH, MDH, FR and SDH. SaEE possesses a remarkable anthelmintic activity against C. cotylophorum. It may serve as an alternative for anthelmintic chemotherapeutic agents to avoid their toxic side effects and development of resistance in a safe and ecofriendly manner. In depth field trials of plant based anthelmintics along with best farm management practices can play a great role in parasite control strategies and in enhancing productivity of livestock farming.

**Table 1:** In vitro effect of SaEE on the enzymes involved in Carbohydrate metabolism of C. cotylophorum

| Conc. mg/ml* | % inhibition (mean ± SD of n = 5) at various periods of incubation** | Table 1a PK and PEPCK |
|--------------|---------------------------------------------------------------|-----------------------|
|              | PK                                                                            | PEPCK                                                                 |
|              | 2h     | 4h     | 8h     | 2h     | 4h     | 8h     |
| 0.005        | 9.95±0.03 | 13.72±0.04 | 30.72±0.03 | 9.08±0.10 | 26.57±0.08 | 47.97±0.06 |
| 0.01         | 11.75±0.01 | 16.84±0.03 | 48.33±0.05 | 14.47±0.08 | 38.51±0.04 | 56.11±0.01 |
| 0.05         | 17.26±0.01 | 23.04±0.05 | 59.89±0.01 | 21.98±0.08 | 45.44±0.09 | 62.37±0.13 |
| 0.1          | 20.55±0.06 | 32.33±0.12 | 65.37±0.17 | 30.01±0.07 | 52.38±0.12 | 66.80±0.18 |
| 0.5          | 24.18±0.07 | 46.12±0.13 | 76.92±0.19 | 44.16±0.01 | 63.70±0.06 | 90.67±0.11 |

| Conc. mg/ml* | % inhibition (mean ± SD of n = 5) at various periods of incubation** | Table 1b LDH |
|--------------|---------------------------------------------------------------|-------------|
|              | Oxidation                                  | Reduction    |
|              | 2h    | 4h    | 8h    | 2h    | 4h    | 8h    |
| 0.005        | 9.70±0.05 | 23.06±0.09 | 59.21±0.01 | 9.08±0.10 | 26.57±0.08 | 47.97±0.06 |
Table 1c Cdh

| Concentration (mg/ml) | % Inhibition (mean ± SD of n = 5) at various periods of incubation** |
|-----------------------|---------------------------------------------------------------------|
|                       | Oxidation | Reduction |
|                       | 2h | 4h | 8h | 2h | 4h | 8h |
| 0.005                 | 19.59±0.07 | 45.40±0.24 | 61.12±0.08 | 7.50±0.17 | 40.49±0.22 | 61.09±0.18 |
| 0.01                  | 31.44±0.11 | 49.27±0.01 | 74.44±0.01 | 12.58±0.28 | 43.73±0.14 | 75.94±0.40 |
| 0.05                  | 40.00±0.03 | 55.26±0.10 | 83.71±0.04 | 23.68±0.13 | 56.23±0.24 | 79.69±0.17 |
| 0.1                   | 46.23±0.13 | 64.21±0.13 | 87.72±0.14 | 33.62±0.22 | 63.85±1.21 | 88.46±0.23 |
| 0.5                   | 53.22±0.14 | 72.18±0.16 | 98.77±0.18 | 59.03±0.35 | 75.06±0.70 | 92.91±0.39 |

Table 1d mMDH

| Concentration (mg/ml) | % Inhibition (mean ± SD of n = 5) at various periods of incubation** |
|-----------------------|---------------------------------------------------------------------|
|                       | Oxidation | Reduction |
|                       | 2h | 4h | 8h | 2h | 4h | 8h |
| 0.005                 | 5.54±0.03 | 32.25±0.06 | 62.96±0.17 | 15.40±0.14 | 35.12±0.01 | 54.00±0.07 |
| 0.01                  | 15.25±0.04 | 40.45±0.06 | 69.46±0.24 | 19.22±0.07 | 44.05±0.06 | 58.51±0.05 |
| 0.05                  | 22.96±0.03 | 43.05±0.14 | 78.34±0.19 | 23.48±0.04 | 51.87±0.05 | 65.73±0.06 |
| 0.1                   | 35.79±0.01 | 54.49±0.03 | 81.77±0.16 | 27.38±0.03 | 55.55±0.03 | 72.68±0.07 |
| 0.5                   | 53.40±0.06 | 64.67±0.12 | 93.41±0.04 | 42.66±0.11 | 60.22±0.13 | 84.29±0.18 |

Table 1e FR and SDH

| Concentration (mg/ml) | % Inhibition (mean ± SD of n = 5) at various periods of incubation** |
|-----------------------|---------------------------------------------------------------------|
|                       | FR | SDH |
|                       | 2h | 4h | 8h | 2h | 4h | 8h |
| 0.005                 | 28.60±0.18 | 41.10±0.18 | 63.28±0.02 | 12.79±0.05 | 37.33±0.06 | 58.02±0.04 |
| 0.01                  | 31.65±0.27 | 47.25±0.13 | 71.93±0.01 | 22.43±0.03 | 49.82±0.08 | 69.44±0.01 |
| 0.05                  | 37.29±0.01 | 54.14±0.04 | 79.10±0.03 | 35.42±0.02 | 55.20±0.11 | 73.52±0.12 |
| 0.1                   | 45.78±0.01 | 68.13±0.16 | 83.24±0.01 | 38.00±0.15 | 63.61±0.09 | 76.08±0.03 |
| 0.5                   | 62.72±0.14 | 70.28±0.11 | 87.39±0.21 | 48.41±0.08 | 67.34±0.14 | 81.46±0.05 |

* Inhibitory effects of the extracts among the different concentrations of the respective plant are significantly different for each duration of incubation (Bonferroni test; P < 0.05)

** Inhibitory effects of the extracts among the different hours of incubation is significantly different for each concentration of the respective plants (Bonferroni test; P < 0.01)

References

[1] Sackett, D., Holmes, P., Abbott, K., Jephcott, S. and Barber, M. 2006: Assessing the Economic Cost of Endemic Disease on the Profitability of Australian Beef Cattle and Sheep Producers. Miscellaneous publication of Meat and Livestock Australia Limited. 087.

[2] Soliman, M.F. and Zalat, S.M. Prevalence and Intensity of Nematodirus Sp. And Eimeria Sp. Infections in the Domestic Goats of St. Katherine’s Protectorate (Sinai, Egypt): Relations with Some Ecological and Biological Factors. Egyptian Journal of Biotechnology. 2003. 5; 78-85.

[3] Tsotetsi, A.M. and Mbati, P.A. Parasitic Helminthes of Veterinary Importance in Cattle, Sheep and Goats on Communal Farms in the Northeastern Free State, South Africa. Journal of South African Veterinary Association. 2003. 74 (2) 45-48.

[4] Waller, P.J. and Prichard, R.K., 1985: Drug Resistance in Nematodes. In: Campbell, W.C., Rew, R.S. (Eds.), Chemotherapy of Parasitic Infections, Phenum, New York, USA. 339-362.
[5] Kaemmerer, K. and Butenkotter, S. *The Problem of Residues in Meat of Edible Domestic Animals after Application or Intake of Organophosphate Esters*. 1973. 46; 1.

[6] Satyavati, G.V., Raina M.K. and Sharma, M., 1976: *Medicinal Plants of India*. Vol. I. Indian Council of Medical Research, New Delhi. 201-206.

[7] Lewis, W.H. and Elvin-Lewis, M.P.H. 1977: *Medicinal Botany Plants Affecting Man’s Health*. Wiley – Interscience. New York.

[8] Huang, Y., Ho, S.H., Lee, H.C. and Yap, Y.L. *Insecticidal Properties of Eugenol, Isoeugenol and Methyleugenol and Their Effects on Nutrition of Sitophilus zeamais Mostch. (Coleoptera: Curculionidae) and Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae)*. Journal Stores Products Research. 2002. 38; 403-412.

[9] Pandey, R.C. and Dwivedi, B.K. *Comparative Study of Different Plant Extracts for their Nematicidal Potential*. Current Nematology. 2000. 11; 39-43.

[10] Srivastava, A.K., Srivastava, S.K. and Syamasundar, K.V. *Bud and Leaf Essential Oil Composition of Syzygium Aromaticum from India and Madagascar*. Flavour and Fragrance Journal. 2003. 20 (1) 51-53.

[11] Debjit bhownik, Sampath Kumar, K.P., Akhilesh Yadav, S., Srivastava, Shravan Paswan and Amit sankar Dutta. *Recent Trends in Indian Traditional Herbs Syzygium aromaticum and its Health Benefits*. Journal of Pharmacognosy and Phytochemistry. 2012. 1 (1).

[12] Kumar and Singh. *Activity of Allium Sativum, Ferula Asafoetida and Syzygium Aromaticum against Fasciola Gigantica*. Journal of Biology and Earth Sciences. 2014. 4 (1) B57-B65.

[13] Manoj Dhanraj and Veerakumari. *In Vitro Effect of Syzygium Aromaticum on the Motility and Acetylcholinesterase of Cotylophoron Cotylophorum*. Indian Journal of Veterinary and Animal Sciences. 2014. 43 (3) 187-194.

[14] Martin, R.J. *Modes of Action of Anthelmintic Drugs*. Veterinary Journal. 1997. 154; 11-34.

[15] Tielen, A.G.M. *Energy Generation in Parasitic Helminths*. Parasitology. 1994. 10; 346-352.

[16] Skelly, P.J., Kim, J.W., Cunningham, J. and Shoemaker, C.B. *Cloning Characterization and Functional Expression of CDNA Encoding Glucose Transporter Proteins from the Human Parasite, Schistosoma Mansoni*. The Journal of Biological Chemistry. 1994. 269; 42-47.

[17] Thompson, D.P. and Geary, T.G. 1994: *The Structure and Function of Helminthes Surface*. In: *Biochemistry and Molecular Biology of Parasitres*. Eds. Marr, J.J. and Muller, M. Academic Press, London. 203.

[18] Barrett, J., 1981: *Biochemistry of Parasitic Helminths*. Macmillan Press, London. 308.

[19] Kaur, R. and Sood, M.L. *The Effects of DL-Tetramisole and Rafoxanide on Tricarboxylic Acid Cycle Enzymes of Haemonchus Contortus, In Vitro*. Veterinary Parasitology. 1983. 13; 333-340.

[20] Tielen, A.G.M. *Energy Generation in Parasitic Helminths*. Parasitology. 1994. 10; 346-352.
[21] Veerakumari, L., 1996: In Vitro Studies on the Effect of Some Anthelmintics on Cotylophoron cotylophorum (Fischoeder, 1901) (Digenea: Paramphistomidae). A Structural and Biochemical Analysis. Ph.D. thesis submitted to the University of Madras, Chennai.

[22] Harborne, J.B., 1998: Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. 3rd Edition. Chapman & Hall, London. 302.

[23] Fry, M., Bazil, C. and Jenkins, D.C. A Comparison of Mitochondrial Electron Transport in the Intestinal Parasitic Nematodes Nippostrongylus Brasiliensis and Ascardia Galli. Comparative Biochemistry and Physiology. 1983. 75B; 451-453.

[24] McManus, D.P. and Smyth, J.D. Intermediary Carbohydrate Metabolism in Protoscoleces of Echinococcus Granulosus (Horse and Sheep Strains) and E. Multiocularis. Parasitology. 1982. 84; 351-366.

[25] Priya, P. and Veerakumari, L. In Vitro Effect of Acacia Concina on the Key Enzymes of Carbohydrate Metabolism of Cotylophoron cotylophorum (Digenea: Paramphistomidae). Biomedicine. 2011. 31 (3) 329-333.

[26] Yoshida, A. and Freese, E. Lactate Dehydrogenase from Bacillus Subtilis. In: Methods in Enzymology. Wood, W.A. (ed.), Vol. XLI. Academic Press, New York. 1975. 304.

[27] Veerakumari, L. and Munuswamy, N. In Vitro Effect of Some Anthelmintics on Lactate Dehydrogenase Activity of Cotylophoron cotylophorum (Digenea: Paramphistomidae). Veterinary Parasitology. 2000. 91; 129-140.

[28] Yoshida, A. L-Malate Dehydrogenase from Bacillus Subtilis. In: Methods in Enzymology. Lowenstein J.M. (Ed.), Vol. XIII, Academic Press, New York. 1969. 141-145.

[29] Sanadi, D.R. and Fluharty, A.L. On The Mechanism of Oxidative Phosphorylation. VII- The Energy Requiring Reduction of Pyridine Nucleotide by Succinate and Energy Yielding Oxidation of Reduced Pyridine Nucleotide by Fumarate. Biochemistry. 1963. 2; 523-528.

[30] Singer, T.P., 1974: Determination of Activity of Succinate, NADH, Choline and?-Glycerophosphate Dehydrogenase. In: Methods in Biochemical Analysis. Glick, D. (ed.), Vol. 22, Interscience Publications. John Wiley and Son, New York. 133-161.

[31] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein Measurement with the Folin Phenol Reagent. The Journal of Biological Chemistry. 1951. 193; 265-275.

[32] Bueding, E. and Fisher, J. Biochemical Effects of Niridazole on Schistosoma Mansoni. Molecular Pharmacology. 1970. 6 (5) 532-539.

[33] Schulman, M.D., Ostlind, D.A. and Valentino, D. Mechanism of Action of MK-401 against Fasciola Hepatica: Inhibition of Phosphoglycerate Kinase. Molecular Biochemistry and Parasitology. 1982. 5 (3) 133-45.

[34] Donahue, M.J., Masaracchia, R.A. and Harris, B.G. The Role of Cyclic AMP–Mediated Regulation of Glycogen Metabolism in Levamisole– Perfused Ascaris Suum Muscle. Molecular Pharmacology 1983. 23; 378-383.
[35] Veerakumari, L., Lahmingchhuanmawii, K. and Ashwini, R. Effect of Punica Granatum Ethanol Extract on the Carbohydrate Metabolism of Cotylophoron Cotylophorum. International Journal of Biological Sciences. 2014.

[36] Jasra, N., Sanyal, S.N. and Khera, S. Effect of Thiabendazole and Fenbendazole on Glucose Uptake and Carbohydrate Metabolism in Trichuris Globulosa. Veterinary Parasitology. 1990. 35; 201-209.

[37] Rahman, M.S. and Bryant, C. Studies of Regulatory Metabolism in Moniezia Expansa: Effects of Cambendazole and Mebendazole. International Journal of Parasitology. 1977. 7; 403-409.

[38] Lloyd, G.M. and Barrett, J. Fasciola Hepatica: Inhibition of Phosphoenolpyruvate Carboxykinase, and End-Product Formation by Quinolinic Acid and 3-Mercaptopicolinic Acid. Experimental Parasitology. 1983. 56; 259-265.

[39] Srivastava, J.K., Gupta, S. and Katiyar, J.C. Effects of Methyl [5][4-(2-Pyridinyl)-1-Piperazinyl [Carbonyl]-1H-Benzimidazol-2-Yl] Carbamate on Energy Metabolism of Ancylostoma Ceylanicum and Nippostrongylus Brasiliensis. Indian Journal of Experimental Biology. 1989. 27; 735-738.

[40] Navaneetha, L.K. and Veerakumari, L. Effect of Allium Sativum on the Phosphoenolpyruvate Carboxykinase and Pyruvate Kinase Activity of Haemonchus Contortus in Vitro. Pharmacognosy Magazine. 2009. 5 (20) 430-432.

[41] Kumar, M., Pathak, K.M.L. and Pachauri, S.P. Clinicopathological Studies on Naturally Occurring Bovine Fascioliasis in India. British Veterinary Journal. 1982. 138; 241-246.

[42] Swarup, D., Pachauri, S.P., Sharma, B. and Bandhopadhyay, S.K. Sero Diagnosis of Fasciola Gigantica in Buffaloes. Veterinary Parasitology. 1987. 24; 67-74.

[43] Maiti, S.K., Rao, V.N., Ali, S.L., Dutta, G. and Mishra, A. Haematobiochemical and Therapeutic Aspects of Biliary Amphistomiasis in Buffalo. Indian Journal of Veterinary Medicine. 1999. 19; 49-51.

[44] Ozcelik, S., Bakir, S. and Bakir, M. The in Vitro Effect of Albendazole on Fasciola Hepatica and Host Liver Lactate Dehydrogenase Enzymes. Turkiye-Prazitoloji-Dergisi. 1992. 16; 6-10.

[45] Veerakumari, L. and Lakshmi, K.N. In Vitro Effect of Allium Sativum on Lactate Dehydrogenase Activity of Haemonchus Contortus. Journal of Veterinary Parasitology. 2006. 20; 93-96.

[46] Tejada, P., Sanchez–Moreno, M., Monteoliva, M. and Gomez–Banqueri, H. Inhibition of Malate Dehydrogenase Enzymes by Benzimidazole Anthelmintics. Veterinary Parasitology. 1987. 24; 269–274.

[47] Oztop, A.Y., Saygi, G. and Oztop, H.N. In Vitro Effects of Some Anthelmintics on the Malate Dehydrogenase and Lactate Dehydrogenase Enzyme Activities of Taenia saginata. Turkish Journal of Medical Sciences. 1999. 29; 365-370.

[48] McCracken, R.O. and Stillwell, W.H. A Possible Biochemical Mode of Action for Benzimidazole Anthelmintics. International Journal of Parasitology. 1991. 21 (1) 99-104.
[49] Swargiary, A., Roy, B., Giri, B.R. and Rohang, B. A Comparative Study on the Anthelmintic Efficacy of Some Medicinal Plants of North–East India: Alteration in the Glycolytic Enzymes of Fasciola Buski, a Giant Intestinal Fluke. Asian Pacific Journal of Tropical Medicine. 2013. 412-420.

[50] Lwin, T. and Probert, A.J. Effect of Certain Fasciolicides on Malate Dehydrogenase Activity in Fasciola Hepatica: A Possible Biochemical Mode of Action of Hexachlorophene and Oxyctolozanide. Journal of Pesticide Science. 1975. 6; 121-128.

[51] Probert, A.J., Sharma, R.K., Singh, K. and Saxena, R. The Effect of Five Fasciolicides on Malate Dehydrogenase Activity and Mortality of Fasciola Gigantica, Fascioloips Buski and Paramphistomum Explanatum. Journal of Helminthology. 1981. 55; 115-122.

[52] Maule, A.G. and Marks, N.J. Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology. CABI. 2006. 448.

[53] Prichard, R.K. Mode of Action of the Anthelmintic Thiabendazole in Haemonchus Contortus. Nature. 1970. 228; 684-685.

[54] Malkin, M.F. and Camacho, R.M. The Effect of Thiabendazole on Fumarate Reductase from Thiabendazole-Sensitive and Resistant Haemonchus Contortus. Journal of Parasitology. 1972. 58; 845-846.

[55] Kaur, R. and Sood, M.L. The Effects of DL-Tetramisole and Rafoxanide on Tricarboxylic Acid Cycle Enzymes of Haemonchus Contortus, In Vitro. Veterinary Parasitology. 1972. 13; 333-340.

[56] Barrowman, M.M., Marriner, S.E. and Bogan, J.A. The Fumarate Reductase System as a Site of Anthelmintic Attack in Ascaris suum. Bioscience Reports. 1984. 4; 879-883.

[57] Kumari, Y.S. Effest of Tolzan on Carbohydrate Metabolism and Protein Metabolism of an Acanthocephalan Parasite Pallisentis Nagpurenis Parasitising the Fresh Water Fish Channa striatus.

[58] Lemke, T.L., Williams, D.A., Roche, V.F. and Zito, S.W. Principles of Medicinal Chemistry. Seventh Edition Copyright Lippincott Williams & Wilkins, 351 West Camden Street, Baltimore. 2013. MD 21201.

[59] Turrens, J.F. The Enzyme-NADH Fumarate Reductase in Trypanosomatids – A Potential Target against Parasitic Diseases. Molecular and Cell Pharmacology. 2012. 4 (3) 117-122.

[60] Van den Bossche, H. and Janssen, P.A.J. The Biochemical Mechanism of Action of the Antinematodal Drug Tetramisole. Biochemical Pharmacology. 1969. 18; 35-42.

[61] Parvathi, J. and Aruna, K. Correlation of Hyperglycemia and Succinate Dehydrogenase Activity during Hymenolepiasis in Mice and Treatment with Praziquantel. IOSR. Journal of Pharmaceutical sciences. 2012. 1 (1) 022-028.

[62] Skuce, P.J. and Fairweather, I. The Effect of the Hydrogen Ionophore Clopobel upon the Pharmacology and Ultrastructure of the Adult Liver Fluke Fasciola Hepatica. Parasitology. 1990. 76; 241-250.
[63] Boczoni, K., Uszynska, Z., Rodriguez-caabeiro, F., Criado-fornello, A. and De armas-serra, C. The Effect of Some Anthelmintics on Trichinella Spiralis and Trichinella Pseudospiralis Mitochondrial Energy-Generating Pathways. Research and Reviews in Parasitology. 1991. 51 (1-4) 61-63.

[64] Swan, G.E. The Pharmacology of Halogenated Salicylanilides and Their Anthelmintic Use in Animals. Journal of South African Veterinary Association. 1999. 70 (2) 61-70.

[65] Chen, M., Zhai, L., Christensen, S.B., Theander, T.G. and Kharazmi, A. Inhibition of Fumarate Reductase in Leishmania Major and L. Donovani by Chalcones. Antimicrobial Agents and Chemotherapy. 2001. 45 (7) 2023-2029.

[66] Saz, H.J. Comparative Energy Metabolism of Some Parasitic Helminths. Journal of Parasitology. 1970. 56; 634-642.