Anthelmintic Efficacy of Ethanol Extract of Albizia lebbeck and Trachyspermum ammi on the Glutathione-s-transferase of Cotylophoron cotylophorum

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ABSTRACT

Sheep is an economically important livestock species, mostly reared for meat and wool production. Infection with gastrointestinal helminths has been identified as one of the causes for the production loss in sheep rearing, which arise primarily through severe weight loss, poor meat, milk and wool production, impaired reproductive performance, mortality, carcass and offal condemnation. Paramphistomosis caused by amphistomes constitutes a major group of diseases, causing considerable economic loss to livestock industry in India. Chemical control of helminths coupled with improved management has important parasitic control strategy throughout the world. However increasing problems of development of resistance in helminths against anthelmintics led to the proposal of screening medicinal plants for their anthelmintic activity. Glutathione-s-transferase (GST) is multifunctional enzyme that participates in the detoxification of endogenous and exogenous toxic metabolites which is fatal to the parasites. In the present investigation, effect of ethanol extracts of Albizia lebbeck (A/EE) and Trachyspermum ammi (TaEE) on GST activity against the paramphistome Cotylophoron cotylophorum was studied in vitro. The parasites were exposed to five sub-lethal concentrations of A/EE and TaEE for 2, 4 and 8h. Maximum inhibition of GST activity was observed in 0.5 mg/ml concentration after 8h of exposure. Inhibition of GST activity was dose and time dependent. Decrease in GST lead to accumulation of toxic metabolites which is lethal to the parasites.

Keywords: Cotylophoron cotylophorum, Albizia lebbeck, Trachyspermum ammi, Glutathione-s-transferase.

I. INTRODUCTION

Cotylophoron cotylophorum is a diegnetic trematode that parasitizes the rumen and reticulum of livestock. The immature parasites are responsible for destroying the mucosal walls of the alimentary tract on their way to growing into adults by the fervent tissue obliteration (Soulsby, 2006; Millar et al., 2012). Helminth parasites adversely affect the absorption and utilization of proteins, minerals and vitamins as well as upset the general metabolism of the host by causing diarrhea, anaemia and liver disorders often leads to death of the animal (Anand et al., 2000). The control of gastrointestinal parasites has traditionally relies on grazing management and anthelmintic drugs treatment. Anthelmintic drugs have been used either prophylactically or curatively to control gastrointestinal parasites. Current large scale sheep and goat production relies heavily on the application of chemical anthelmintics (Hein and Harrison, 2005). But the management practices are very poor (Wolstenholme et al., 2004). This has resulted in development of resistance to various chemical drugs. Also synthetic anthelmintic treatments are often impracticable in developing countries due to relatively high price of these anthelmintics (Tariq and Tantry, 2012). In this regard, several medicinal plants have been investigated...
for their anthelmintic properties. In the present study anthelmintic efficacy of Albizia lebbeck and Trachyspermum ammi was investigated based on its effect on GST, the enzyme involved in detoxification. 

Albizia lebbeck belongs to the family Mimosaceae is commonly called Vaagai in Tamil. A. lebbeck contains alkaloids, flavonoids, tannins, saponins which have therapeutic value (Mohammad faisal et al., 2012; Rahul et al., 2010). It possess biological activities such as antipyretic, analgesic, estrogenic, anti-inflammatory, antimicrobial and antioxidant activity (Resmi et al., 2006; Mohamed Farag et al., 2013).

Trachyspermum ammi commonly known as Omum in Tamil belongs to the family apiaceae. T. ammi exhibits anti-fungal, anti-microbial, anti-aggregatory, anthelmintic, anti-inflammatory, anti-oxidant and anti-spasmodic activity (Srivastava, 1988; Sivropoulou et al., 1996; Krishnamoorthy and Madalageri, 1999; Joshi, 2000; Singh and Singh, 2000; Kamal Jeet et al., 2012 and Sadiq et al., 2012).

Glutathione-S-transferase is a major detoxification enzyme in parasitic helminthes (Rao et al., 2000; Saeed et al., 2013). The biological role of GST in parasite is important for understanding the host-parasite relationship and any change in their functions could have therapeutic implications. Because of its role in protecting the cell against the immune-mediated lipid peroxidation, GST is considered as one of the vital targets for anthelmintic drugs (Singh and Irshadullah, 2003). The present investigation is designed to elucidate the effect of ethanol extracts of bark of A. lebbeck and seeds of T. ammi on the Glutathione-S-transferase of Cotylophoron cotylophorum.

**II. METHODS AND MATERIAL**

**In vitro maintenance of Cotylophoron cotylophorum:**

Cotylophoron cotylophorum were collected from the rumen of infected sheep, slaughtered at Perambur abbatior, Chennai. Adult live flukes were collected, washed thoroughly in physiological saline and maintained in Hedon-Fleig solution, which is the best medium for in vitro maintenance (Veerakumari, 1996). 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.

**Preparation of plant extracts:**

Albizia lebbeck (Bark) and Trachyspermum ammi (seeds) were collected from Lakshmi stores at Chennai, and were authenticated in the Department of Botany, Pachaiyappa’s college, Chennai and vouchered specimens are deposited in the herbarium of Pachaiyappa’s College, Chennai-30. The extraction of plant materials was done following the method of Harborne (1998). A. lebbeck and T. ammi were coarsely powdered and soaked serially in hexane, chloroform, ethyl acetate and ethanol. Aqueous extract was also prepared. The ethanol extract was filtered using Whatman filter paper No.1 and concentrated using, rotary evaporator (EQUITIRON). The concentrated extracts were completely dried to remove the last traces of the solvents using Lyodel Freeze Dryer (DELVAC).

**Estimation of Glutathione-S-transferase**

Activity of glutathione S-transferase (GST, EC 2.5.1.18) was assayed following the procedure of Habig et al. (1974). Glutathione S-transferase catalyses the conjugation of glutathione reduced (GSH) thiolate anion with a multitude of second substrate like 1-chloro-2,4- dinitrobenzene (CDNB). The conjugation of CDNB with GSH was measured by disappearance of free sulphydryl groups at 340 nm.

The sample for the enzyme was prepared by homogenizing 100 mg of the parasite in 1 ml of 0.2 M Tris-HCl buffer (pH 7.8). The homogenate was centrifuged at 1000 rpm for about 5 min. To 0.05 ml of the supernatant, 0.4 ml of 0.2 M Tris-HCl buffer (pH 7.8), 1.2 ml of water, 0.1 ml of 1.5 mM CDNB were added and incubated in water bath at 37°C for 10 min. After incubation 0.1 ml of 1.5 mM GSH was added. The change in absorbance was measured against a reagent blank at 340 nm at 30 sec interval for 5 min.

The protein content in the sample was estimated following the procedure of Lowry et al. (1951). The enzyme activity was calculated using the millimolar extinction coefficient of 9.6 of CDNB-GSH conjugate and was expressed as μmoles of CDNB-GSH conjugate formed/ min/ mg protein.
Statistical analyses

Statistical analyses were performed with the Statistical program for the social sciences SPSS version 16.0. The significance of drug induced inhibition in GST activity of the parasites was assessed using Analysis of Variance (ANOVA) for different concentrations of ethanol extracts of A. lebbeck and T. ammi. The term significant had been used to indicate differences for which P ≤ 0.05.

III. RESULTS AND DISCUSSION

RESULTS

Table 1. Effect of AlEE on GST of C. cotylophorum

<table>
<thead>
<tr>
<th>Concentration (mg/ml)*</th>
<th>% inhibition (mean ± S.D n = 5) at various periods of incubation **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>0.1</td>
<td>08.77 ± 0.12</td>
</tr>
<tr>
<td>0.2</td>
<td>13.86 ± 0.06</td>
</tr>
<tr>
<td>0.3</td>
<td>22.41 ± 0.06</td>
</tr>
<tr>
<td>0.4</td>
<td>35.86 ± 0.01</td>
</tr>
<tr>
<td>0.5</td>
<td>42.85 ± 0.03</td>
</tr>
</tbody>
</table>

*Inhibitory effects of the extract among the different concentrations are significantly different for each duration of incubation (P<0.05) using Bonferroni test

**Inhibitory effects of the extract among the different hours of incubation is significantly different for each concentration (P<0.01) using Bonferroni test

Table 2. Effect of TaEE on GST of C. cotylophorum

<table>
<thead>
<tr>
<th>Concentration (mg/ml)*</th>
<th>% inhibition (mean ± S.D n = 5) at various periods of incubation **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>0.1</td>
<td>10.32 ± 0.19</td>
</tr>
<tr>
<td>0.2</td>
<td>19.79 ± 0.03</td>
</tr>
<tr>
<td>0.3</td>
<td>27.47 ± 0.09</td>
</tr>
<tr>
<td>0.4</td>
<td>38.11 ± 0.02</td>
</tr>
<tr>
<td>0.5</td>
<td>46.82 ± 0.03</td>
</tr>
</tbody>
</table>

*Inhibitory effects of the extract among the different concentrations are significantly different for each duration of incubation (P<0.05) using Bonferroni test

**Inhibitory effects of the extract among the different hours of incubation is significantly different for each concentration (P<0.01) using Bonferroni test

IV. DISCUSSION

The present study elicits the deleterious effects of A. lebbeck and T. ammi ethanol extracts on Glutathione-S-transferase (GST) of Cotylophoron cotylophorum. GST is a multifunctional enzyme that participate in the detoxification of endogenous toxic substances including pharmacologically active compounds (Habig et al.,
GST enzyme have been considered to play a major part in drug metabolism where they contribute to cell survival by detoxification of foreign compounds (Klassen, 1996). In the present investigation, GST inhibition was found to be 85.17% in A/EE-treated parasites and 80.90% in TαEE-treated parasites at 0.5 mg/ml after 8h of incubation. Similar inhibitory effect of GST activity by ethanolic extracts of Areca cae techu and Syzygium aromaticum has been reported in C. cotylophorum (Manoj Dhanraj and Veerakumari 2015). Agneszka et al. (2012) suggested that targeting the GST in anthelmintic therapy may break the defense mechanism of parasites.

Similarly Fakae et al. (2000) reported that phytochemicals from piliostigma thonningii, Ocimum gratissimum, Nuclea latifolia and Alstonia boonei possesses a potential inhibitory effect on GST of Ascaris suum and onchocerca volvulus. Singh and Irshadullah (2003) reported the inhibition of GST activity of Fasciola gigantica treated with closantel, bithionol and refoxanide. Farahnak et al., (2006) reported that activity of GST is suppressed in F. gigantica treated with triclabendazole. Phytochemicals from Cinnamomum verum, C. Aromaticum, Allium sativum, Coriandrum sativum and Cymbopogan citrates have potential to inhibit the GST in Brugia malayi (Shamina et al., 2010).

GST enzymes of helminths parasites may protect the parasites against exogenous free radical damage or xenobiotics as a result of immune effector mechanism from the host directed at the parasite (Brophy and Pritchard, 1994). Coles and Kadlubar (2003) reported that GST catalyzes the nucleophilic addition of reduced glutathione (GSH) to numerous endobiotic and xenobiotic electrophilic substrates, usually promoting their inactivation, degradation, and excretion. In addition, these enzymes are thought to play a role in protecting DNA from oxidative damage (Little et al., 2006). Significant inhibition in the activities of GST, the enzyme involved in detoxification, was observed in A/EE and TαEE-treated flukes. Inhibition of GST might be fatal to the parasite due to the accumulation of toxic metabolites, as GST involved in the detoxification process. The present study discloses the anthelmintic potential of A/EE and TαEE and paves the way for including these plants in the armoury of anthelmintic herbal medicines to combat Cotylophoron cotylophorum infection in livestock.

V. REFERENCES


