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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* (*Al*EE) and *Trachyspermum ammi* (*Ta*EE) on GST activity against the paramphistome *Cotylophoron cotylophorum* was studied *in vitro*. The parasites were exposed to five sub-lethal concentrations of *Al*EE and *Ta*EE 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 abbatoir, 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 37ºC 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 $P \leq 0.05$.

III. RESULTS AND DISCUSSION

RESULTS

Ethanol extracts of *T. ammi* and *A. lebbeck* significantly inhibited the glutathione-s-transferase activity of *Cotylophoron cotylophorum*. Inhibition was dose and time dependent. GST activity was decreased by 85.17% in flukes treated with 0.5mg/ml of *Al*EE at 8h (Table 1). *Ta*EE-treated flukes showed rapid reduction in GST activity with maximum inhibition of 46.82, 69.33 and 80.90% (Table 2) after 2, 4 and 8 h of incubation, at 0.5 mg/ml concentration. Both the plant extracts significantly reduced the GST activity in *C. cotylophorum*.

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

Concentration (mg/ml)*	% inhibition (mean ± S.D n = 5) at various periods of incubation **		
	2 h	4 h	8 h
0.1	08.77 ± 0.12	38.25 ± 0.09	53.90 ± 0.13
0.2	13.86 ± 0.06	49.92 ± 0.04	62.80 ± 0.06
0.3	22.41 ± 0.06	54.25 ± 0.01	79.28 ± 0.01
0.4	35.86 ± 0.01	59.57 ± 0.03	82.67 ± 0.05
0.5	42.85 ± 0.03	68.27 ± 0.05	**85.17 ± 0.02**
*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

Concentration (mg/ml)*	% inhibition (mean ± S.D n = 5) at various periods of incubation **		
	2 h	4 h	8 h
0.1	10.32 ± 0.19	36.48 ± 0.03	56.52 ± 0.04
0.2	19.79 ± 0.03	44.56 ± 0.02	67.91 ± 0.04
0.3	27.47 ± 0.09	55.09 ± 0.09	71.06 ± 0.04
0.4	38.11 ± 0.02	62.34 ± 0.05	76.26 ± 0.03
0.5	46.82 ± 0.03	69.33 ± 0.07	**80.90 ± 0.03**
*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 Glutathoine-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.*,

1974). 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 *Al*EE-treated parasites and 80.90% in *Ta*EE-treated parasites at 0.5 mg/ml after 8h of incubation. Similar inhibitory effect of GST activity by ethanolic extracts of *Areca caetechu* 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, Nauclea 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 *Cinnamoum 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 *Al*EE and *Ta*EE-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 *Al*EE and *Ta*EE and paves the way for including these plants in the armoury of anthelmintic herbal medicines to combat *Cotylophoron cotylophorum* infection in livestock.

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