GLUTATHIONE-S-TRANSFERASE OF HAEMONCHUS CONTORTUS - A VULNERABLE TARGET FOR ALLIUM SATIVUM AND ANDROGRAPHIS PANICULATA

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ABSTRACT

Haemonchosis has been identified as major gastrointestinal parasitic disease in livestock caused by Haemonchus contortus. It is an important bloodsucking parasite of ovines and causes an insidious drain on production and high mortality in all classes of livestock. Drug resistance necessitated the exploration of alternative control methods. Therefore, there is a need for developing cheaper, less toxic and eco-friendly novel drugs. Indigenous medicinal plants offer an important alternative source. Glutathione-S-transferase (GST) are major metabolic enzymes and may play an important role in the parasite’s survival. GST’s are able to scavenge the products of lipid peroxidation and to metabolize toxic products, including anthelmintics. The effect of ethanol extract of Allium sativum (AsEE) and chloroform extract of Andrographis paniculata (ApCE) on GST activity of the nematode H. contortus was studied. The worms were exposed to various sublethal concentrations of AsEE and ApCE for 2, 4 and 8h. GST was assayed in control and drug-treated worms. Maximum inhibition of GST activity was observed at 0.5 mg/ml of AsEE and ApCE after 8 h of exposure. Inhibition of GST activity was dose and time dependent. Reduction in GST leads to accumulation of toxic metabolites which ultimately kill the parasites. The present study thus enlightened the anthelmintic effect of AsEE and ApCE, which can be used to combat nematode infection in livestock.

KEYWORDS: Allium sativum, Andrographis paniculata, Haemonchus contortus and Glutathione-S-transferase.
INTRODUCTION
Gastrointestinal parasites are the world wide problem manifested by reduced weight, lowered meat and milk production.[1] Furthermore, competition for the nutrients and tissue damage during feeding and migration could cause severe clinical signs such as anorexia, anaemia, diarrhoea and oedema associated with poor performance and mortality particularly in young, aged and immune suppressed animals.[2] Gastrointestinal (GI) nematodes are a major threat to sheep productivity and endanger animal welfare Worldwide particularly in developing countries. Haemonchosis caused by H. contortus, mostly a disease of grazing animals has been shown to induce stress in lambs[3] which may get aggravated due to transportation. Anthelmintic drugs are used to treat helmintic parasitic infections. Chemotherapy is a major treatment modality used for control of haemonchosis.[4] Chemotherapeutic agents are expensive and cause immense side effects to the animals and are non-biodegradable. Chemical control of helminths coupled with improved farm management has been an important worm control strategy.[5] However, in recent years the more important GI nematodes of sheep and goat have developed resistance against the broad-spectrum anthelmintics.[6,7]

Plant derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs.[8] The discovery and development of new drug will open new vitas to serve humanity from unconquered diseases. An estimate suggests that about 13,000 plant species worldwide are known to have used as drugs. The natural plant extracts were frequently screened for new drug discovery.[9] Most of the research on plant extracts is being focused on identifying bioactive compounds from plants against nematodes.

*Allium sativum* commonly known as garlic belongs to the family Alliaceae. It is an indigenous dietary component, is also used widely in home remedies and pharmacotherapy against debilitated pathologies because of its anti-oxidant[10], anti-cardiovascular[11] and anti-hyperglycaemic[12] activities. It has also proposed to treat asthma, candidiasis, cold and possess anti-bacterial effect against food borne pathogen like *Salmonella*, *Shigella* and *Stapylococcus aureus*.[13] The anthelmintic activity of aqueous and alcoholic extract of *A. sativum* against *Fasciola gigantica*, *Gigantocotyle explanatum* and adult *Cotylophoron cotylophorum* was reported by several researchers.[14-16]
Andrographis paniculata commonly called as ‘King of Bitters’ is one of the species belonging to the family acanthaceae. In Ayurvedic medicine the plant is used to treat inflammation, pain and dispose toxins from body and used as remedy for the cold, fever and detoxification, since ancient times.\cite{17} A. paniculata has a broad range of pharmacological effects including anthelmintic, anti-microbial, and immuno stimulant.\cite{18-21} Its major constituents are diterpenoids, flavanoids and derivative compounds such as andrographolide, the major active compound, mostly studied for its bioactivities. Andrographolide exhibits multiple pharmacological properties and is a potential chemotherapeutic agent.\cite{22} Andrographolide is abundant in leaves and can be easily isolated from the crude plant extracts as crystalline solid.\cite{23,24}

Glutathione-S-transferases (GST) are widely distributed as isoenzymes in the plant and animal kingdoms.\cite{25} GST have been detected in a range of helminths, where it may be one of the major detoxification enzymes.\cite{26} GST may potentially favour parasite survival by neutralizing the toxins acting against them may repair host-induced damage.\cite{27} Therefore, inhibition of Glutathione-S-transferase activity is the most important target for anthelmintic drugs. In the present investigation anthelmintic activity of ethanol extract of Allium sativum and chloroform extract of Andrographis paniculata against the nematode Haemonchus contortus was evaluated in vitro based on their effect on Glutathione-S-transferase.

MATERIALS AND METHODS

Collection and in vitro maintenance of H. contortus

Adult H. contortus were collected from the abomasum of sheep, slaughtered at Perambur slaughter house, Chennai. The worms were washed in physiological saline and maintained in Hedon-Fleig solution (pH 7.0) at 37º, which is the best medium for in vitro maintenance.\cite{28}

Preparation of plant extracts

The bulbs of Allium sativum was purchased from a local shop and the leaves of A. Paniculata were collected from cultivated places of Vellore District, Tamil Nadu, India. A. Paniculata was identified and authenticated by a botanist in the Department of Botany, Captain Srinivasa Moorthy Drug Research Institute for Ayurveda and Siddha, Arumbakkam, Chennai. The vouchered specimens are deposited at Unit of Parasitology, Pachaiyappa’s College, Chennai - 600 030. The bulbs of Allium sativum was made into paste and soaked in hexane, chloroform, ethyl acetate, ethanol and water in an aspirator bottle and the leaves of A. paniculata were cleaned, shade dried and coarsely powdered soaked serially in hexane, chloroform, ethyl.
acetate, ethanol and water in an aspirator bottle. The extraction was done by cold percolation method after 48 h. The filtrate was collected by passing the mixture through Whatman filter paper No.1 and concentrated by using Rotary Evaporator (EQUITRON). The concentrated extracts were dried to remove the solvents using Lyodel freeze Dryer (DELVAC, Chennai).

**Exposure of H. contortus to A. sativum and A. paniculata**

Adult *H. contortus* were incubated in five different sub-lethal concentrations (0.005, 0.01, 0.05, 0.1 and 0.5 mg/ml) of AsEE and ApCE for 2, 4 and 8h. Simultaneously, control was also maintained in Hedon-Fleig solution without the plant extract.

**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.*[^30] GST 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 at 340 nm.

The sample for the enzyme was prepared by homogenizing 100 mg of the parasite in 1.5% KCl. 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 6.8), 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.*[^31] The enzyme activity was calculated using the millimolar extinction coefficient of 9.6 of CDNB-GSH conjugate was expressed as μ moles of CDNB-GSH conjugate formed/min/mg protein.

**Statistical analyses**

The experimental results were expressed as mean ± standard deviation. Each value is expressed as mean of triplicate experiments. Statistical analyses were performed by ANOVA using SPSS version 20 for different concentration of AsEE and ApCE.
RESULTS AND DISCUSSION

AsEE and ApCE significantly inhibited the GST activity of *H. contortus*. Dose and time dependent inhibition in GST activity was observed in drug-treated parasites. Inhibition in GST activity by AsEE and ApCE at 0.5 mg/ml concentration was found to be 92.11% and 93% after 8h respectively (Table 1).

In the present study, the GST activity of *H. contortus* was significantly inhibited following treatment with AsEE and ApCE. Certain anthelmintics including oltripraz in *Schistoma mansoni*, Rafoxanide, Closantel and bithionol in *Fasciola gigantica* have been reported to inhibit the GST activity.\[^{32,33}\] Gupta and Rathur\[^{34}\] suggested that xenobiotics such as diethylcarbamazine, inhibited GST of *Setaria cervi*, which could play an important role in parasite’s survival. Fakae *et al.*\[^{35}\] reported the inhibition of GST activity of *Ascaris suum* and *Onchocerca volvulus* following treatment with phytotherapeutic drugs viz. *Piliostigma thonningii*, *Ocimum gratissimum*, *Nauclea latifolia* and *Alstonia boonei*. Similarly, phytochemicals from *Cinnamomum verum*, *Cinnamomum aromaticum*, *Allium sativum*, *Coriandrum sativum*, *Cymbopogon citrates*, *Curcuma longa*, *Strychnos nuxvomica*, *Vanilla planifolia*, *Litsea cubeba*, *Pimenta dioica* illustrated a potential inhibition of GST in *Brugia malayi*\[^{36}\].

It is imperative to identify the key enzymes and biochemical pathways that are pivotal to the parasites survival in the host’s hostile environment, including their oxidative stresses and immune responses. These enzymes should provide excellent biochemical targets for developing effective chemotherapies and vaccines.\[^{37,38}\] GSTs are major metabolic enzymes and may play an important role in the parasite’s survival. GST has been detected in a range of helminths, where it may be one of the major detoxification enzymes.\[^{39}\] GST secreted by the helminth parasites favours the survival of the parasite by neutralizing the toxins including the anthelmintics and also by scavenging the products of lipid peroxidation. These biological functions make GST’s as vulnerable molecular targets for new antifilarial drugs.\[^{40}\] GST isozymes are potential protective antigens against fascioliasis and schistosomiasis.\[^{41-46}\]

The current investigation clearly discloses the inhibitory effect of AsEE and ApCE on GST activity of *H. contortus*. Inhibition of GST’s have chemotherapeutic application.\[^{47}\] The decrease in GST could lead to accumulation of toxic metabolites like aldehydic products of lipid peroxidation, which ultimately kill the parasites. Thus GST is the vulnerable target for
ethanol extract of A. sativum and chloroform extract of A. paniculata. This study suggests the possible use of A. sativum and A. paniculata to combat haemonchosis in livestock.

Table 1: Effect of AsEE and ApCE on GST activity of H. contortus.

<table>
<thead>
<tr>
<th>Concentration mg/ml*</th>
<th>% inhibition (mean ± SD of n=5) at various periods of incubation**</th>
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<tbody>
<tr>
<td></td>
<td>2h</td>
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<tr>
<td>AsEE</td>
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<tr>
<td>0.005</td>
<td>24.44 ± 0.29</td>
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<tr>
<td>0.01</td>
<td>32.90 ± 0.29</td>
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<tr>
<td>0.05</td>
<td>39.31 ± 0.29</td>
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<tr>
<td>0.1</td>
<td>41.81 ± 0.20</td>
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<tr>
<td>0.5</td>
<td>43.99 ± 0.23</td>
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<tr>
<td>ApCE</td>
<td></td>
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<tr>
<td>0.005</td>
<td>26.57 ± 0.16</td>
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<tr>
<td>0.01</td>
<td>33.70 ± 0.16</td>
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<tr>
<td>0.05</td>
<td>38.09 ± 0.16</td>
</tr>
<tr>
<td>0.1</td>
<td>42.42 ± 0.16</td>
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<tr>
<td>0.5</td>
<td>51.14 ± 0.16</td>
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*Inhibitory effects of the extracts among the different concentrations of the respective plants are duration of incubation (P < 0.05) using Duncan principle comparisons.

**Inhibitory effects of the extracts among the different hours of incubation is significantly different for each concentration of the respective plants (P < 0.01) Duncan principle comparisons.

CONCLUSION

Glutathione-S-transferase is the major detoxification enzyme in helminths. Ethanol extract of A. sativum and chloroform extract of A. paniculata significantly inhibited the activity of GST of H. contortus. The inhibition of GST in H. contortus following treatment with AsEE and ApCE amply demonstrated that GST is the vulnerable drug target for AsEE and ApCE and may contribute to the anthelmintic activity of the plant extract against H. contortus and also ensure the restriction of emergence of drug-resistance strains.

ACKNOWLEDGEMENTS

We gratefully acknowledge University Grant Commission (UGC), for funding this project.

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