Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 1361-1367
Received: 25-07-2018
Accepted: 26-08-2018

Om Prakash
Veterinary Polytechnic, Surajpur, Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh, India

S Gomathinayagam
Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

Tirunelveli Jeyagopal Harikrishnan
Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

Muthusamy Raman
Transboundary Research Platform for Veterinary Biological, Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

V Pandiyan
Department of Veterinary Biochemistry, Madras Veterinary College, Chennai, Tamil Nadu, India

P Azhahianambi
Transboundary Research Platform for Veterinary Biological, Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu, India

Correspondence
Om Prakash
Veterinary Polytechnic, Surajpur, Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, Chhattisgarh, India

Is L-buthionine sulfoximine reverse the benzimidazole resistance in *Haemonchus contortus* of small ruminants?

Om Prakash, S Gomathinayagam, Tirunelveli Jeyagopal Harikrishnan, Muthusamy Raman, V Pandiyan and P Azhahianambi

Abstract
Anthelmintic resistance (AR) in parasites of livestock is an emerging problem in many parts of the World. Hence, finding new classes of anthelmintic drugs or anthelmintic resistance reversal agents is the need of the hour to sustain the global livestock production. The aim of present study was to study the Benzimidazole (BZ) resistance reversal property of L-buthionine sulfoximine (L-BSO) using *in vitro* egg hatch assay (EHA). Thiabendazole (TBZ) alone and combination of TBZ and 200µM, 500µM and700µML-BSO were used with proper control and the hatching percentage and ED_{50}values were recorded. It was found that significant (P<0.001) reversal of BZ resistance occurred when *H. contortus* was treated with L-BSO (>500µM) and the effect was dose dependent. Hence, L-BSO might potentially be used as a BZ resistance reversal agent to treat BZ resistant nematode parasites of livestock

Keywords: *Haemonchus*, benzimidazole, buthionine sulfoximine, small ruminants

Introduction
The *Haemonchus contortus*, commonly known as the barber's pole worm, parasitic nematode, a major cause of loss of production in sheep and goat industry. This parasite can inflict a considerable amount of damage to a flock or herd in a short span of time. Treatment and control of this gastrointestinal parasite have been successful using various anthelmintics, such as benzimidazoles, levamisole, and ivermectin, which exert selection pressure resulting in the development of anthelmintic resistance (Torres-Acosta *et al*., 2012) [56]. Unraveling the anthelmintic resistance mechanisms employed by *H. contortus* will help us to find solutions to overcome the anthelmintic resistance and use better treatment and control methods. The effect of glutathione (GSH) on the eggs of *H. contortus* susceptible/ resistance to anthelmintics was investigated using *in vitro* egg hatch assay (EHA). The modulators or GSH analogue like Diethylemaleate, D, L-buthionine sulfoximine and patulin induce an unexpected decrease in the susceptibility of egg to thiabendazole (Kerboeuf and Aycaedi, 1999) [31]. L-BSO increases the toxicity of nifurtimox, benzidazole to the epimastigote, trypomastigote and amastigote stages of *Trypanosoma cruzi* (Faundez *et al*., 2005) [19]. The enzyme activity of *Burgia malayi*, the causative agent of lymphatic filariasis is irreversibly inhibited by the BSO (Hussein and Walter, 2005) [29]. Buthionine sulfoximine is a potent and specific inhibitor of γ-glutamylcysteine synthetase and inhibits glutathione biosynthesis and causes depletion of cellular glutathione levels (Griffith and Meister, 1979) [25]. Glutathione S- transferase (GST) which is part of parasite detoxification system associated with the establishment of parasitic nematode infections within the gastrointestinal environment of the mammalian host (Rossum *et al*, 2004) [53]. On the above hypotheis the present study was conducted with the objective of is BSO potentiate the activity of TBZ in H. *contortus.*

Material and Methods
Collection of Parasites and Harvesting of Eggs
Abomasums of sheep and goat were collected in normal saline from the slaughter house, Perumbur, Chennai, India and transported to the laboratory. Abomasums were cut open along greater curvature and manual removal of adult *H. contortus* was carried out from the abomasums content and mucosal fold. The worms were washed two times with normal saline and female worms were separated and incubated in normal saline for an hour at 37 ^{0}C for release of eggs. After incubation, normal saline was collected in centrifuge tube and centrifuged (Eppendorf, 5810R, Germany) at 2000 rpm for 5 minutes to sediment the eggs. The supernatant was poured off and sediment was examined for the presence of eggs. The concentration of egg was adjusted to50 eggs/µl.

Assessment of benzimidazole resistance by EHA

Assessment of benzimidazole resistance by egg hatch assay (EHA) was performed as using harvested eggs from the slaughter house, Perumbur, Chennai, India using pure thiabendazole -99 percent (Sigma Aldrich- T8904, USA) in five concentration of 0.05, 0.1, 0.3, 0.5, and 1.0 µg/ml as per the procedure of World Association for Advancement of Veterinary Parasitology (WAAVP) proposed by Coles *et al.*, 1992 [12] with slight modification (Lourde Raj, *et al*., 2006; Laksmipriya, 2012) [41, 36].

Assessment of reversal effect of buthionine sulfoximine by EHA

The effect of BSO was performed as similar to egg hatch assay, in this test different concentrations (200µM, 500µM and 700µM) of BSO (B2515, Sigma Aldrich, USA) added along with thiabendazole.

Estimation of Intracellular glutathione concentration

The total intracellular glutathione (GSH) was determined by a calorimetric method described by Greech *et al*., 1999 [24]. TBZ resistant eggs and larvae of *H. contortus* were used in this assay**.** One gram of eggs and larvae were exposed to 1.0 µg/ml of TBZ and 200µM, 500µM and 700µM of BSO at 2, 4, and 6 hours intervals. The observance was read at 413nm against a blank and the amount of glutathione is expressed as nano mole per gram of larvae by UV-VIS mini-1240, spectrophotometer (A109035i, Shimadzu Asia Pacific Pte Ltd, Singapore).

Statistical Analysis

Data analysis was carried out using SPSS 20 version software (SPSS 20.0; Inc., Chicago, Illinois, USA). To analysis the mean hatching percentage and intracellular glutathione level between resistant populations before and after treatment with BSO, data were subjected to a multivariate analysis (ANOVA) with Tukey's post-host test. The differences were considered statistically significant when $P<0.001$. The ED_{50} were calculated using probit analysis.

Result

In the present study a total of 1003 abomasal samples of sheep and goat were examined at the slaughter house, Perambur, Chennai, India. Out of which 534 samples were found to contain male and female worms of *Haemonchus contortus* on dissection. Prevalence of *H. contortus* in small ruminants slaughtered in Perambur slaughter house, Chennai, Tamil Nadu, India was 53.2% recorded.

Assessment of resistance by Egg hatch assay (EHA)

The assessment of resistance and susceptible worms were based on the ability of eggs to hatch at concentration greater than 0.1µg/ml of thiabendazole (TBZ). In present study, samples were found to be resistant to TBZ by EHA, where larvae could be seen even at concentrations higher than 1.0 µg/ml of TBZ. The mean hatching percentage at different concentrations, *i.e*. control, 0.05, 0.1, 0.3, 0.5 and 1.0 µg/ml was recorded and analysed (Table 1). Using probit analysis ED_{50} value for benzimidazole resistance worm was 0.193µg/ml.

Table 1: Comparative egg hatching percentage (Mean ±SEM) after *in vitro* egg hatch assay (EHA) to see the anthelmintic resistance and reversal of anthelmintic resistant in *Haemonchus contortus* in sheep and goat treatment with thiabendazole (Alone) and a combination of thiabendazole and 200, 500 and 700µM L- buthionine sulfoximine (L-BSO) compared with control

Treatment	Comparative egg hatching percentage (Mean ±SEM)			
	No L-BSO (Alone TBZ)	L-BSO 200µM	L-BSO 500µM	L-BSO 700µM
Control (No TBZ)	82.44 ±1.59	78.02 ±1.31	77.31±1.29	79.75 ±1.14
TBZ 0.05 µg/ml	74.78 ±1.58	62.53 ±1.64	52.73±1.57	36.70 ±1.77
TBZ 0.1 µg/ml	62.04 ±1.90	54.34±1.96	40.04±1.88	26.29 ±1.18
TBZ 0.3 µg/ml	48.80 ±2.82	40.09±2.53	34.24 ±2.25	22.48±1.68
TBZ 0.5 µg/ml	37.56 ±2.16	24.80 ±1.93	16.39 ±1.39	4.53 ±0.86
TBZ 1.0 µg/ml	4.02±0. 64	2.80 ±0.39	1.21 ± 0.27	1.02 ±.23
ED_{50}	0.193µg/ml	0.137 µg/ml	0.077 µg/ml	0.033µg/ml
Result	Resistant	Resistant	Susceptible (Reversed)	Susceptible (Reversed)

*Level of significance: $P<0.001$

Assessment of reversal effect of buthionine sulfoximine

The mean percentage of egg hatch at different concentration of TBZ *i.e*. 0.05, 01, 0.3, 0.5 and 1.0µg/ml with combination of 200µM, 500µ and 700µM were recorded and It was significantly ($P<0.001$) reduced in all concentrations. Using probit analysis the ED_{50} values after the combination of TBZ and 200 µM, 500 µM and 700 µM concentrations of reversal agent BSO, as 0.137, 0.077 and 0.033µg/ml TBZ were recorded respectively.

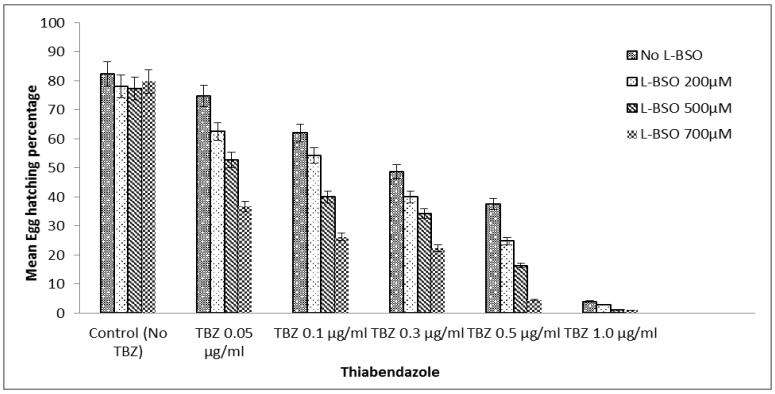

Fig 1: Thiabendazole Resistance and effect of BSO in Thiabendazole resistance

Estimation of intracellular glutathione

Intracellular glutathione has been estimated in eggs and larvae of TBZ resistant *H. contortus*, before and after exposure to different concentration of 200, 500, and 700µµM of BSO and OD values were taken at 412nm in2, 4, 6 hour intervals. It was found that the glutathione level was significantly (P<0.001) reduced in all concentrations (Table. 2). Regression coefficient (R square) between intracellular reduced glutathione (GSH) and egg hatching percentage at 1.0µg/ml of TBZ in 2, 4, and 6 hours intervals were 0.372, 0.615 and 0.354 calculated respectively.

Table 2: Reduced intracellular glutathione (Mean±SEM) in *Haemonchus contortus* when treatment with thiabendazole (1.0 µg/ml) alone and combination of thiabendazole and L-BSO 200 µM, L-BSO 500 µM and L-BSO 700 µM, at two, four and six hours intervals

Treatment	Intracellular glutathione (nmol/g) (Mean± SEM)		
	Two hours	Four hours	Six hours
TBZ (1.0 µg/ml)	39.09±1.08	24.65 ±0.61	14.04 ±0.82
TBZ+ L-BSO 200 µM	28.94±1.23	15.30 ±1.05	7.52 ±0.61
TBZ+ BSO 500 µM	18.02±0.69	8.08 ±0.19	2.14 ±0.30
TBZ+ BSO 700 µM	8.03±0.39	4.01±0.40	1.17 ±0.97

Level of significance: P < 0.001

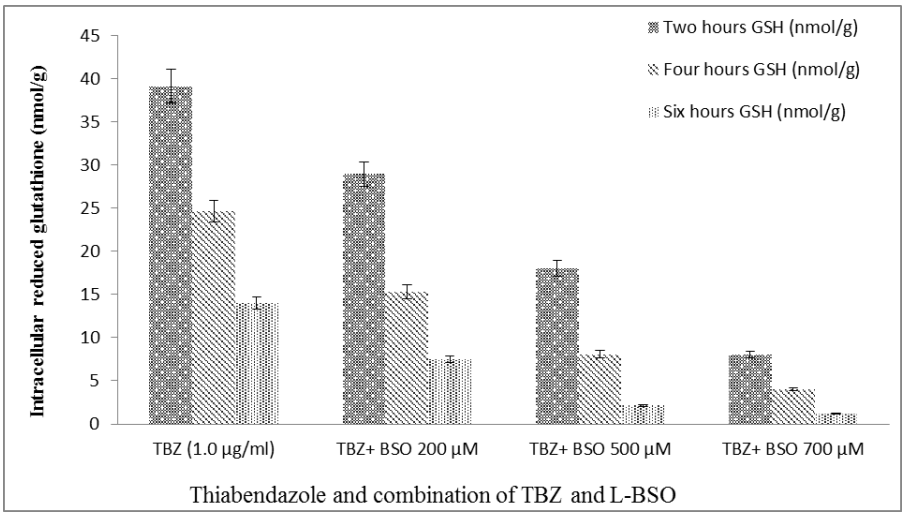

Fig 2: Estimated intracellular reduced glutathione (nmol/g)in *H. contortus* of sheep/goat after treatment with thiabendazole alone and combination of thiabendazole 200µM L-BSO, 500µM L-BSO and 700 µM L-BSO at two, four and six hour interval

Discussion

The previous studied about prevalence of *H. contortus* reported by scientist time to time. Fakae (1990) [18] conducted survey in the Nigerian derived savanna (from August 1987 to July 1988) on the seasonal fluctuations in the composition of *Haemonchus contortus* burden of naturally infected West African Dwarf sheep and goats and found that the incidence of *H. contortus* infection was high 77.8–100% with no definite seasonal distinction. *H. contortus* infection lowers in the summer season as compared to winter and rainy season Garg *et al*. (2003) [20]. Yadav *et al*. (2006) [60] recorded that the seasonal variation throughout the year and was highest during the rainy season (88.54%) followed by summer (83.15%) and winter (76.01%). Shugufta *et al*. (2005) [54] studied the incidence of gastrointestinal nematodes in sheep of Kashmir valley. Five types of nematodes viz., strongyles, *Trichostrongylus* spp., *Haemonchus* spp., *Nematodirus* spp. and *Marshallagia* spp. were identified. The seasonal prevalence of infections indicated that the nematode infection (overall) was highest in summer (67.14%) and lowest in winter (44.31%). Al- shaibani *et al*. (2008) [1] observed for 12 months and *H. contortus* (24.6%) were found to be predominantly of gastrointestinal nematode parasites. Qamar *et al*. (2008) [50] found overall the highest (43.69%) seasonal prevalence in sheep and Goats was recorded during summer; followed by autumn (38.46%), spring (37.12%), while the lowest (28.79%) was recorded during winter. Lashari *et al*. (2015) [37] reported the prevalence of *Fasciolia hepatica*, *Avitellina centripunctata*, *Haemonchus contortus* and *Trichuris globulosa* was 21.41, 12.23, 6.50 and 5.73%, respectively and suggest that the age, sex, body weight and breed are important factors which influence the prevalence of gastrointestinal parasites. Tasawar *et al*. (2010) [55] reported the overall prevalence of *Haemonchus contortus* was 77.7% in sheep at Government Research Centre for Conservation of Sahiwal Cattle (RCCSC) Jehangirabad, District Khanewal from February 2007 to June 2007. Mesele *et al*. (2014) [46] perfomeda cross sectional was conducted to estimate the prevalence of haemonchosis in small ruminants through examination of 613 Abomasum of small ruminants, 355 sheep and258 goats. The overall prevalence in this study was 38.6%, with a prevalence of 22.8%, and 15.8% were recorded for sheep and goats respectively. similarly number of studied about prevalence of haemonchosis in sheep and goat conducted time to time with 57.8%, 9.18% (Degheidy *et al*. 2014) [13], 40.9% (Gebresilassie and Tadele, 2015);insheep and goats 67.2% and 56.6%respectively(Bulbul *et al*. 2015), 12.1% (Boukhari *et al*. 2016) [7] and 38.0% (Castle, 2017) [11]. Coles *et al*. (1992) [12] given the procedure of egg hatch assay and eggs hatching with an ED_{50} value in excess of 0.1 µg TBZ per ml were indicative of benzimidazole resistance. Varady and Corba (1999) [59] found egg hatch assay to be sensitive and accurate to determine benzimidazole resistance in *H. contortus*. They demonstrated that the ED_{50} in resistant strain was different from the susceptible strain by resistant factors. Calvete *et al*. (2012) [10] also found 11 per cent of

TBZ resistant nematodes in sheep farms in Northeast Spain using egg hatch assay. The mean percentage of egg hatch in 42 resistant samples was above the discriminating dose of 0.1 µg per ml of TBZ and larvae hatched even at higher concentration of TBZ. This is similar to the report of Le Jambre (1976) [38] who showed that TBZ resistant strains hatched in higher concentration of TBZ than non-resistant strains. Taylor *et al*. (2002) [57] reported eggs from susceptible individuals rarely hatch at concentrations greater than 0.1µg / ml of thiabendazole. In the previous studied many scientist and researchers reported the anthelmintic resistance by egg hatch assay with range of ED_{50} Value as 0.586 (Arunachalam *et al*. 2005) [2] ;0.627, 0.678 and 0.388 µg / ml (Easwaran *et al*. 2009) [16, 17] and 0.8 and 0.6 µg/ml of benzimidazole (Lourde raj *et al.* 2006) [41]; 17.9% Lakshmipriya (2012) [36]; ED50 for egg hatch was 0.196 with lower and upper limit ofED_{50} of 0.051 and 0.329 (Dinesh, 2013) [14]; 0.059 µg/ml (Rialch *et al*. (2013) [51]; 0.299µg/ml of albendazole (Minakshisundram *et al*. 2014) [45]; The ED_{50} for egg hatch was 0.196, indicating suspected resistance to benzimidazole anthelmintics (Kumbhakar *et al*. 2015) [35]; Egg hachability 19.66% (Goncalves *et al*., 2016) [23].

Buthionine sulfoximine (BSO) was used as reversal agent in the present study and found significant reversal effect which was dose depended. The reversal of resistance occurred when TBZ resistance eggs were treated with BSO and resulted in inhibition of egg hatching. Beugnet *et al.* (1997) [5] performed egg hatch assay (EHA) and demonstrated the relationship between P-glycoproteins (p-gp) and benzimidazole resistance through the use of the p-gp inhibitor verapamil, a calcium channel blocker. They had shown that, in the presence of verapamil, the toxicity of the drug increased and that benzimidazole resistance could be partially reversed. Kerboeuf *et al*. (1999) [31] examined membrane drug transport mechanism of resistance to anthelmintics by flow cytometry using rhodamine 123, a p-gp transport probe and found a higher level of green fluorescence in eggs of resistant *H. contortus*, indicating high levels of expression of pgp in resistant eggs. Riou *et al.* (2003) [52] confirmed that cholesterol could modulate p-gp activity on nematode eggs and change the level of resistance to anthelmintics. They suggested changes in membrane lipid contents could provide another way to improve the reversion of resistance and to increase the efficacy of anthelmintics. Kerboeuf *et al.* (2008) [32] suggested that the combination of anthelmintic targeting nematodes with an inhibitor of p-gp efflux pumps had a significant effect on both egg excretion and the number of worms coming from anthelmintics resistant nematodes. They opined that this discovery would open up new perspectives in nematode control by maintaining a good efficiency of the treatment, while reducing the doses of active compound. They analyzed the functional consequences of the localization for xenobiotic transport and drug resistance in nematodes and compared with results obtained in vertebrates. They suggested that understanding of such mechanisms was crucial in overcoming the failure of drug treatments due to the development of resistance. In this study addition of BSO to TBZ has inhibited hatching of resistant eggs by increasing toxicity of TBZ. Blackhall *et al*. (2008) [32] studied an association between a specific allele of p-gp and survival of benzimidazole treatment. They suggested that by reducing the amount of drug to reach its target, p-gp could act as a general defensive mechanism against xenobiotics. Bartley *et al*. (2009[a]) [3] showed the influence of various p-gp interfering compounds on the efficacy of ivermectin sensitive and resistant nematode isolates. They also demonstrated that in the presence of p-gpinterfering agents, the *in vitro* susceptibility to ivermectin of both sensitive and resistant isolates of *T. circumcincta* and *H. contortus* was increased. Bartley *et al*. (2009[b]) [4] described the increased sensitivity of resistant larvae to ivermectin after the co-incubation with pluronic 85 but *in vivo* co-administration of ivermectin with this p-gp modulator to sheep did not show an improved efficacy against resistant *H. contortus.* Lifschitz *et al*. (2010[a]) [39] showed, by field trial done in Argentina that the efficacy of both ivermectin and moxidectin against resistant *Cooperia* spp. in cattle tended to increase after their co-administration with loperamide (LPM) as a p-gp modulator. Lifschitz *et al*. (2010[b]) [40] reported the effects of loperamide (LPM), a p-gp modulating agent, on both ivermectin kinetic behaviour and anthelmintic activity in infected lambs. Described that the raft-like structures (RLSs) in egg shell colocalized with a large proportion of the p-gp. They analyzed the functional consequences of the colocalization for xenobiotic transport and drug resistance in nematodes and compared with results obtained in vertebrates. They suggested that understanding of such mechanisms was crucial in overcoming the failure of drug treatments due to the development of resistance. Godoy (2010) [22] studied that the expression of *Hc*Pgp-A, in transfected LLC-PK1 cells, and to see the effect of ivermectin and moxidectin on inhibition of rhodamine 123 transport by the transfected cells. Rhodamine 123 was actively transported by *Hc*Pgp-A. Ivermectin was four fold more potent at inhibiting rhodamine 123 transport by *Hc*Pgp-A than was moxidectin. The result provided the first information that MLs can inhibit the transport of Pgp substrates by a parasitic nematode ABC transporter and may indicate an active role for *H. contortus* Ppgs in ML resistance. Heckler *et al*. (2014) [26] evaluated the *in vitro* effect of eight P-gp modulating drugs to potentiate IVM efficacy against an IVM-resistant field isolate of *H.placei.* The association of IVM with cyclosporin-A, ceftriaxone, dexametha-sone, diminazene aceturate, quercetin, trifluoperazine, verapamil, or vinblastine resulted in increased IVM (10−4M) efficacy of 5.1, 49.06, 76.42, 3.31, 28.85, 13.74, 45.64% and 43.61%, respectively.

Hussein *et al.* 1996 [28] reported that effect of DL- buthionine-S, R sulfoximine (BSO), a selective glutathione (GSH) depleting agent, on the GSH synthesis of *Ascaris suum.* The GSH concentration of reproductive and muscle tissues of *A. suum* was 8.5 ± 0.3 and 14.3 ± 1.3 nmol/mg protein, respectively and after treatment of parasites with BSO for 24 h, the GSH content of reproductive tissue was totally depleted as compare to untreated control. Faundez *et al*. (2005) [19] found that L - buthionine-S, R sulfoximine (BSO), increased the toxicity of nifurtimox and benzimidazole towards the epmastigaote, tryapomastigote and mastigote form of *Trypanosoma cruzi.* BSO at 500µm decreased the total glutathione-derived thiol by 70 to 80% in 48 hours. Hussein, *et al.,* (2005) [29] the Treatment of the *Brugia malayi* with different concentrations of 20, 100, 200 and 500 µM BSO lowered the GSH content by 70%, 47%, 35% and 23%, respectively. The effect of BSO is dose dependent. Buthionine sulfoximine (S-(n-buty1) homocysteine sulfoximine) is a potent and specific inhibitor of y-glutamylcysteinesynthetase; administered to animals orincorporated into tissue culture media it inhibits glutathione biosynthesis and causes depletion of cellular glutathione levels.

Glutathione, the most abundant intracellular nonproteinsulihydryl compound, has various cellular functions, mainly related to the thiolgroupof the cysteine

residue. The reduced glutathione (GSH), as a cosubstrate of glutathione peroxidase, plays an essential protective role against oxygen-reactive species that may be generated under various conditions. This protective mechanism results in increased formation of oxidized glutathione (GSSG), which is actively transported across the cell membrane, so that its intracellular concentrations are kept low. Drug resistance has been correlated to increased GST and GSH level in certain nematodes like *Haemonchus contortus* (Kwalek *et al*. 1984) [30]. Ullah *et al*., (2017) [58] estimated reduced glutathione as total acid soluble sulfhydryl concentrations colorimetrically using Ellman's reagent [5, 5'-dithiobis-(2-nitrobenzoic acid) or DTNB] according to thymoquinone and curcumin effect on *Fasciola gigantica.* The level of reduced glutathione was significantly inhibited by both curcumin as well as thymoquinone at the highest concentration (60 µM) used. The present study showed the increase GSH level in resistance *H. contortus* and after treatment with GSH inhibitors BSO level of GSH was decreased.

The our studied showed the relationship between egg hatching percentage and reduced glutathione with regression of coefficient at 2,4,and 6 hours interval after treating with thinbendazole and L-BSO. When reduced intracellular glutathione is responsible for is 61.5% hatching of egg when treated with 500µM L-BSO.

Conclusion

The present experiment was conducted to see the potentiation of thiabendazole efficacy by buthionine sulfoximine (BSO). BSO can be used as AR reversal agent along with BZ drugs. Enhances the TBZ toxicity by reducing the GSH concentration. Suppressing the antioxidant/redox potential of the worms.

Acknowledgement

The Dean, Madras Veterinary College, Tamil Nadu Veterinary and animal Science University, Chennai, India, 600 007 for providing facilities to conduct research work.

References

1. AL-Shaibani IRM, Phulan MS, Arijo A, Qureshi TA. Epidemiology of ovine gastrointestinal nematodes in Hyderabad district. Pakistan. Pakistan Veterinary Journal, 2008; 28(3):125-130.
2. Arunachalam K, Raman M, Jeyathialakan N, Karunanidhi K. Multiple anthelmintics resistance in gasterointestinal nematodes from Mecheri sheep in an organized farm in Tamil Nadu. Indian Journal of Animal Science. 2005; 75(2):203-205
3. Bartley DJ, Dupuy, Alvinerie JM, Jackson F, Lespine A. Theinfluence of ketoconazole and Pluronic85 on the efficacy and pharmacokinetics of ivermectin in lambs infected with anthelmintic resistant *Haemonchus contortus.* In: Proceedings of 22nd International Conference of the World Association for the Advancement of Veterinary Parasitology, CS2.2, 2009[a], 9.
4. Bartley DJ, McAllister H, Bartley Y, Dupuy J, Menez C, Alvinerie M *et al*. P-glycoprotein interfering agents potentiate ivermectin susceptibility in ivermectin sensitive and resistant isolates of *Teladorsagia circumcinata* and *Haemonchus contortus.* Parasitology, 2009; 136:1081-1088.
5. Beugnet F, Gautney M, Kerboeuf D. Partial *in vitro* reversal of benzimidazole resistance by the free living stages of *Haemonchus contortus* with verapamil, Veterinary Record. 1997; 141:575-576
6. Blackhall WJ, Prichard RK, Beech RN. P-glycoprotein selection in strains of *Haemonchus contortus* resistant to benzimidazoles. Veterinary Parasitology, 2008; 152:101-107
7. Boukhari MI, Elfadil AAM, Omer FA, Shuaib YA. Prevalence and Risk Factors of *Haemonchus contortus* in Sheep in Khartoum State, the Sudan IOSR Journal of Agriculture and Veterinary Science. 2016; 9(2):77-83
8. Bulbul, KH, Baruah N, Saleque A Seasonal prevalence of haemonchosis in beetal goats in an organized farm of assam. International Journal of Recent Scientific Research. 2015; 6(6):4468-4471
9. Calveta C, Calaviaa R, Ferrerb LMJ, Ramosb J, Lacastab D, Uriartea J Management and environmental factors related to benzimidazole resistance in sheep nematodes in Northeast Spain Veterinary Parasitology. 2012; 184:193-203
10. Calvetea C, Calaviaa R, Ferrerb LM, Ramosb JJ, Lacastab D, Uriartea J. Management and environmental factors related to benzimidazole resistance in sheep nematodes in Northeast Spain. Veterinary Parasitology, 2012; 184:193-203
11. Castle K. Prevalence of the *Haemonchus sp.* parasite in Oregon Cattle, degree of Honors Baccalaureate of Science in Zoology. A THESIS submitted to Oregon State University Honors College, 2017.
12. Coles GC, Bauer C, Borgsteede FHM, Greets S, Klei TR, Taylar MA *et al*. World association for advancement of Veterinary Parasitology (WAAVP) method for the detection of anthelmintics resistance in nematodes of Veterinary importance. Veterinary Parasitology, 1992; 44:35-44
13. Degheidy NS, Al-Malki JS, Al-Omari FI. Some Epidemiological Studies of Caprin Heamonchosis in Taif. Saudi Arabia, International Conference on Advances in Agricultural, Biological & Environmental Sciences (AABES-2014) Oct 15-16, 2014 Dubai (UAE), 2014.
14. Dinesh. Studies on the status of anthelmintic resistance in ruminants in Jashpur District of Chhattisgarh M.V.Sc. Thesis, Submitted to Chhattisgarh Kamdhenu Vishwavidyalaya, Durg, India, 2013.
15. Durrani AZ, Kamal N, Khan MS. Serodiagnosis of haemonchosis in small ruminants, J Anim. Pl. Sci. 2007; 17(3-4):20
16. Easwaran C, Harikrishnan TJ, Raman M. Multiple anthelmintics resistance in gastrointestinal nematodes of sheep in southern, India Vet. Archiv. 2009; 79:611-620
17. Easwaran, C, Harikrishnan TJ, Raman M. multiple anthelmintic resistance in gastrointestinal nematodes of Sheep in southern indie Veterinarski Arhiv. 2009; 79(6):611-620.
18. Fakae BB. Seasonal changes and hypobiosis in *Haemonchus contortus* infection in the West African Dwarf sheep and goats in the Nigerian derived savanna. Veterinary Parasitology. 1990; 36(1-2):123-130.
19. Faundez M, Pino L, Letelier P, Ortiz C, Lopez CS, ferreria J *et al*. Buthionine sulfoximine increases the toxicity of nitrotimox and benznidazole to *Trypanosoma cruzi.* Antimicrobial Agents Chemotherapy. 2005; 49(1):126-130
20. Garg G, Sharma DK, Agrawal RD, Raut PK. Epidemiology of *Haemonchus contortus* infection in goat

in semi-arid region of India. Journal of Veterinary Parasitology. 2003; 17(1):57-60.
21. Gebresilassie L, Tadele BA. Prevalence of Ovine Haemonchosis in Wukro, Ethiopia Journal of Parasitology Research, 2014, 2015, 1-5
22. Godoy P. Functional assay of *Haemonchus contortus* P-glycoprotein-A and interaction with macrocyclic lactones Degree of Master of Science, Thesis, Submitted to Institute of Parasitology McGill University Montreal, Canada, 2010.
23. Gonçalves FMF, Debiage RR, Yoshihara E, Regildo Gonçalves da Silva M, Porto PP *et al*. Anthelmintic and antioxidant potential of *Fagopyrum esculentum* Moench *in vitro.* African Journal of Agricultural Research. 2016; 11(44):4454-4460.
24. Greech KV, Davey RA, Davey MA. The relationship between modulation of MDR and Glutathion in overexpressing humen leukemia cells. Biochemical Pharmacology. 1998; 53:1283-1289
25. Griffith OW, Meister A. Potent and specific inhibition of glutathione by buthionine sulfoximine (S-n-butyl homocysteine sulfoximine) Journal of Biochemistry, 1979; 254:7558-7560
26. Heckler RP, Almeida GD, Santos LB, Borges DGL, Neves JPL, Onizuka MKV *et al*. P-gp modulating drugs greatly potentiate the *in vitro* effect ofivermectin against resistant larvae of Haemonchus placei, Veterinary Parasitology (Article In Press), 2014.
27. Hussein AS, Walter RD. Purification and characterization of Y-glutamylcysteine synthetase from *Ascatissuum*. Molecular Biochemical Parasitology. 1995; 72:57-64
28. Hussein AS, Walter RD. Inhibition of glutathione synthesis of *Ascarissuum* by buthionine Sulfoximine Parasitol Res. 1996; 82:372-374
29. Hussein A. Brugiamalayi: Depletion of Glutathione by Buthionine Sulfoximine, An-Najah University Journal of Research, (N. Sc.) 2005, 19.
30. Kawalek JC, Rew RS, Heavner J. Glutathione-S-transferase, a possible drug metabolizing enzyme, in *Haemonchus contortus*; comparative activity of a cambendazole-resistant and susceptible strain. International Journal of Parasitology. 1984; 14:173-175.
31. Kerboeuf D, Aycaedi J. Unexpected increased thiabendazole tolerance in *Haemonchus contortus* resistant to anthelmintics by modulation of glutathione activity, Parasitological Research, 1999; 85(8-9):713-718
32. Kerboeuf D, Blackhall W, Kaminsky R, Von Samson, Himmelstjerna G. P- glycoprotein in helminths: function and prospectives for anthelmintics treatment and reversal of resistance Int. Journal of Antimicrobial Agents, 2008; 22:332-346
33. Krecek RC, Waller PJ. Towards the implementation of the "basket of options" approach to helminth parasite control of livestock: Emphasis on the tropics/subtropics Vet. Parasitol. 2006; 139:270-282
34. Kripali Pant, Rajput MKS, Jitendra Kumar, Shivani Sahu, Vandna, Rajkumari *et al*., Prevalence of helminthes in small ruminants in Tarai region of Uttarakhand, Veterinary World, 7, 265-266
35. Kumbhakar NK, Sanyal PK, Rawte D, Kumar D, Kerketta AE, Pal S. Efficacy of pharmacokinetic interactions between piperonylbutoxide and albendazole against gastrointestinal nematodiasis in goats, Journal of Helminthology, 2015, 1-6.
36. Lakshmipriya. Assessment and *in vitro* reversal of anthelmintic resistance in *haemonchus contortus*. M.V.Sc. Thesis, Submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, 2012.
37. Lashari1 MH, Tasawar Z, Akhtar MS, Chaudhary MS, Sial N. Prevalenceof *Haemonchus contortus* in local goats of D. G. Khan. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4(5):190-196
38. Le Jambre LF. Egg hatch assay as an *in vitro* assay for thiabendazole resistance in nematodes Veterinary Parasitology. 1976; 2(4):385-391
39. Lifschitz A, Entrocasso C, Alvarez L, Lloberas M, Ballent M, Manazza G *et al*. Interference with p-glycoprotein improves ivermectin activity against adult resistant nematodes in sheep. Veterinary Parasitology, 2010[a]; 172:291-298.
40. Lifschitz A, Sallovitz J, Imperiale F, Suarez V, Cristel S, Ahoussou S *et al*. Modulation of p-glycoprotein enhances ivermectin and moxidectin systemic availabilities and their efficacy against resistant nematodes in cattle. Experimental Parasitology. 2010[b]; 125(2):172-178.
41. Lourde Raj, Gomathinayagam S, Dhinkar R. Detection of benzimidazole resistance by egg hatch assay Tamil Nadu. Journal of Veterinary and Animal Science. 2006; 2(1):18-20
42. Loyacano AF. Effect of gastrointestinal nematode and liver fluke infections on weight gain and reproductive performance of beef heifers Veterinary. Parasitology. 2002; 107:227-234
43. Luersen K, Walter RD, Muller S. *Plasmodium falciparum*i nfected red blood cells depend on a functional glutathione de novo synthesis attributable to an enhanced loss of glutathione,. Biochemistry. 2000; 346:545-552
44. Maingi N. Resistance to thiabendazole, fenbendazole and levamisole in *Haemonchus* and *Trichostrongyle* species on a Kenyan farm Veterinary Parasitology. 1991; 39:285-291.
45. Meenakshisundaram A, Anna T, Harikrishnan TJ. Prevalence of drug-resistant gastrointestinal nematodes in an organized sheep farm. Veterinary World. 2014; 7(12):1113-1116.
46. Mesele K, Yisehak TR, Nesibu A. Prevalence of Haemonchosis in Sheep Slaughtered at Abergele Export Abattoir. Acta Parasitologica Globalis. 2014; 5(2):115-119.
47. Miller JE, Bahirathan M, Lemarie SL, Hembry FG, Kearney MT, Barras SR. Epidemiology of gastrointestinal nematode parasitism in Suffolk and Gulf Coast Native sheep with special emphasis on relative susceptibility to *Haemonchus contortus* infection Veterinary Parasitology. 1998; 74:55-74.
48. Nahar L, Sarder MJU, Mondal MMH, Faruque MO, Rahman M. prevalence of haemonchosis of goats at rajshahi district in Bangladesh, Bangladesh Journal of Veterinary Medicine. 2015; 13(1):29-36
49. Prichard RK, Hall CA, Kelly JD, Martin ICA, Donald AD. The problem of anthelmintics resistance in nematodes. Australian Veterinary Journal. 1980; 56:239-251
50. Qamar MF, Maqbool A, Khan MS, Ahmad N, Munee MA. Epidemiology of *Haemonchosis* in sheep and Goats under different managemental conditions, Veterinary World. 2008; 2(11):413-417

51. Rialch A, Vatsya S, Kumar RR. Detection of benzimidazole resistance in gastrointestinalnematodes of sheep and goats of sub-Himalyan region of northern India using different tests. Veterinary Parasitology, 2013;198:312-318
52. Riou M, Guegnard F, Sizaert PY, Le Vern JY, Kerboeuf D. Modulation of the multidrug resistance (MDR) system in the nematode Haemonchus contortus by changing cholesterol contents; effect on resistance to anthelmintic. Journal of Antimicrobial Chemotherapy, 2003; 52:180-187.
53. Rossum AJ, Jefferies JR, Rijsewijk FAM, LaCourse EJ, Teesdale-Spittle P, Barrett John *et al*. Binding of Hematin by a New Class of Glutathione Transferase from the Blood-Feeding Parasitic Nematode *Haemonchus contortus*, Infection and Immunity, 2004, 2780-2790.
54. Shugufta N, Jeelani SG, Hakeem M. Incidence of gastrointestinal nematodes in sheep in Kashmir valley. Journal of Veterinary Parasitology, 2005; 19(1):27-29.
55. Tasawar Z, Ahmad S, Lashari HM, Hayat CS. Prevalence of *Haemonchus contortus* in Sheep at Research Centre for Conservation of Sahiwal Cattle (RCCSC) Jehangirabad District Khanewal, Punjab, Pakistan. Pakistan Journal of Zoology. 2010; 42(6):735-739
56. Torres-Acosta JFJ, Mendoza-De-Gives P, Aguilar-Caballero AJ, Cuéllar-Ordaz JÁ. Anthelmintic resistance in sheep farms: update of the situation in the American continente. Vet. Parasitol. 2012; 189:89-96.
57. Taylor MA, Hunt KR, Goodyear KL. Anthelmintic resistance detection methods. Veterinary Parasitology. 2002; 103:183-194.
58. Ullah R, Rehman A, Zafeer MF, Rehman L, Khan YA, Khan MAH *et al*. Anthelmintic Potential of Thymoquinone and Curcumin on *Fasciola gigantica*. PLoS ONE. 2017; 12(2):e0171267. doi:10.1371/journal.pone.0171267
59. Varady M, Corba J. Comparison of six *in vitro* tests in determining benzimidazole and levamisole resistance in *Haemonchus contortus* and *Ostertagia circumcincta* of sheep, Veterinary Parasitology. 1999; 80:239-249
60. Yadav A, Khanjuria JK, Raina AK. Seasonal prevalence of gasterointestinal parasite in sheep and goats of Jammu. Journal of Veterinary Parasitology, 2006; 20(1):0971-6157.