Applied Biological Research 26(4): 474-481; (2024)
DOI: 10.48165/abr.2024.26.01.55

THYMOQUINONE AND ALBENDAZOLE AS UNCOMPETATIVE INHIBITORS OF GLUTATHIONE-S-TRANSFERASE IN *Moniezia expansa*: IMPLICATIONS FOR ANTHELMINTHIC THERAPY

Hana Hussein M.S. Wardah[1,2], Humera Aslam[1], Anjum B. Ahmed[1], Malik Irshadullah[1]* and S.M.A. Abidi[1]

[1]*Section of Parasitology, Department of Zoology, Aligarh Muslim University, Aligarh - 202 002 (India)*
[2]*Biology Department, Dhamar University, Dhamar - 00967 (Yemen)*
*****e-mail: *malikirshadullah@yahoo.co.in*

(Received 2 October, 2024; accepted 20 November, 2024)

ABSTRACT

The infection of *Moniezia expansa* is common among sheep and goat in the Indian sub-continent, and little is known about their detoxification ability. Therefore, in this study specific activity of glutathione-S-transferase (GST), along with its kinetic parameters like K_m, V_{max}, pH and temperature optima were investigated before and after treatment of worms with thymoquinone (TQ), and albendazole (ALBZ). Worms were collected from the infected small intestine of goats (*Capra hircus)* washed in Hanks' medium and incubated separately in RPMI 1640 medium with and without 120 µM TQ and ALBZ at 37ºC for 4 h. The specific activity of GST in the somatic extract of *M. expansa* was found to be 59.12 µ mole min^{-1} mg^{-1} protein. The optimum temperature and pH were found to be 25°C and 6.5, respectively. It was observed that the rate of reaction increased with the increasing concentration of both GSH and CDNB; and maximum velocity (V_{max}) of enzyme was recorded at 2 mM concentration for both the substrates. The K_m value was higher while V_{max} was lower in both TQ and ALBZ treated worms than control, indicating that both TQ and the ALBZ act as uncompetitive inhibitors of GST in *M. expansa* and appeared to be equally effective in inhibiting the GST activity, possibly through conformational changes of enzyme. This type of inhibition may greatly affect the detoxification ability of the worms and, therefore, their survival in the host; hence, GST of *M. expansa* could be exploited for therapeutic intervention and thymoquinone could be further tested at the *in vivo* level for dose standardization and field application.

0Keywords: Albendazole, glutathione-S-transferase, *Moniezia expansa*, cestode, thymoquinone

INTRODUCTION

Monieziasis, caused by *Moniezia expansa*, is one of the most important diseases of small ruminants and is quite prevalent throughout the world (Iacob *et al.*, 2020; Bi *et al.*, 2023). It causes intestinal disorders, responsible for declining health of ruminants, and thereby causes huge economic losses to the sheep and goat industry (Nagarajan *et al.*, 2022; Kumar and Kaur, 2023). The life cycle of *M. expansa* involves oribatid mites as intermediate host and ruminants as definitive hosts. The ruminants acquire infection by ingesting mites infected with *Moniezia* cysticercoids, which emerge in the small intestine and develop into the adult. Moderate infection with *Moniezia* causes mild pathogenicity, whereas heavy infection often leads to poor growth rate, pot-belly, diarrhea, anemia, intestinal obstruction and poor quality of wool and even death of the ruminant host (Yan *et al.*, 2013; Kumar and Kaur, 2023). In order to prevent the losses caused by monieziasis, great attention is needed to control this parasite. The control of helminths is usually based on indiscriminate use of anthelminthic,

which results into multiple drug resistance among parasites that poses a major problem in the production of sheep and goat (Bihaqi *et al.,* 2020; Fissiha and Kinde, 2021; Mpofu *et al.,* 2022). Therefore, many alternative control strategies like rotational grazing, nutritional management and use of plant products containing natural anthelminthic are being used to control gastrointestinal helminths that have been found quite effective (Sahoo and Khan, 2016; Mpofu *et al.,* 2022).

Helminths possess a limited ability to detoxify xenobiotics due to the lack of cytochrome P-450 dependent detoxification system and therefore, glutathione-S-transferase (GST, EC 2.5.1.18) appears to be the major phase II detoxification enzyme in this group of parasites (Mordvinov and Pakharukova, 2022). GST has been associated with the detoxification of both exogenously and endogenously derived toxic compounds and anthelminthics through their enzymatic and ligand binding capabilities (Brophy, *et al.,* 1989; Torres-Rivera and Landa, 2008). This enzyme not only neutralizes toxins by conjugating them with glutathione but could also passively bind with toxic ligands; and therefore, act as biochemical sponges (Brophy and Prichard, 1994). This enzyme has been widely studied in mammalian tissues and in many parasitic helminths (Singh and Irshadullah, 2003; Rashid and Irshadullah, 2019; Aslam *et al.,* 2023), where it has been suggested that high level of GST may decrease the effective concentration of drugs and thereby decrease their toxicity.

The effects of various known anthelminthics and plant products on GST activity as well as the dynamic behaviour of this enzyme through its kinetics properties have been investigated by many workers in different helminth parasites (Singh and Irshadullah, 2003; Ullah *et al.,* 2017; Maldonado *et al.,* 2018; Rehman *et al.,* 2020; Farhat *et al.,* 2022), but little information is available on *M. expansa*. Due to emerging anthelmintic resistance among parasites it has become necessary to search for alternative therapeutic compounds as well as some novel targets. Therefore, the present study was undertaken to evaluate the effect of thymoquinone and albendazole on the kinetic parameters of GST of *M. expansa.*

MATERIALS AND METHODS

Parasite collection and homogenate preparation

The present study was conducted in Aligarh (India) during 2022-2023. A total of 20 samples of *M. expansa* were collected throughout the year from the small intestine of the naturally infected goats (*Capra hircus*), freshly slaughtered at the Aligarh abattoir and immediately brought to the laboratory. The worms were washed three times with Hanks' balanced salt solution (HBSS), blot-dried on Whatman filter paper and then homogenized in 100 mM phosphate buffer (pH 7.4) using Potter Elvehjem tissue homogenizer, followed by centrifugation at 12,000 rpm for 20 min at 4^{o}C in an Eppendorf AG centrifuge 5430 (Germany). The supernatant was collected gently, stored at -80°C in aliquots and used for the estimation of specific GST activity along with its pH and temperature optima. A total of 3 different biological samples were used for this study.

Estimation of GST activity

The GST activity was measured following the method of Habig *et al.* (1974) by using 1.0 mM, 1-chloro-2,4-dinitrobenzene (CDNB) and 1.0 mM reduced glutathione (GSH) as substrates. The total 3 mL assay mixture contained 2.64 mL of 100 mM potassium phosphate buffer (pH 6.5), 0.01 mL of 1.0 mM CDNB and 0.3 mL of 1.0 mM GSH. The enzyme reaction was initiated by adding 0.05 mL sample and the increase in absorbance was recorded at an interval of 30 sec for 3 min at 340 nm on a UV-visible spectrophotometer (Taurus Scientific Instruments, USA). A complete assay mixture without sample was used as control. The specific enzyme activity was expressed as unit mg^{-1} protein. One unit of enzyme activity is defined as the amount of enzyme that catalyses the formation 1µ mole CDNB-glutathione conjugate min^{-1} under specified assay conditions. The molar extinction coefficient of 0.0096 μM^{-1} cm^{-1} for CDNB was used for the calculation of enzyme activity. The protein content

of homogenate was determined by the dye binding assay of Spector (1978) using bovine serum albumin as the standard.

Effect of temperature and pH on the activity of GST

The effect of temperature and pH on the GST activity of *M. expansa* was studied by using standard assay conditions (Habig *et al.*, 1974). In order to find out the dependence of enzyme on temperature, the reaction mixture (without sample) was incubated at different temperatures, ranging from 10 to 45ºC for 10 min and then the enzyme assay was performed after the addition of sample. Furthermore, to find out the pH optima of enzyme, the activity was analyzed by using 100 mM phosphate buffer of pH ranging from 3.5 to 9.5. The experiment was performed in triplicates.

Treatment with thymoquinone and albendazole

Both thymoquinone (TQ) and albendazole (ALBZ) was dissolved separately in dimethyl sulfoxide (DMSO) which is a safe drug solvent (Ahmad and Nizami, 1983). Two sets of experiments were performed. In first set, the worms were incubated with TQ and in second set with ALBZ. Freshly isolated worms measuring about 1 m length was incubated separately in triplicate (one worm in each replicate) with 25 mL RPMI 1640 medium containing either 120 µM TQ or 120 µM ALBZ for 4 h at 37ºC. For control, the worms were incubated without test compounds in RPMI 1640 medium containing only DMSO at the final concentration of 0.06% (v/v). After incubation, both the treated and untreated worms were washed, homogenized and the homogenates were used for the determination of kinetic parameters in 3 independent experiments.

Kinetic parameters of GST

The kinetic parameters were determined in the somatic extract of untreated and treated worms with TQ and ALBZ by using two sets of assays. In first set, the CDNB concentration was maintained at 1mM while GSH concentration varied from 0.25 to 4 mM to determine the K_m and V_{max} for GSH, whereas in second set the concentration of GSH was fixed at 1 mM and CDNB concentration varied from 0.25 mM to 4 Mm to determine the K_m and V_{max} for CDNB. The data were plotted using Graph Pad Prism software version 5 and excel version 2409. The K_m and V_{max} values for CDNB and GSH were determined from the double reciprocal plot of Lineweaver and Burk (1934).

RESULTS AND DISCUSSION

The present study revealed that the specific activity of GST in the somatic extract of *M. expansa* was 59.12 µ mole min^{-1} mg^{-1} protein. The experimental data showed that the reaction catalysed by GST is of second order as the initial rate of reaction depends on the concentration of both substrates. The high GST activity in somatic extract of *M. expansa* could be due to the increased production of GST to neutralize the toxic substances and to protect the parasite against host immune responses since this enzyme is considered as toxin neutralizing and immune defence protein (Precious and Barrett, 1989; Brophy and Prichard, 1994). Similarly, many workers have reported considerable amount of GST in various helminths like *Setaria cervi* (Srivastava *et al.*, 1994), *Ascaris suum* (Douch and Bouchanan, 1978), *Fasciola hepatica* (Howell *et al.*, 1988), *Fasciola gigantica* (Singh and Irshadullah, 2003; Ullah *et al.*, 2017; Rehman *et al.*, 2020), *Gigantocotyle explanatum* (Farhat *et al.*, 2022), *Trichuris ovis* (Bi, 2023) and in the protoscoleces of *Echinococcus granulosus* (Morello *et al.*, 1982; Aslam, 2023). These authors (loc. cit.) have suggested that high level of GST may decrease the effective concentration of drugs via its binding capability and thereby decreasing the toxicity of drugs. Helminth parasites have enzymatic antioxidant system like mammals that are being used to defend themselves against host generated free radicals to avoid lipid peroxidation and protein oxidation, and thereby generation of hydroperoxides and carbonyl, which are cytotoxic and cause damage to various

biological structure and consequently causing death of parasites (Chiumiento and Bruschi, 2009). The ubiquitously distributed GST, a multifunctional xenobiotic metabolising enzyme is involved in the detoxification of exogenously (xenobiotics) and endogenously derived toxins including hydro-peroxides and carbonyl by conjugating them with glutathione and is also involved in leukotriene and prostaglandins synthesis, transport of molecules, regulation of signal and transcription processes (Torris-Rivera and Landa, 2008; Matouskova *et al.*, 2016). Furthermore, it has been reported that helminth GSTs conjugates various xenobiotics or bind with anthelminthics, and are selectively targeted by different inhibitors/drugs (Brophy and Prichard, 1994; Matouskova *et al.*, 2016).

The specific activity of GST was also analysed at different temperatures and pH to determine the temperature and pH optima and the results are presented in Fig. 1a, b. It revealed that the specific activity of enzyme increased with increase in temperature from 10 to 25ºC and pH from 3.5 to 6.5, and beyond these values the activity decreased. The temperature and pH optima were recorded at 25˚C and 6.5, respectively. In previous studies the maximum activity of GST has also been recorded as 25˚C in *Teladorsagia circumcincta* (Umair *et al.*, 2020), *Spirometra mansoni* (Chen *et al.*, 2022), *Haemonchus contortus* and *Ancylostoma caninum* (Zhan *et al.*, 2005), whereas maximum activity of GST 1 and GST2 were reported at 35 and 40˚C, respectively, in *Taenia crassiceps* (Maldonado *et al.*, 2018). Similar to our finding, optimal GST activity was recorded at pH 6.5 in *Taenia solium* (Nava *et al.*, 2007). In contrast, the optimum pH was recorded as 8.5 and 7.5 for GST1 and GST2, respectively, for *T. crassiceps* (Maldonado *et al.*, 2018).

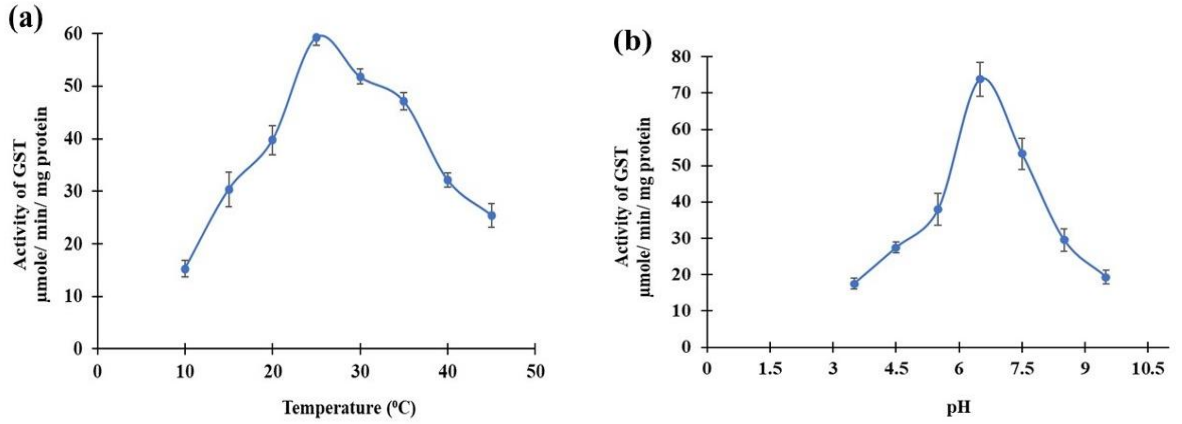

Fig. 1: Effect of temperature and pH on the activity of glutathione-S- transferase of *M. expansa.* The enzyme was assayed by varying either temperature at pH 6.5, a) or pH at 25^{0}C, b) by using 1 mM CDNB and GSH as a substrate and co-substrate, respectively. Each point represents the mean ± SD of 3 different independent assays.

The effect of thymoquinone and albendazole on the kinetic parameters of GST was studied by using different concentrations of GSH and CDNB as substrates. It was observed that the specific activity of GST in both untreated and treated worms increased with increase in substrate concentration, and followed Michaelis-Menten kinetics. The K_m and V_{max} of both the substrates were calculated from the Lineweaver-Burk double reciprocal plots (Figs. 2 and 3). The calculated values before and after treatment are given in Table 1. The results revealed that the rate of reaction increased with the increase in the concentration of both GSH and CDNB, and apparent maximum velocity (V_{max}) of enzyme was recorded at 2 mM concentration for both the substrates, which is similar to those reported by Singh and Irshadullah (2003) for *F. gigantica.* Contrary to our findings, maximum velocity of GST was reported at 4.0 and 1.6 mM in *Spirometra mansoni* (Chen *et al.*, 2022) and 1.5 and 2.5 mM in *Setaria digitata* (Srinivasan *et al.*, 2011) for GSH and CDNB, respectively. The GST catalyses the bi-substrate reaction, and K_m and V_{max} varies for both substrates. In present study, the K_m and V_{max} values were 0.52 mM and 97.08 µ mol min^{-1} mg^{-1} protein for GSH and 0.58 mM and 80 µ mol min^{-1} mg^{-1} protein for CDNB, respectively, in *M. expansa.* In contrast, lower K_m (0.16 mM) and V_{max} (0.15 µ mol min^{-1}

mg^{-1}) values for GSH while higher K_m (14 mM) and lower V_{max} (27 µ mol min^{-1} mg^{-1}) for CDNB was reported for GST in the microsomal fraction for *T. solium* (Nava *et al.*, 2007) and higher K_m (26.5 mM) values for GSH in the cytoplasmic extract of *T. solium* GST (Plancarte *et al.*, 2004).

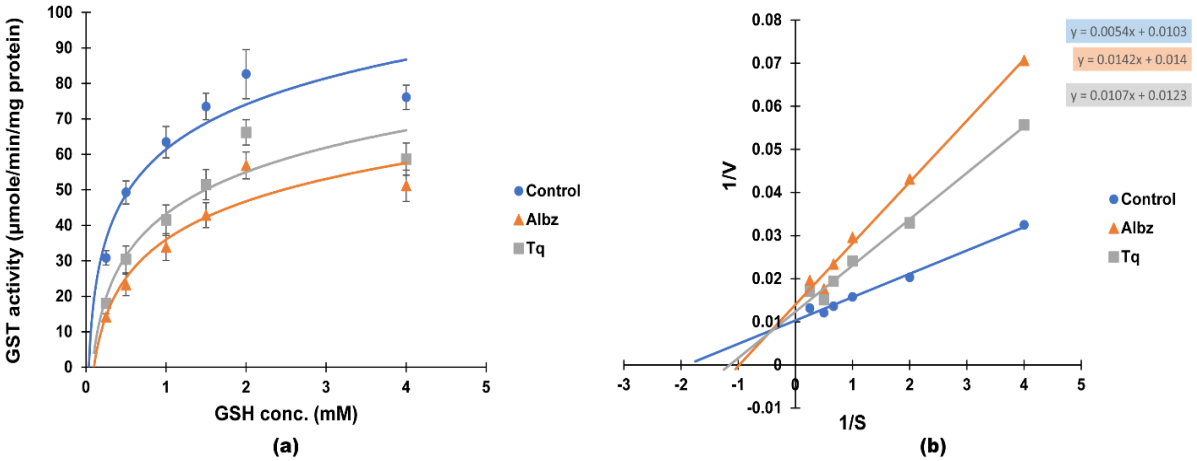

Fig. 2: (a) Determination of kinetic parameters of GST in untreated (control) and treated *Moniezia expansa* with thymoquinone (TQ) and albendazole (ALBZ), using different concentrations (0.25-4.0 mM) of glutathione (GSH) while keeping 1-chloro-2,4-dinitrobenzene (CDNB) concentration constant (1 mM); (b) The double reciprocal plot of Lineweaver-Burk (1934).

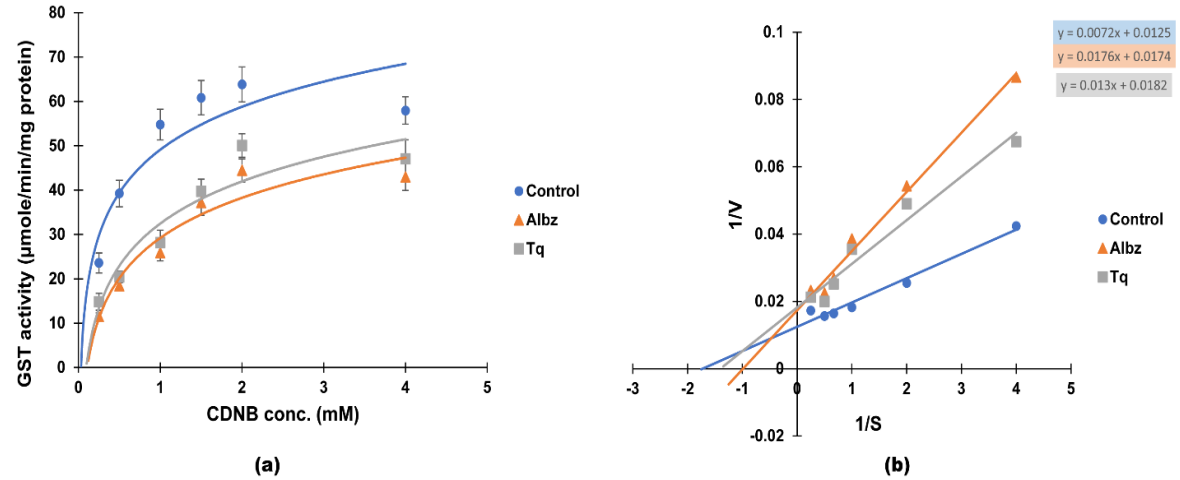

Fig. 3: a) Determination of the kinetic parameters of GST in untreated (control) and treated *Moniezia expansa* with thymoquinone (TQ) and albendazole (ALBZ), using different concentrations (0.25 - 4.0 mM) of 1-chloro-2,4-dinitrobenzene (CDNB) while keeping glutathione (GSH) concentration constant (1 mM); b) The double reciprocal plot of Lineweaver-Burk (1934)

Table 1: The K_m and V_{max} values of GSH and CDNB in untreated and treated worms with thymoquinone and albendazole

Moniezia expansa	K_m (mM)			V_{max} (µ mole min^{-1} mg^{-1} protein)		
	Control	Thymoquinone	Albendazole	Control	Thymoquinone	Albendazole
GSH	0.52	0.86	1.01	97.08	81.30	71.43
CDNB	0.58	0.71	1.01	80.00	54.95	57.41

The K_m value was found higher while V_{max} was lower in both TQ and ALBZ treated worms than control, indicating that both TQ and LBZ act as uncompetitive inhibitors of GST in *M. expansa*,

thereby altering the catalytic efficiency and substrate binding affinity of enzyme. According to Silverstein (2019), the molecular crowding-induced changes in the stability of specific enzyme conformations can alter both K_m and V_{max}, thus leading to the changes in the catalytic efficiency of enzyme as also observed in the presence of both ALBZ and TQ in the present study. Similarly, Baars *et al.* (1978) have reported higher V_{max} and K_m values of GST for treated rat liver with tetrachloro-dibenzo-p-dioxin (enzyme-inducing agent) as compared to control. Both the kinetic parameters of GST were found altered after the treatment of worms with TQ and ALBZ. The K_m values for both substrates were higher for ALBZ than TQ-exposed worms whereas, the V_{max} value was higher in TQ-exposed worms for GSH, while lower for CDNB (Table 1), indicating that the drug-substrate interaction might be occurring in a complex manner.

Conclusion: Both TQ and ALBZ appeared to be equally effective in inhibiting the activity of GST enzyme in *Moniezia expansa* that might compromise the detoxification ability of worms, rendering the worms more vulnerable to the host immune responses. Therefore, considering strong *in vitro* antihelminthic potential of thymoquinon, *in vivo* studies can be undertaken in future in order to evaluate and standardise the effective dose of TQ against moneiziasis.

Acknowledgments: The authors are thankful to the Chairperson, Department of Zoology, Aligarh Muslim University, Aligarh, for providing the laboratory facilities. The author H.H.M.S. Wardah is thankful to Dhamar University, Yemen for providing financial assistance.

Conflict of interest: The authors have no relevant financial or non-financial conflicts of interest to disclose.

Author's contribution: All authors contributed to the study's conception and design. The methodology validation was done by Malik Irshadullah and S.M.A. Abidi. The material collection and experimental work was performed by Hana Hussein M.S. Wardah. Anjum B. Ahmed assisted in the treatment of worms. Data analysis, preparation of figures and tables were done by Hana Hussein M. S. Wardah and Humera Aslam. Hana Hussein M.S. Wardah wrote the first draft of the manuscript. Prof. Malik Irshadullah and Prof. S.M.A. Abidi performed the supervision and checked the final draft of article. All authors read and approved the final manuscript.

Ethics approval: Not applicable.

REFERENCES

Ahmad, M. and Nizami, W.A. 1983. Dimethyl sulfoxide-a safe drug solvent for *in vitro* screening against cestode parasite. *Annals of the New York Academy of Sciences*, **411:** 347-351**.**

Aslam, H. 2023. *Analysis of Antioxidant Enzymes and Efficacy of some Plant Extracts on the Metacestode of Echinococcus granulosus.* Ph.D. thesis, Aligarh Muslim University, Aligarh, India.

Aslam, H., Bi, S. and Irshadullah, M. 2023. Analysis of antioxidant enzymes and oxidative stress markers in the liver of naturally infected Indian water buffalo (*Bubalus bubalis*) with cystic echinococcosis. *Journal of Parasitic Diseases*, **47**: 340-348.

Baars, A.J., Jansen, M. and Breimer, D.D. 1978.The influence of phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin on glutathione S-transferase activity of rat liver cytosol. *Biochemical Pharmacology*, **27**: 2487-2494.

Bi, S. 2023. *Epidemiological, Biochemical and Pathological Studies on "Trichuris ovis" a Gastro-intestinal Helminth Parasite of Small Ruminants*. Ph.D. thesis, Aligarh Muslim University, Aligarh, India.

Bi, S., Aslam, H. and Irshadullah, M. 2023. Epidemiological studies of gastrointestinal helminths in goats (*Capra aegagrus hircus*) of Aligarh, Uttar Pradesh, North India. *Applied Biological Research*, **25**: 409-416.

Bihaqi, S.J., Allaie, I.M., Banday, M.A.A., Sankar, M., Wani, Z.A. and Prasad, A. 2020. Multiple anthelmintic resistance in gastrointestinal nematodes of Caprines on Mountain Research Centre for Sheep and Goat at Kashmir valley, India. *Parasite Epidemiology and Control*, **11**: e00163. [*https://doi.org/10.1016/j.parepi.2020.e00163*].

Brophy, P.M. and Pritchard, D.I. 1994. Parasitic helminth glutathione S-transferases: An update on their potential as targets for immuno-and chemo-therapy. *Experimental Parasitology*, **79**: 89-96.

Brophy, P.M., Southan, C. and Barrett, J. 1989. Glutathione transferases in the tapeworm *Moniezia expansa*. *Biochemical Journal*, **262**: 939-946.

Chen, W.Q., Liu, S.S., Cheng, C., Cui, J., Wang, Z.Q. and Zhang, X. 2022. Molecular characteristics of glutathione transferase gene family in a neglect medical *Spirometra* tapeworm. *Frontiers in Veterinary Science*, **9**: 1035767. [*https://doi.org/10.3389/fvets.2022.1035767*].

Chiumiento, L. and Bruschi, F. 2009. Enzymatic antioxidant systems in helminth parasites. *Parasitology Research*, **105**: 593-603.

Douch, P.G.C. and Buchanan, L.L. 1978. Glutathione conjugation of some xenobiotics by *Ascaris suum* and *Moniezia expansa*. *Xenobiotica*, **8**: 171-176.

Farhat, F., Wasim, S. and Abidi, S.M.A. 2022. Antihelminthic effect of thymoquinone against biliary amphistome, *Gigantocotyle explanatum*. *Experimental Parasitology*, **243**: 108421. [*https://doi.org/10.1016/j.exppara.2022.108421*].

Fissiha, W. and Kinde, M.Z. 2021. Anthelmintic resistance and its mechanism: A review. *Infection and Drug Resistance*, **14:** 5403-5410. [*https://doi.org/10.2147/IDR.S332378*].

Habig, W.H., Pabst, M.J. and Jakoby, W.B. 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, **249**: 7130-7139.

Howell, M.J., Board, P.G. and Boray, J.C. 1988. Glutathione S-transferases in *Fasciola hepatica*. *The Journal of Parasitology*,**74**: 715-718.

Iacob, O.C., El-Deeb, W. M., Pasca, S.A. and Turtoi, A.I. 2020. Uncommon co-infection due to *Moniezia expansa* and *Moniezia benedeni* in young goats from Romania: Morphological and histopathological analysis. *Annals of parasitology*, **66**: 501-507.

Kumar, S. and Kaur, H. 2023. Molecular characterization of *Moniezia denticulata* (Rudolphi, 1810) and its distinction from *M. expansa* infecting sheep and goats raised in the north and north-western regions of India. *Parasitology*, **150**: 831-841.

Lineweaver, H. and Burk, D. 1934. The determination of enzyme dissociation constants. *Journal of the American Chemical Society*, **56**: 658-666.

Maldonado, G., Nava, G. and Plancarte, A. 2018. Two glutathione transferase isoforms isolated from juvenile cysts of *Taenia crassiceps*: Identification, purification and characterization. *Journal of Helminthology*, **92**: 687-695.

Matoušková, P., Vokřál, I., Lamka, J. and Skálová, L. 2016. The role of xenobiotic-metabolizing enzymes in anthelmintic deactivation and resistance in helminths. *Trends in Parasitology*, **32**: 481-491.

Mordvinov, V. and Pakharukova, M. 2022. Xenobiotic-metabolizing enzymes in trematodes. *Biomedicines*, **10**: 3039. [*https://doi.org/10.3390/biomedicines10123039*].

Morello, A., Repetto, Y. and Atias, A. 1982. Characterization of glutathione S-transferase activity in *Echinococcus granulosus*. *Comparative Biochemistry and Physiology. Part B. Comparative Biochemistry*, **72**: 449-452.

Mpofu, T.J., Nephawe, K.A. and Mtileni, B. 2022. Prevalence and resistance to gastrointestinal parasites in goats: A review. *Veterinary World*, **15:** 2442. [*https://doi.org/10.14202%2Fvetworld.2022.2442-2452*].

Nagarajan, G., Thirumaran, S.M.K., Pachaiyappan, K., Thirumurugan, P., Rajapandai, S., Rajendiran, A.S., Velusamy, R., Ram Vannish, M. and Kanagarajadurai, K. 2022. First report on molecular

identification of *Moniezia expansa* in sheep from Mannavanur, Palani hills, Tamil Nadu, India. *Acta Parasitologica*, **67**: 1626-1633.

Nava, G., Robert, L. and Plancarte, A. 2007. Characterization of *Taenia solium* cysticerci microsomal glutathione-S-transferase activity. *Parasitology Research*, **101**: 1373-1381.

Plancarte, A., Rendon, J.L. and Landa, A. 2004. Purification, characterization and kinetic properties of the *Taenia solium* glutathione S-transferase isoform 26.5 kDa. *Parasitology Research*, **93**: 137-144.

Precious, W.Y. and Barrett, J. 1989. The possible absence of cytochrome P-450 linked xenobiotic metabolism in helminths. *Biochimica et Biophysica Acta,* **992**: 215-222.

Rashid, S. and Irshadullah, M. 2019. Evalustion of antioxidant and oxidant status of goats (*Capra eagagrus hircus*) naturally infected with *Haemonchus contortu. Journal of Helminthology,* **94:** e36. [*doi: 10.1017/S0022149X19000117*].

Rehman, A., Ullah, R., Gupta, D., Khan, M.H., Rehman, L., Beg, M.A., Khan, A.U. and Abidi, S.M.A. 2020. Generation of oxidative stress and induction of apoptotic like events in curcumin and thymoquinone treated adult *Fasciola gigantica* worms. *Experimental Parasitology*, **209**: 107810. [*doi: org/10.1016/j.exppara.2019.107810*].

Sahoo, A. and Khan, F. 2016. Nutritional and biological control synergism against gastrointestinal nematodes in small ruminants. *Journal of Veterinary Science and Animal Husbandry*, **4**: 104. [*10.15744/2348-9790.4.104*].

Singh, S. and Irshadullah, M. 2003. Kinetic studies and *in vitro* effect of anthelmintics on glutathione S-transferase of *Fasciola gigantica*. *Journal of Veterinary Parasitology*, **17**: 27-31.

Silverstein, T.P. 2019. When both Km and Vmax are altered, Is the enzyme inhibited or activated? *Biochemistry and Molecular Biology Education*, **47**: 446-449.

Spector, T. 1978. Refinement of the Coomassie blue method of protein quantitation: A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 μg of protein. *Analytical Biochemistry*, **86**: 142-146.

Srivastava, A.K., Tripathi, R.P., Khan, A.R., Bhaduri, A.P., Singh, S.N. and Chatterjee, R.K. 1994. Glutathione-S-transferase activity in *Setaria cervi* females and effect of new antifilarial compounds. *Indian Journal of Parasitology*, **18**: 127-133.

Srinivasan, L., Mathew, N., Karunan, T. and Muthuswamy, K. 2011. Biochemical studies on glutathione S-transferase from the bovine filarial worm *Setaria digitata*. *Parasitology Research*, **109**: 213-219.

Torres-Rivera, A. and Landa, A. 2008. Cooperative kinetics of the recombinant glutathione transferase of *Taenia solium* and characterization of the enzyme. *Archives of Biochemistry and Biophysics*, **477**: 372-378.

Ullah, R., Rehman, A., Zafeer, M.F., Rehman, L., Khan, Y.A., Khan, M.H., Khan, A.U. and Abidi, S.M.A. 2017. Anthelmintic potential of thymoquinone and curcumin on *Fasciola gigantica. Plos One*, **12**: 171-267.

Umair, S., Bouchet, C.L., Deng, Q., Palevich, N. and Simpson, H.V. 2020. Characterization of a *Teladorsagia circumcincta* glutathione transferase. *Molecular and Biochemical Parasitology*, **239**: 111-316.

Yan, H., Bo, X., Liu, Y., Lou, Z., Ni, X., Shi, W., Zhan, F., Ooi, H. and Jia, W. 2013. Differential diagnosis of *Moniezia benedeni* and *M. expansa* (Anoplocephalidae) by PCR using markers in small ribosomal DNA (18S rDNA). *Acta Veterinaria Hungarica*, **61**: 463-472.

Zhan, B., Liu, S., Perally, S., Xue, J., Fujiwara, R., Brophy, P., Xiao, S., Liu, Y., Feng, J., Williamson, A., Wang. Y., Bueno, L.L., Mendez, S., Goud, G., Bethony, J.M., Hawdon, J.M., Loukas, A., Jones, k. and Hotez, P.J. 2005. Biochemical characterization and vaccine potential of a heme-binding glutathione transferase from the adult hookworm *Ancylostoma caninum*. *Infection and Immunity*, **73**: 6903-6911.