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Evaluation of antioxidant and oxidant status of goats (*Capra aegagrus hircus*) naturally infected with *Haemonchus contortus*

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Research Paper

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Abstract

The present study aimed to assess the antioxidant and oxidant status of goats naturally infected with *Haemonchus contortus*. Based upon the parasite burden, infection in goats was categorized as heavy (> 500 worms), mild (100–500 worms) or low (< 100 worms). Abomasal tissues from non-infected and infected goats were used for the determination of catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), aspartate (AST) and alanine (ALT) aminotransferases, acid (ACP) and alkaline (ALP) phosphatases, superoxide content (O_2^-), protein carbonyl (PC), malondialdehyde (MDA) and reduced glutathione (GSH). A significantly higher level of CAT, GST and GR activity and a lower level of GPx activity were recorded in infected compared to non-infected tissue. A significant increase in the level of AST, ALT, ALP and ACP was found in the abomasal tissue of the infected animals, which was related to the worm burden. The oxidative stress markers were also altered, with a significant decline in GSH levels, whereas MDA, PC and O_2^- concentrations showed a marked increase. In conclusion, it has been demonstrated that haemonchosis in goats resulted in considerable oxidative stress, which was directly related to the worm burden.

Introduction

Among the parasites that constrain the survival and productivity of sheep and goats, the gastrointestinal nematodes rank highest, with *Haemonchus contortus* being of paramount importance (Perry *et al.*, 2002). *Haemonchus contortus* causes notable morbidity and mortality as well as production losses in the animals due to their blood-feeding behaviour. Both adult and fourth-stage larvae are haematophagous and thus cause severe anaemia, losses in weight, milk, meat and wool production, and often death, particularly in young animals (Rashid and Irshadullah, 2014). It has been estimated that each worm is responsible for about 0.05 ml of blood loss per day, either by ingestion or seepage from lesions (Urquhart *et al.*, 2000).

Haemonchus contortus is one of the most important parasites of sheep and goats and is quite prevalent throughout the world (Burke *et al.*, 2016; Rashid and Irshadullah, 2018). During the establishment of parasites in their microhabitat, chains of dynamic interactions occur at the host–parasite interface. The host reacts against parasites in a number of ways, including the production of reactive oxygen species (ROS) that are generated by the host during normal aerobic metabolism, as well as by activated leukocytes via oxidative burst (Callahan *et al.*, 1988; Chiumiento and Bruschi, 2009). The production of ROS by immune effector cells considerably increases due to parasitic infections and is thought to play a role in killing or expulsion of parasites from their host, thereby preventing the establishment of infection (Callahan *et al.*, 1988; Smith and Bryant, 1989; Batra *et al.*, 1993; Ben-Smith *et al.*, 2002). Oxidative stress develops due to an imbalance between the generation and removal of ROS within the organism, which causes oxidative damage to all major classes of biomolecules, leading to protein oxidation, lipid peroxidation, DNA modification/strand breakage and depolymerization of polysaccharides (Southern and Powis, 1988; Chiumiento and Bruschi, 2009). Biological effects of ROS on these intracellular targets depend upon the concentration of ROS (Weydert and Cullen, 2010) and the damage may be due to depletion of antioxidant defence, an increase in ROS production, or both (Halliwell and Gutteridge, 2007).

Oxidative damage has been attributed to many helminth parasites: *Fasciola hepatica* (Kolodziejczyk *et al.*, 2005; Siemieniuk *et al.*, 2008), *Dicrocoelium dendriticum* (Deger *et al.*, 2008), *Trichinella spiralis* (Othman *et al.*, 2016); *Taenia saginata* (Łuszczak *et al.*, 2011), *Echinococcus granulosus* (Heidarpour *et al.*, 2012, 2013a, b) and *Schistosoma mansoni* (de Oliveira *et al.*, 2013). To our knowledge, there is no report on the status of oxidative stress and antioxidant defence in goats infected with *H. contortus*. Most of the above-mentioned studies have been carried out on experimental animals, but it is debatable whether experimental model animals can be used for pathological studies, given the difference in responses of the

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host. Based upon the pathological responses in sheep and rabbits infected with *F. hepatica*, Le Bars and Banting (1976) suggested that rabbits cannot be used as a model for the study of pathological effects. Thus in the present study we investigated various biochemical and oxidative stress parameters in goats naturally infected with *H. contortus*. This study may provide information on the oxidative response of infected animals and the pathogenesis of the disease.

Materials and methods

Collection and preparation of samples

Gastrointestinal tracts (GI) were collected from goats (*Capra aegagrus hircus*) slaughtered at the local abattoir (Aligarh, India), labelled individually, and brought to the laboratory. The GI tracts were opened and *H. contortus* were collected from each sample. The worms were counted manually. Based upon the number of worms present in the individual host, the host's infection status was categorized as low (< 100 worms), mild (100–500 worms) or heavily infected (> 500 worms).

Small pieces of infected and non-infected abomasa were washed several times with Hanks' balanced salt solution (HBSS), premaintained at 37 ± 2°C to remove debris, blotted on filter paper and homogenized separately in a Potter Elvehjem homogenizer, in 0.1M ice-cold phosphate buffer (pH 7.4). The homogenates were sonicated (Ultrasonic processor, 5 mm probe) in an ice bath for 3 × 1 minute at 30 s intervals and then centrifuged at 9000 g for 15 minutes at 4°C. After centrifugation, supernatants were collected and stored at −20°C in aliquots and were used for the estimation of antioxidant and oxidant parameters and other biochemical variables. In the present study, only tissues from those hosts that had only *H. contortus* infection were analysed. The samples of those animals that had concurrent infections of other helminths were discarded. A total of nine infected goats were used, with three in each category of infection.

Biochemical analysis

Analysis of antioxidant enzymes

The level of antioxidant enzymes, i.e. catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx), was estimated by standard spectrophotometric methods as described by Aebi (1974), Habig *et al.* (1974), Carlberg and Mannervik (1985) and Paglia and Valentine (1967), respectively.

Analysis of marker enzymes

Aspartate (AST) and alanine (ALT) aminotransferases were analysed using a Span diagnostic enzyme kit (Surat, India). The assay principle of these enzymes is based on the method of Reitman and Frankel (1957). The activity of acid (ACP) and alkaline (ALP) phosphatases was determined by the method of Bergmeyer *et al.* (1974).

Analysis of oxidant parameters

The concentrations of superoxide anion (O_2^-), protein carbonyl (PC), malondialdehyde (MDA) and reduced glutathione (GSH) were estimated by the methods of Green and Hill (1984), Levine *et al.* (1990), Ohkawa *et al.* (1979) and Jollow *et al.* (1974), respectively. Protein concentration in the samples was determined by the dye-binding method of Spector (1978).

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by post-hoc Tukey's HSD multiple comparison tests using the statistical software R v. 2.15.1(R Development Core Team, 2012). The confidence level was held at 95% and $P \leq 0.05$ was considered significant.

Results

Antioxidant enzymes

The activity of CAT, GST and GR was found to increase whereas GPx activity declined in the infected tissues as compared to non-infected tissues (fig. 1). A significant increase in the activities of CAT and GST was recorded in mild and heavily infected animals, whereas there were no differences in the values of these enzymes in animals with low or no infection. The activity of GR and GPx was significant in all the groups compared with the non-infected animals.

Marker enzymes

The levels of AST, ALT and ALP were found to be significantly higher in the low, mild and heavily infected tissues compared to the controls (fig. 2). The level of ACP was also found to be significantly higher in the mild and heavily infected groups compared to the control, but the differences were insignificant in cases of low infection.

Oxidant parameters in the tissue

The results for oxidative stress markers such as superoxide anion (O_2^-) concentration, lipid peroxidation, protein oxidation and glutathione level are shown in fig. 3. A rise in the level of O_2^- was observed in the infected tissue, which was directly related to the number of parasites in the individual infected host (fig. 3a). The increase in O_2^- was significant in cases of mild ($P < 0.05$) and heavy infection ($P < 0.001$), but insignificant in cases of low infection. A significant rise in MDA level, an index of lipid peroxidation, was seen in all the groups (fig. 3b). An increase in the protein carbonyl level, a parameter of oxidative damage to proteins, was also found to be elevated in the infected tissue (fig. 3c). Reduced glutathione levels were also altered; a drastic decline was seen in all the groups analysed when compared with the non-infected tissue (fig. 3d).

Discussion

Parasitic infection is responsible for a variety of biochemical changes, depending upon the species of the parasites, the invasion sites and the worm burden (Esmaeilnejad *et al.*, 2012; de Oliveira *et al.*, 2013; Ortolani *et al.*, 2013). The present study of the alteration in antioxidant enzymes and oxidative stress of goats infected with *H. contortus* provides reliable biochemical evidence for the generation of circulating oxidative stress. Increased free radical generation (O_2^-), enhanced lipid peroxidation and increased oxidation products of proteins, coupled with changes in the activity of the enzymatic (CAT, GST, GR and GPx) and non-enzymatic (GSH) antioxidant defences, were detected in naturally infected goats. However, the present study cannot be compared with previous reports, as no published report is available for goats infected with *H. contortus*.

Cells contain a variety of antioxidant mechanisms that play a central role in protection against ROS (Halliwell, 1991). The

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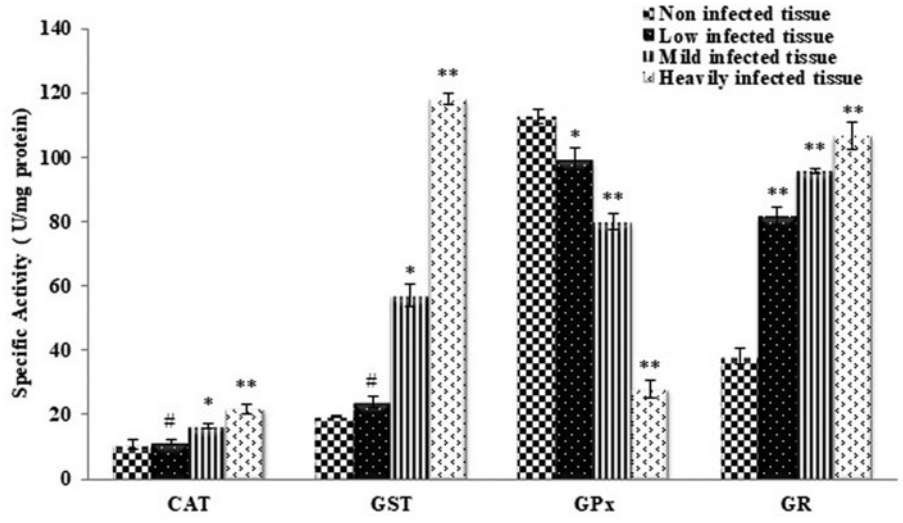

Fig. 1. Activities of antioxidant enzymes in non-infected host tissue and host tissue infected with *Haemonchus contortus.* CAT, catalase; GST, glutathione S-transferase; GR, glutathione reductase; GPx, glutathione peroxidase. All values are the mean ± S.E. of three replicates. Statistical analysis was performed by one-way ANOVA followed by the post-hoc Tukey's test; #, non-significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.001$.

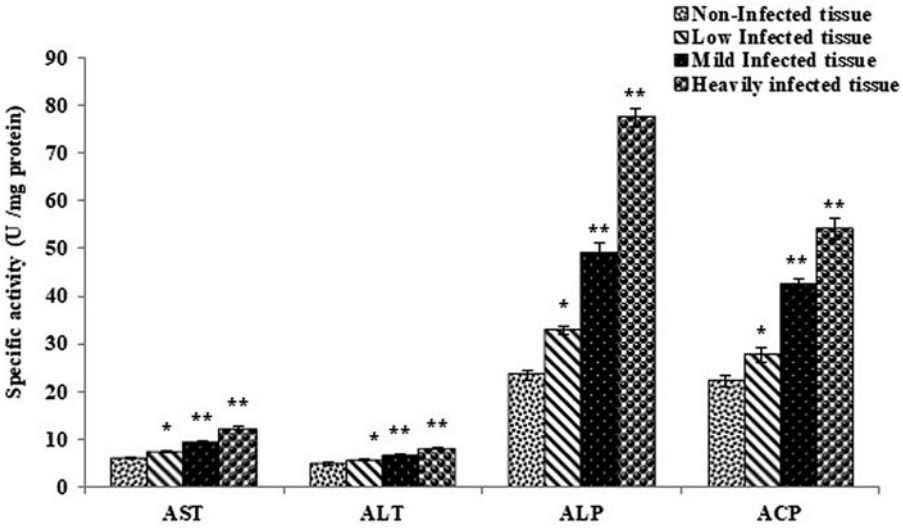

Fig. 2. Marker enzymes of non-infected host tissue and host tissue infected with *Haemonchus contortus*. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ACP, acid phosphatase. All values are the mean ± S.E. of three replicates. Statistical analysis was performed by one-way ANOVA followed by the post-hoc Tukey's test; #, non-significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.001$.

increase in the activity of CAT, GR and GST in infected animals may be explained by the fact that oxidants may activate gene expression through antioxidant responsive elements (Rushmore *et al.*, 1991). A significant increase in catalase activity was found in the animals infected with *H. contortus*, as also reported in hamsters infected with *D. dendriticum* (Sanchez-Campos *et al.*, 1999), in rat liver infected with *F. hepatica* (Kolodziejczyk *et al.*, 2005) and in mice liver infected with *S. mansoni* (de Oliveira *et al.*, 2013). The up-regulation in CAT activity is expected due to excessive peroxide production by leukocytes, as also suggested by de Oliveira *et al.* (2013). We observed significantly increased levels of GR activity in *H. contortus* infected tissues, whereas Kolodziejczyk *et al.* (2005) reported a significantly reduced level of GR activity in liver infected with *F. hepatica.* An increased level of GST in the infected animal may be due to toxic secretions by the parasites and the host's inflammatory response, as this enzyme is responsible for the neutralization of toxins (Brophy and Pritchard, 1994). Similarly, an increase in GST activity has been reported in hamsters infected with *D. dendriticum* (Sanchez-Campos *et al.*, 1999), in mice infected with *Ancylostoma caninum* (Gollapudi and Vardhani, 2013) and in the muscles of mice infected with *T. spiralis* (Derda *et al.*, 2004). GPx activity was found to be lower in the infected animals compared to the non-infected group, which is similar to the findings of Heidarpour *et al.* (2012) and Kolodziejczyk *et al.* (2005) for camels infected with *E. granulosus* and rats infected with *F. hepatica*, respectively. In contrast, an increase in the activity of GPx was reported in cattle infected with *E. granulosus* (Heidarpour *et al.*, 2013a), in hamsters infected with *D. dendriticum* (Sanchez-Campos *et al.*, 1999), in sheep infected with *F. hepatica* (Benzer and Temizer–Ozan, 2003), in skeletal muscle of cattle infected with *T. saginata* (Łuszczak *et al.*, 2011) and in the liver of sheep infected with *D. dendriticum* and *F. hepatica* (Deger *et al.*, 2008). The superoxide dismutase (SOD) activity has also been found to be significantly reduced in abomasal tissue infected with *H. contortus* (Rashid and Irshadullah, 2014).

Reactive oxygen species (ROS) are produced by phagocytes with the aim of destroying the parasite; however, the host's innate defence mechanism is not specific, so the destruction of its own tissues is possible. This reaction may lead to pathological processes. The level of O_2^- was found to be higher in infected animals and is related to the worm burden. Similarly, an increased level of

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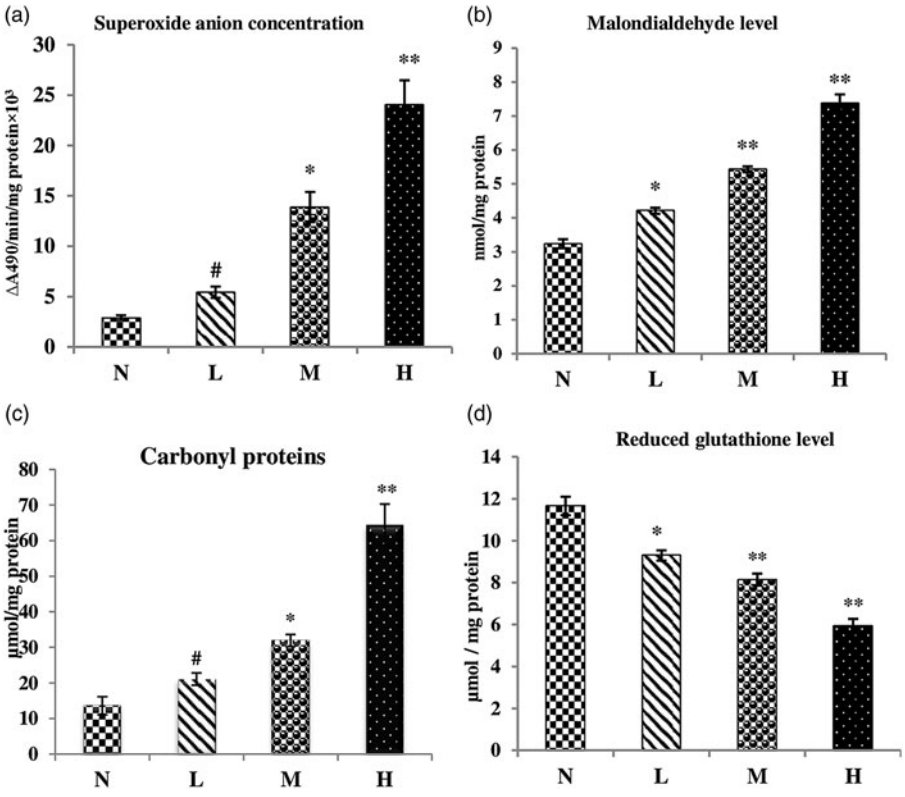

Fig. 3. Alteration in the level of the oxidant parameters in non-infected host tissue and host tissue infected with *Haemonchus contortus*. N, non-infected host tissue; L, low infection of host tissue; M, mildly infected host tissue; H, heavily infected host tissue. All values are the mean ± S.E. of three replicates. Statistical analysis was performed by one-way ANOVA followed by the post-hoc Tukey's test; #, non-significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.001$.

O_2^- has been reported in animals infected with *F. hepatica* (Abo-Shousha *et al.*, 1999). Lipid peroxidation was significantly enhanced in the tissue infected with *H. contortus*. Lipid peroxidation results in the disarrangement and, ultimately, disruption of cell membranes, which leads to cell death. During helminth infections, lipid peroxidation has been found to increase (Kolodziejczyk *et al.*, 2005; Heidarpour *et al.*, 2012, 2013b; de Oliveira *et al.*, 2013). Protein carbonyls are not only good markers for oxidative damage but are also oxidizing agents, which enhance oxidative damage to a membrane protein, principally in the form of disulphide skeleton protein adducts, causing denaturation and instability of the cytoskeleton (McMillan *et al.*, 2005; Dalle-Donne *et al.*, 2006). The increased carbonyl protein in the infected tissue confirms enhanced release of free radicals in *H. contortus*-infected animals. Siemieniuk *et al.* (2008) studied the oxidative modifications of rat liver cells due to *F. hepatica* infection and reported a significant increase in protein oxidation and lipid peroxidation. De Oliveira *et al.* (2013) reported an elevated level of protein carbonyls in the tissue of mice infected with *S. mansoni*. The increase in carbonyl proteins in the present study is directly related to the worm burden, as also reported by Saleha *et al.* (2011). The glutathione level was significantly reduced; it may be connected with the enhanced oxidation of GSH into glutathione disulphide catalysed by free radicals. As glutathione is the most important cellular antioxidant and plays a major role in protecting the cells against oxidative stress (Shan *et al.*, 1990), it has been postulated that loss of GSH may compromise cellular antioxidant defences and lead to the accumulation of reactive oxygen species (Halprin and Ohkawara, 1967). Furthermore, it was shown that administering GSH to experimentally infected rats greatly reduced the damage to membrane lipids of the liver tissue (Maffei Facino *et al.*, 1993). Glutathione peroxidase and GSH represent a major pathway for metabolizing hydrogen peroxide and lipid peroxides. Therefore, depletion of GSH and suppression of glutathione peroxidase activity, as we have observed in the present study, may result in the accumulation of peroxides to toxic levels. Similarly, Kolodziejczyk *et al.* (2006) reported a decline in the level of GSH and GPx activity in the serum of rats infected with *F. hepatica*.

The elevated level of marker enzymes (AST, ALT, ACP and ALP) in infected tissues indicated some damage or changes in membrane permeability. Similar results have been reported by Khan *et al.* (2013) and Kolodziejczyk *et al.* (2005) in fish liver infected with *Clinostomum complanatum* and rat liver infected with *F. hepatica*, respectively. Deger *et al.* (2008) also reported a higher level of ALT and AST in the liver of sheep naturally infected with *D. dendriticum* and *F. hepatica* as compared to the control.

Observations in the present study on biochemical changes caused by haemonchosis in goats carry great importance as they may indicate the extent of damage to the abomasal mucosa, and thereby help in better understanding the host–parasite interactions. During *H. contortus* infection, it was observed that there were significant alterations in oxidative markers, indicators of cell lesion of the abomasum, which was confirmed by an increase in the marker enzymes, and consequently, there were changes in levels of antioxidants with the purpose of cell protection. It can be concluded that *H. contortus* plays a critical role as an oxidative stressor in goats.

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Conflict of interest. None.

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