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# Effect of fenbendazole and ivermectin on antioxidant status of goats suffering from gastrointestinal nematodes

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**Abstract**
A total of 146 goats having FAMACHA score from 3 to 5 were examined for estimating the occurrence of gastrointestinal (GI) nematodosis in Tarai region around Pantnagar. Hundred and three goats were found positive for GI nematodosis. Out of these 103 goats, 18 goats were separated and divided randomly in three groups. The animals of Group I: (n=6) were treated with fenbendazole @ 5 mg/kg body wt. po; Group II: (n=6) were given ivermectin @ 0.2 mg/kg body wt. po. The animals of Group III: (n=6) were the control without any treatment. Using standard procedures, the serum obtained were assayed for estimation of antioxidant values and lipid peroxidation *viz* glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD). On 21$^{st}$ day post treatment, goats of group I & II showed significant reduction in lipid peroxidation however values of CAT, SOD & GSH-Px increased significantly. Values of group III showed elevated values of malondialdehyde (MDA) & reduced levels of GSH-Px, SOD and CAT.

**Keywords:** Ivermectin, GI nematodosis, oxidative stress, free radicals, anti-oxidant

## Introduction
Helminth infection of the gastrointestinal tract is a significant contributing factor to decreased goat productivity on a global scale, especially under grazing conditions. Clinically, gastrointestinal parasitic infections in goats present with symptoms such as enteritis, anemia, emaciation, dehydration, and even death.

As per Tariq *et al.*, (2010) [23], gastrointestinal (GI) parasitism has emerged as primary constraint and major threat to the small ruminant industry, leading to production losses and death in severe cases. The host-parasite relationship with nematode parasites results in substantial damage at the site of attachment, manifesting as enteritis, anaemia, emaciation, dehydration, and death. These changes significantly impact the growth, body weight, yield, and reproductive performance of the animals, resulting in economic losses for the farmers (Sharma *et al.*, 2014) [21]. As per Brunsdon (1988) [1], the GI nematodes cause severe losses in pasture-based livestock farming.

In the sub-clinical form, worms continuously feed on blood (Maiti *et al.*, 1999) [14], causing anemia and hypoproteinemia. Haematological analysis and Serum biochemistry have proved to be the reliable and essential indicators for ascertaining the health status of animals. It also provides insight into the severity of infestation and extent of damage inside the host body, (Otesile *et al.*, 1991) [16]. Imbalance between production of free radicals and antioxidant defences of the body leads to oxidative stress and its estimation has become vital in animal production and management. This assessment has become a supporting tool to evaluate metabolic and nutritional status of infected animals, (Mohebbi-Fani *et al.*, 2012) [15].

The body's antioxidant status plays a critical role in maintaining overall health by serving as an essential defence mechanism against free radicals, which can cause damage to the biological system (Padh, 1991) [18]. Antioxidant enzymes like GSH-Px, SOD and CAT are vital in combating the harmful free radicals (ROS: Reactive oxygen species). SOD changes superoxide radicals into water & hydrogen peroxide, while CAT converts hydrogen peroxide into oxygen and water and GSH-Px helps in destruction of organic peroxides and hydrogen peroxide. Charinya and Sunthamala (2023) [3], reported increase in MDA values in blood in stressed goats. Stress can increase oxidative damage, which leads to MDA and free radical generation, indicative of oxidative stress. Rasha *et al.*, (2020) [19], reported that serum SOD, catalase and

GSH-Px level significantly decreased in heavy infection, in comparison to control whereas significant increase in Serum lipid peroxidation (MDA) level was seen in infected sheep and goats.

Many physiological disturbances are caused by the gut parasites in host body like weight loss, metabolic disturbances, haematological alterations and increased susceptibility to oxidative stress, Khan, *et al.*, (2015) [11].

The Present research was planned in order to study the effect of commonly used anthelmintics on oxidative stress biomarkers in goats suffering from gastrointestinal nematodosis.

## Material and Methods

### Screening and experimental group formation

A total of 146 goats having FAMACHA score from 3 to 5 were examined to determine the prevalence of helminthosis in the Tarai region around Pantnagar through target selective treatment (TST).Out of these 146 goats, 103 were found positive for GI nematodosis. From these 103 goats 18 goats of either sex (n = 18; 9 months to 5 yrs of age weighing between 10-30 kgs) having egg counts (>200) were separated and divided randomly in three groups. The animals of Group I: (n=6) were treated with fenbendazole @ 5 mg/kg body wt. po; Group II: (n=6) were given ivermectin @ 0.2 mg/kg body wt. po. The animals of Group III: (n=6) were the control without any treatment. The FAMACHA system is an affordable and validated method for evaluating the extent of anemia, primarily attributed to *H. contortus* infection. Van Wyk and Bath (2002) found that when farmers used and carried out treatments based on FAMACHA score instead of PCV determinations, there was 58% reduction in the number of treatments required at 10 farms in comparison to previous year.

The present study was conducted at the Dept. of Veterinary Medicine, (CVASc) and Biochemistry Dept. (CBSH), Pantnagar. Approval was taken from Institutional Animal Ethical Committee of college vide proposal No IAEC/C.V.A.Sc./VMD/487 dt 25/10/2021 & dt 28/09/2022.

### Collection and analysis of biochemical parameters

Five millilitres of jugular vein blood was collected in serum vacutainers with clot activators under aseptic condition from each of the goats on 0 day (before treatment), and days 7, 14 and 21 (after treatment) using sterile syringe and needles. Blood after collection was kept at room temperature to facilitate clotting and was centrifuged for separation of serum. Eppendorf tubes were used to transfer the serum samples maintained at 4 °C till they reached the laboratory for estimation of various antioxidant parameters.

### Assessment of antioxidant status

The present investigation focused on the assessment of lipid peroxidation (LPO) and enzymatic antioxidant indices, including GSH-Px, CAT, and SOD, in the serum of goats. To conduct the analysis, we utilized the Activity Assay kit from Real Gene Labs, Ghaziabad, India, following the instructions provided in the user manual. The measurements were taken on day 0 and day 7, 14, and 21.

Estimation of malondialdehyde (MDA) in the blood serum was done for assessment of lipid peroxidation. This marker serves as an indicator of oxidative damage caused by lipid peroxidation.

### Principles of Detection

a) Glutathione peroxidase (GSH-Px) is a crucial peroxidase enzyme widely distributed in the body and its main role is to change reduced glutathione (GSH) into oxidized glutathione (GSSG), thereby converting harmful hydrogen peroxide into non-toxic hydroxyl compounds. The activity of GSH-Px was assessed based on the decrease in absorbance at 412nm, resulting from the formation of a characteristic compound when DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) reacts with GSH.

b) Superoxide dismutase (SOD) is an omnipresent enzyme found in animals, microorganisms, plants and cultured cells. It catalyses the conversion of superoxide anions into hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$), thus playing a crucial role in the biological antioxidant system. To evaluate SOD activity, we utilized the xanthine and xanthine oxidase reaction system, where superoxide anions ($O_2$-.) are generated. These anions reduce nitrogen blue tetrazolium to form blue formazan, which absorbs light at 560nm. By measuring the intensity of the blue colour, we can determine the SOD activity, as higher activity leads to a lighter blue colour due to the inhibition of formazan formation. The enzyme activity of SOD in reaction system is defined as a unit of enzyme activity (U/ml) when the inhibition percentage is 50% in the above xanthine oxidase reaction system.

c) Catalase is a ubiquitous enzyme found in various organisms, including animals, plants, microorganisms, and cultured cells. Its primary function is to scavenge hydrogen peroxide ($H_2O_2$), making it an essential component of the reactive oxygen scavenging system. $H_2O_2$ exhibits a distinct absorption peak at 240nm. By decomposing $H_2O_2$, catalase reduces the absorbance of the reaction solution at 240nm over time. Catalase activity can be determined by calculation of the rate of change in absorbance. One unit of enzyme activity is defined as the amount of enzyme which catalyses degradation of one µmol of $H_2O_2$ in the reaction system per minute every millilitre of serum (plasma).

d) Lipid peroxidase is produced when unsaturated fatty acids of lipids gradually decompose into various complex compounds like malondialdehyde (MDA) due to the activity of oxygen free radicals. Lipid oxidation values are often measured by detecting the level of MDA. A red product with highest absorption peak of 532nm is formed due to condensation of MDA with thiobarbituric acid (TBA). Through colorimetry, lipid peroxide content in the sample can be calculated. Concurrently absorbance at 600nm is measured, and difference in absorbance between 532nm and 600nm is used to calculate the MDA content.

### Statistical analysis

All results were expressed as mean ± standard error of the mean (S.E.M) for statistical analysis. Two-way ANOVA and post-hoc Tukey's test were employed to determine any significant differences among the groups at the same sampling time. Statistical significance level for comparisons was set at $p<0.05$, meaning that P values less than 0.05 were considered significant.

## Results & Discussion

Significant rise in the levels of serum GSH-Px, CAT and SOD were observed in goats treated with Ivermectin and

Fenbendazole on the 21st day post treatment. Conversely non-significant change in values of GSH-Px, SOD, and CAT in comparison to values on 0 day were seen in the untreated control group on the 21st day post treatment. MDA indicates increased oxidative stress as it represents the final outcome of lipid peroxidation in body tissues or biological fluids **(**Halliwell and Chirico, 1993) [7]. Previous studies by Dede *et al.,* (2000) [4] reported increased MDA levels in Akkaraman sheep infected with *Trichostrongylidae, Fasciola spp*. and *Eimeria spp*., while Simsek *et al.,* (2006) [22] and Dimitrijevi´c *et al.* (2012) [5] found increased MDA levels with *Dicrocoelium dendriticum* and *Strongyloides Papillosus* infestations in sheep, respectively. However, the treatment with Ivermectin and Fenbendazole resulted in a lower MDA level compared to the positive control group, indicating improved antioxidant status.

In goats suffering from GI nematodosis, the level of lipid peroxidation decreased significantly ($p$<0.05) on the 21st day after treatment with fenbendazole and ivermectin, while it showed a significant increase in the untreated control group on same day.

Estimation of MDA allows the indirect detection of the level of lipid peroxidation and free oxygen radicals. In this experiment, the MDA levels exhibited an opposite trend to those of GSH-Px, SOD, and CAT. Similarly, various studies (Rashid, 2016; Heidarpour, *et al.,* 2012; Kolodziejczyk, *et al.,* 2006) [20, 9, 13] have reported increased lipid peroxidation during helminth infections. Jaheed *et al., (*2020) [10], reported that, significant decrease in total antioxidant capacity was found in serum biochemistry of infected animals. Values of GSH-Px decreased significantly in goats suffering from GI nematodosis whereas values of malondialdehyde increased significantly.

Effect of Fenbendazole and Ivermectin drug administration on oxidative stress related parameters in goats suffering from GI nematodosis

| Groups | Days | Glutathione peroxidase (mu/ml) | Superoxide dismutase (U/ml) | Catalase (u/ml) | MDA (nmol/ml) |
|---|---|---|---|---|---|
| Group-1 (Fenbendazole) | 0 | 42.833 $^{A}$ | 33.000 $^{A}$ | 23.667 $^{A}$ | 11.250 $^{A}$ |
| | 7 | 40.417 $^{A}$ | 32.900 $^{A}$ | 23.333 $^{B}$ | 11.583 $^{A}$ |
| | 14 | 47.167 $^{B\,b}$ | 34.667 $^{B\,b}$ | 25.833 $^{B\,b}$ | 10.833 $^{B\,ab}$ |
| | 21 | 115.667 $^{C\,c}$ | 84.667 $^{B\,b}$ | 56.667 $^{B\,b}$ | 8.000 $^{B\,b}$ |
| Group-II (Ivermectin) | 0 | 42.833 $^{A}$ | 33.500 $^{A}$ | 26.500 $^{A}$ | 11.100 $^{A}$ |
| | 7 | 42.417 $^{A}$ | 33.333 $^{A}$ | 23.833 $^{B}$ | 11.100 $^{A}$ |
| | 14 | 63.500 $^{A\,a}$ | 41.333 $^{A\,a}$ | 32.000 $^{A\,a}$ | 9.900 $^{B\,b}$ |
| | 21 | 138.500 $^{B\,b}$ | 94.333 $^{A\,a}$ | 69.833 $^{A\,a}$ | 4.800 $^{C\,c}$ |
| Group-III (Control) | 0 | 40.250 $^{A}$ | 32.833 $^{A}$ | 26.100 $^{A}$ | 10.483 $^{A}$ |
| | 7 | 40.583 $^{A}$ | 33.250 $^{A}$ | 26.500 $^{A}$ | 10.833 $^{A}$ |
| | 14 | 42.000 $^{B\,b}$ | 32.333 $^{B\,b}$ | 23.917 $^{B\,b}$ | 12.833 $^{A\,a}$ |
| | 21 | 44.333 $^{A}$ | 34.167 $^{C\,c}$ | 30.167 $^{C\,c}$ | 12.500 $^{A\,a}$ |

Capital letter (A, B, C etc.) Indicates significant difference among the drugs ($p$<0.05)

Small letter (a, b, c etc.) Indicates significant difference among the days ($p$<0.05)

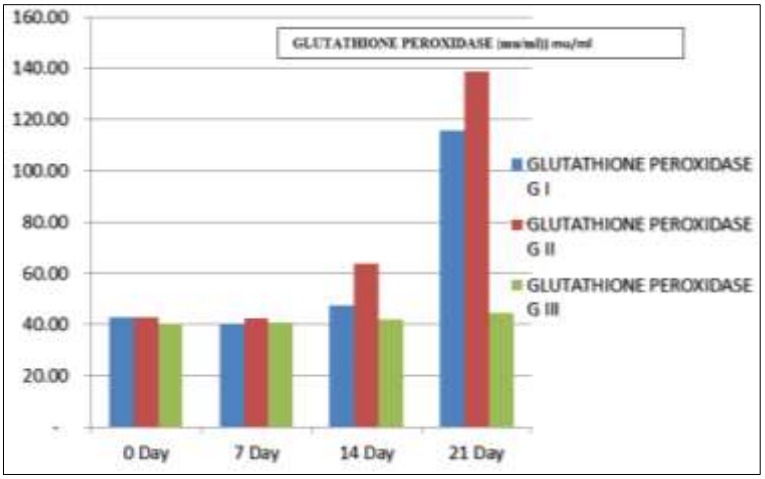


**Fig 1:** Effect of Fenbendazole and Ivermectin drug administration on Glutathione peroxidase in goats suffering from GI nematodosis.

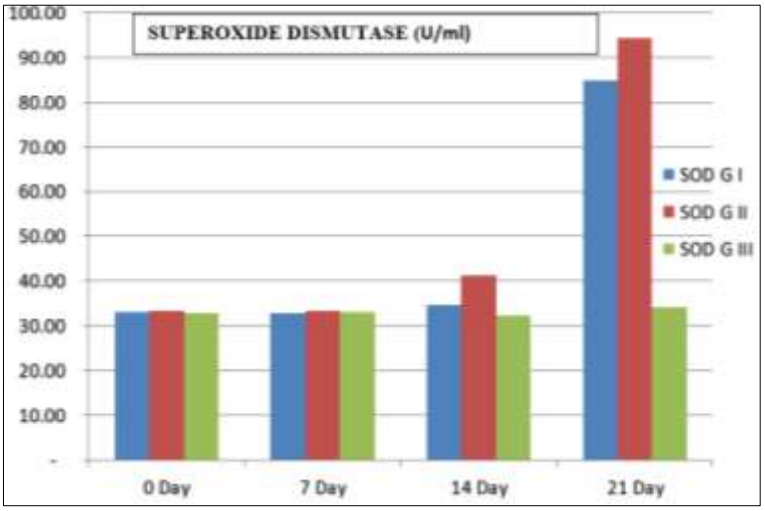


**Fig 2:** Effect of Fenbendazole and Ivermectin drug administration on Superoxide dismutase in goats suffering from GI nematodosis

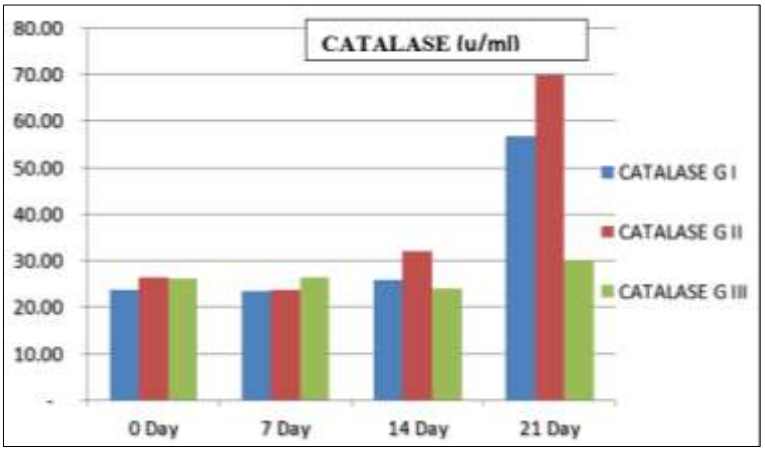


**Fig 3:** Effect of Fenbendazole and Ivermectin drug administration on Catalase in goats suffering from GI nematodosis

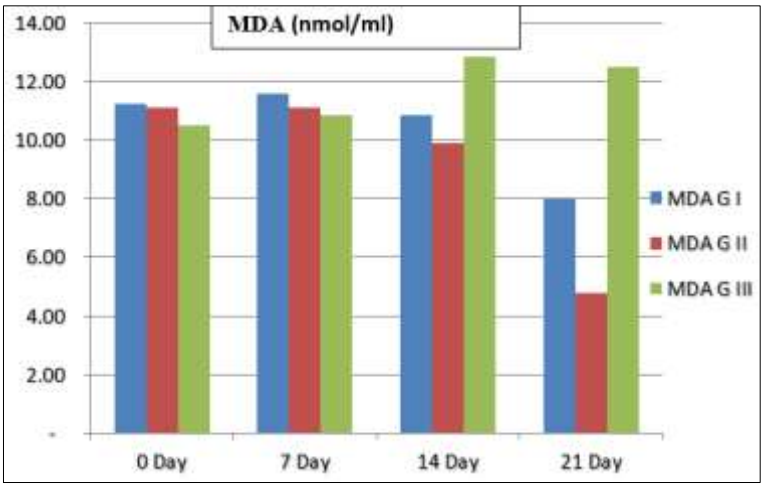


**Fig 4:** Effect of Fenbendazole and Ivermectin drug administration on malondialdehyde in goats suffering from GI nematodosis.

## Conclusion

The present study reveals that nematodosis in goats leads to protein oxidation and oxidative stress. However, treating the infected goats with anthelmintics, reduces the worm load, and improves their antioxidant status. Alteration in serum biochemistry of the infected animal's had strong correlation with the amount of damage and infection severity, Esmaeilnejad *et al*., (2012) [6].

These findings suggest that there is an increase in the level of free radicals coupled with oxidative stress in goats infested with gastrointestinal nematodes. Additionally, the administration of anthelmintics during the early stages of treatment may add to the chemical stress experienced by the animals, Dimitrijevi´c *et al.*, (2012) [5].

Charinya and Sunthamala (2023) [3], reported increase in MDA values in blood in stressed goats. Stress increases the oxidative damage, which leads to generation of MDA and free radicals which is indicative of oxidative stress. Rasha *et al*., (2020) [19], reported that serum SOD, CAT and GSH-Px level significantly decreased in heavy infection, compared to

untreated control whereas MDA level increased significantly in the infected sheep and goats.

After 21 days post-treatment, when the worm burden decreased and the chemical stress from anthelmintics subsided, the serum levels of GSH-Px, SOD, and CAT showed significant increase in goats of both Group I and Group II, in comparison to the untreated control group (Group III). Furthermore, MDA values in Group I and II revealed a significant decrease, indicating that goats treated with anthelmintics were able to achieve and maintain adequate antioxidant compensation, thereby reducing MDA levels.

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**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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