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Biochemical Modulation by Moringa oleifera: Impact on Cytokine Dynamics, Oxidative Stress, and Metabolic Health in Rabbits

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Abstract

Moringa oleifera, a rich source of bioactive phytochemicals, exerts significant immunomodulatory and antioxidant effects in rabbits (*Oryctolagus cuniculus*). This study investigated its influence on cytokine dynamics, oxidative stress markers, and metabolic health. Forty weaned rabbits were assigned to diets containing 0%, 1%, 2%, or 4% Moringa oleifera powder for 10 weeks. Cytokine profiling revealed an reduction of pro-inflammatory mediators (IL-1, IL-6, TNF-α, and IFN-γ) alongside an increase in IL-10, indicating a chemically balanced immune response. Enhanced antioxidant enzyme activity was observed, with increased superoxide dismutase (SOD) and catalase (CAT) levels, coupled with reduced malondialdehyde (MDA), reflecting mitigated oxidative stress. Metabolic analysis demonstrated reduced glucose and cholesterol concentrations, suggesting improved lipid and carbohydrate homeostasis, while total protein levels increased, indicating enhanced protein metabolism. Hematological assessments showed higher white blood cell counts and hemoglobin levels, correlating with improved immune resilience. Growth performance parameters, including body weight gain and feed conversion ratio, exhibited marked improvements, signifying optimized nutrient utilization. These findings underscore the biochemical role of *Moringa oleifera* in modulating immune responses, scavenging reactive oxygen species, and improving metabolic equilibrium. Its supplementation presents a promising strategy for enhancing physiological homeostasis and productivity in rabbits through targeted biochemical pathways.

Keywords: Bioactive compounds, cytokine biochemistry, oxidative stress modulation, immunochemical regulation, metabolic homeostasis, phytochemical nutrition

1. Introduction

In recent years, the exploration of plant-based supplements as alternatives to traditional feed additives in animal nutrition has gained significant momentum. This shift is driven by the increasing demand for sustainable agricultural practices and natural solutions to enhance livestock health and productivity [1]. Among these supplements, Moringa oleifera stands out due to its rich nutritional profile and potent bioactive compounds with notable antioxidant activity.

Native to the sub-Himalayan regions of South Asia, *Moringa oleifera*, often referred to as the "miracle tree," has been used for centuries for its medicinal and nutritional benefits [2]. Its leaves are packed with essential nutrients, including proteins, vitamins, minerals, and bioactive phytochemicals like flavonoids, phenolic acids, and glucosinolates.These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties, making Moringa oleifera a promising dietary supplement in animal agriculture [3].

Rabbits (*Oryctolagus cuniculus*) are important in commercial farming, known for efficient feed conversion and rapid growth [4]. Optimizing rabbits nutrition to improve growth, metabolism, immune function, and antioxidant status is a key focus for producers. Incorporating Moringa oleifera into rabbits diets could be beneficial due to its nutrient density and health benefits. Research on other livestock has shown that Moringa can enhance growth by improving protein utilization and digestion efficiency, critical factors in achieving better feed efficiency and economic returns [5]. Studies on poultry, goats, and cattle have demonstrated improvements in weight gain and feed conversion ratios.

Additionally, the immunomodulatory effects of Moringa oleifera are of interest for their ability to enhance immune responses in animals. Its bioactive compounds stimulate immune cells, increase cytokine production, and support antibody synthesis, which may strengthen immune defenses against pathogens [6]. The antioxidant properties of Moringa, largely attributed to its flavonoids and phenolic acids, also play a crucial role in reducing oxidative stress, which is important for maintaining health and productivity [7]. Livestock supplemented with Moringa have shown increased antioxidant enzyme levels, such as superoxide dismutase (SOD) and catalase (CAT), and reduced malondialdehyde (MDA), indicating improved oxidative stress management.

Despite the promising potential of *Moringa oleifera*, studies on its specific effects on rabbits' growth, metabolic health, antioxidant activity, and immune function are limited. This research aims to address that gap by evaluating the impact of

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Moringa oleifera powder supplementation on rabbits over a set period. Growth metrics, hematological profiles, biochemical markers, antioxidant activity, and cytokine expression will be assessed to provide evidence supporting the use of *Moringa oleifera* in improving rabbits health and productivity in sustainable farming practices.

2. Materials and Methods

2.1 Ethical Approval and Animal Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Umm-Alqura University (Approval No. HAPO-02-K-012-2023-06-1646) and adhered to ethical guidelines for animal use. Rabbits were handled by trained personnel to ensure humane treatment, with all efforts made to minimize suffering throughout the study.

2.2 Preparation of Moringa Powder

Moringa powder was prepared by washing fresh leaves, air-drying them in shaded conditions, grinding them into a fine powder, sieving to remove coarse particles, and storing the powder in airtight containers to maintain its quality. This process ensured the preservation of the leaves' bioactive compounds and nutritional value [8].

2.3 Experimental Animals and Housing Conditions

Forty healthy, weaned rabbits *(Oryctolagus cuniculus*), aged 8 weeks, were obtained from a local breeder. They were housed individually in wire mesh cages under a controlled 12-hour light/dark cycle at a temperature of 21–23°C. Before the experiment, a two-week acclimatization period was provided to ensure adaptation to housing conditions [9].

2.4 Formulation of Experimental Diets

Four experimental diets were formulated: a control diet without *Moringa oleifera* powder and three test diets containing 1%, 2%, and 4% *Moringa oleifera* powder. All diets were isocaloric and isonitrogenous, meeting the nutritional requirements of growing rabbits.

2.5 Feeding Protocol

Rabbits were fed ad libitum twice daily at 08:00 and 16:00, with continuous access to clean drinking water. Feed intake was recorded daily over the 10-week experimental period [10].

2.6 Growth Performance Assessment

Body weight was recorded weekly, and feed conversion ratio (FCR) was calculated as the amount of feed intake per unit of weight gain to evaluate growth efficiency [11].

2.7 Blood Sampling

Blood samples were collected from the **marginal ear vein** of the rabbits using a small needle (23–25 gauge). EDTA-coated tubes were used to collect blood for hematological analysis, while serum samples were obtained by allowing the blood to clot and then centrifuging at 3000 rpm for 10 minutes. The separated serum was stored at -20°C for subsequent biochemical and cytokine analyses .

2.8 Measurements

2.8.1 Hematological Analysis

Hematological parameters, including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin levels, and hematocrit, were analyzed using an automated hematology analyzer to assess immune function and overall physiological status [12].

2.8.2 Serum Cytokine Profiling

At the end of the study, blood samples were collected from the marginal ear vein into EDTA-coated tubes. Serum was separated by centrifugation and stored at -20°C for subsequent analysis. Pro-inflammatory cytokines (IL-1, IL-6, TNF-α, IFN-γ) and the anti-inflammatory cytokine IL-10 were quantified using commercial ELISA kits. Absorbance was measured at 450 nm following the manufacturer's protocols [13].

2.8.3 Metabolic and Biochemical Analysis

Serum biochemical parameters, including glucose, total protein, cholesterol, triacylglycerols, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), were measured using an automated biochemistry analyzer. These markers provided insights into carbohydrate metabolism, protein synthesis, lipid metabolism, and liver function [14].

2.8.4 Oxidative Stress and Antioxidant Biomarkers

Serum oxidative status was evaluated using assay kits for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA). SOD, CAT, and GPx activities were measured to evaluate enzymatic antioxidant capacity. MDA levels was quantified as an indicator of Lipid peroxidation All assays were performed according to standard protocols [15-18].

2.9 Statistical Analysis

Data were analyzed using SPSS software (version 11.0). One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to determine significant differences between treatment groups. Statistical significance was set at $p < 0.05$, and results were expressed as mean ± standard error (SE).

3. Results and Discussion

3.1 Growth performance and Biochemical Metabolic parameters

Dietary supplementation with *Moringa oleifera* powder significantly improved growth performance in rabbits. Weight gain and feed conversion ratio (FCR) were markedly enhanced, indicating better nutrient utilization and growth efficiency. Specifically, the 4% Moringa group exhibited the highest weight gain and the best FCR among all groups, suggesting a dose-dependent effect of Moringa supplementation on growth performance (Figures 1 and 2). This improvement is likely due to the bioactive compounds in Moringa oleifera, such as flavonoids and phenolic acids, which enhance digestion and nutrient

absorption. These findings align with previous studies demonstrating the efficacy of plant-based supplements in promoting growth and feed efficiency in livestock [19].

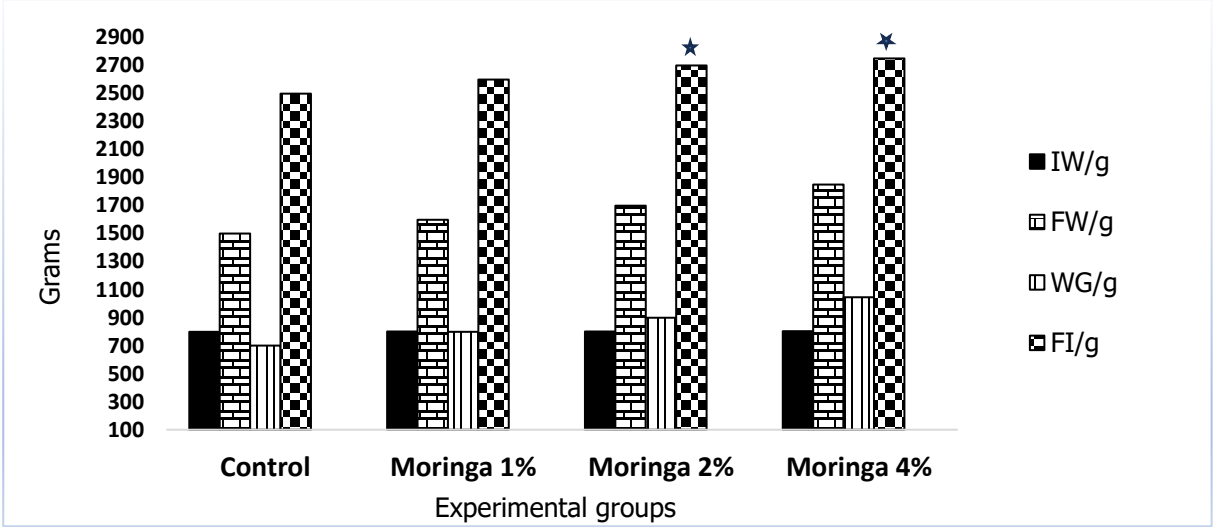

Fig. 1.Growth Performance parameters of Rabbits Supplemented with Moringa powder for 10 weeks, (IW):Initial Weight,(FW):Final Weight,(WG):Weight Gain,(FI):Feed Intake .(★) Significant difference at $P \leq 0.05$.

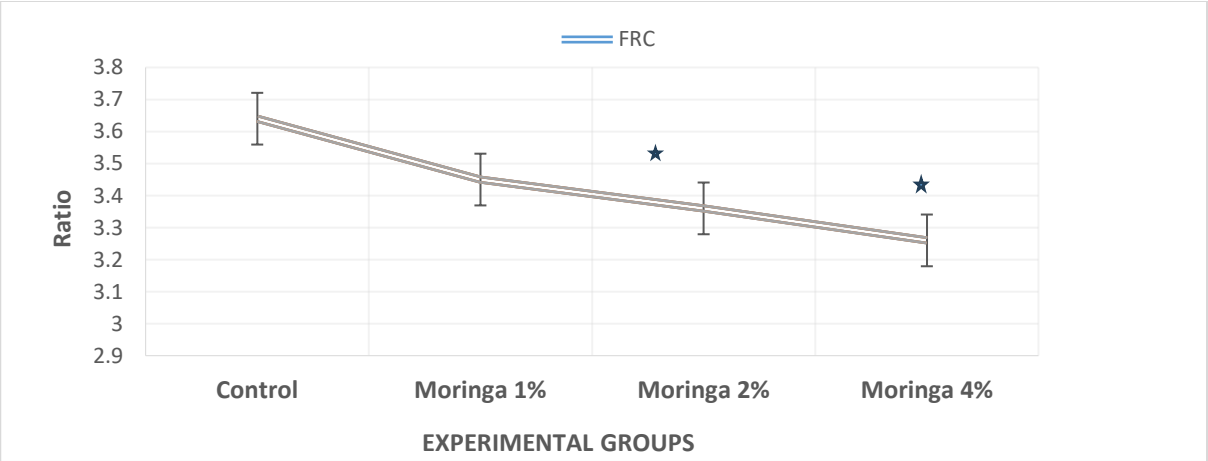

Fig. 2. Feed Conversion Ratio (FRC) of rabbits Supplemented with Moringa powder for 10 weeks. (★) Significance at $P \leq 0.05$ Vs control.

Serum glucose and cholesterol levels were significantly lower ($P \leq 0.05$) in Moringa-supplemented groups (2% & 4%), reflecting improved metabolic health and reduced risk of metabolic disorders (Figure 3). Additionally, total protein levels (Figure 4) were higher in these groups, indicating better protein metabolism and nutritional status. The 4% Moringa group exhibited significant decreased ($P \leq 0.05$) liver enzyme levels (ALT and AST), suggesting hepatoprotective effects. These results are consistent with previous studies reporting the potential of Moringa to mitigate metabolic disorders and protect liver health through its antioxidant properties [20].

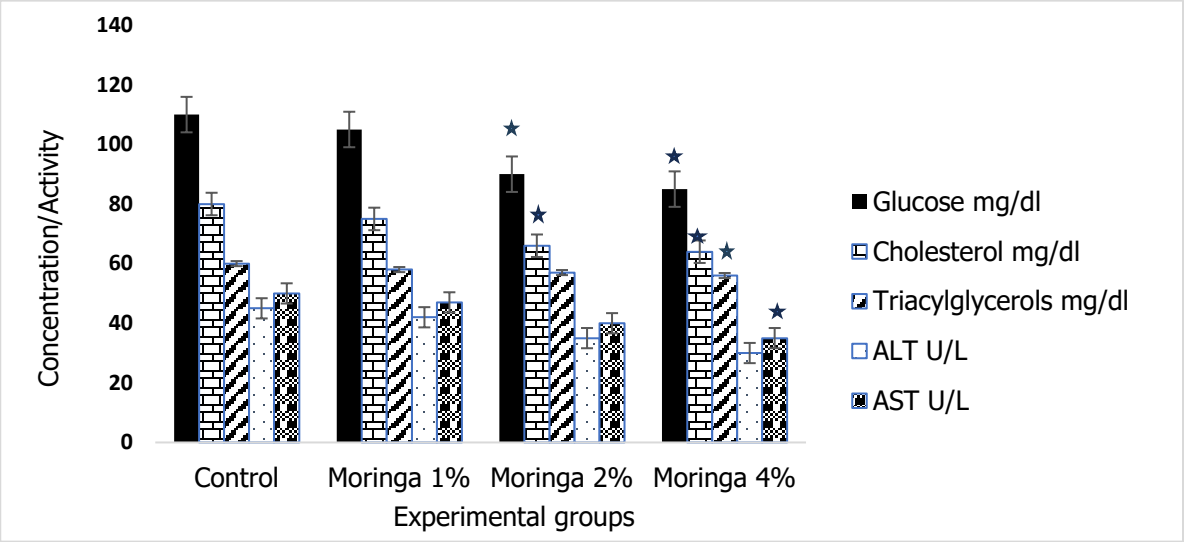

Fig. 3. Biochemical parameters of rabbits supplemented with moringa powder for 10 weeks.ALT(Alanine transferase),AST(Aspartate transferase).(★) Significant difference at P ≤ 0.05 Vs control.

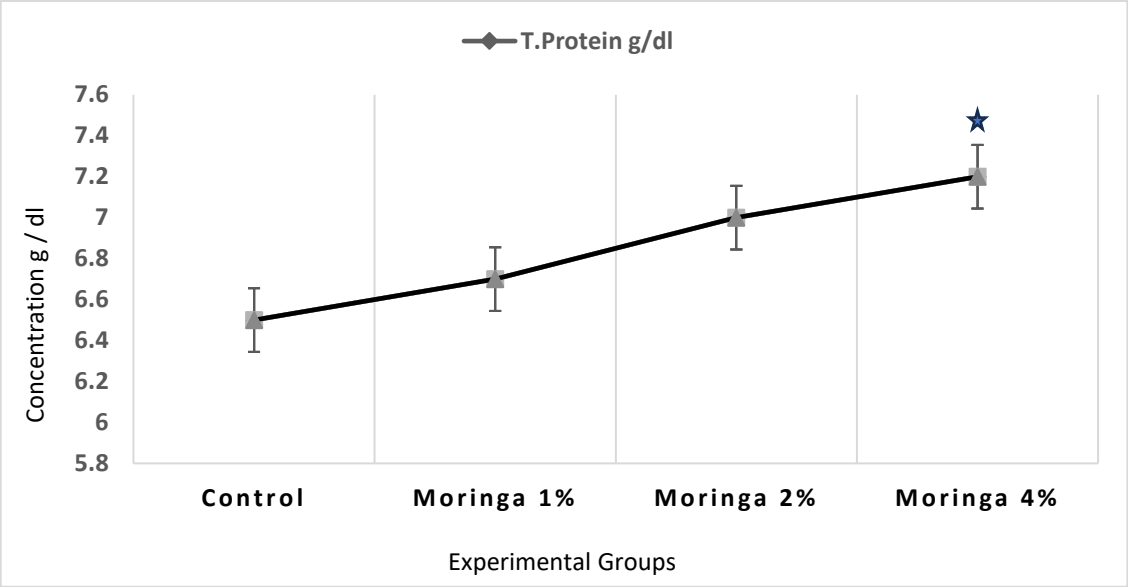

Fig. 4. Total protein levels of rabbits supplemented with moringa powder for 10 weeks. .(★) Significant difference at P ≤ 0.05 Vs control**.**

3.2 Hematological indices

Rabbits in the Moringa-supplemented groups (2% & 4%) showed higher white blood cell (WBCs) counts and hemoglobin levels (P ≤ 0.05), indicating enhanced immune function and better oxygen-carrying capacity (Figure 5).Although red blood cell (RBCs) count and hematocrit levels increased slightly, these changes were not statistically significant. These hematological improvements suggest that Moringa supplementation positively influences blood health, enhancing the rabbits' vitality and resistance to disease. Similar findings were reported by Pstras et al. [21], who observed improved immune parameters in animals receiving dietary plant-based antioxidants.

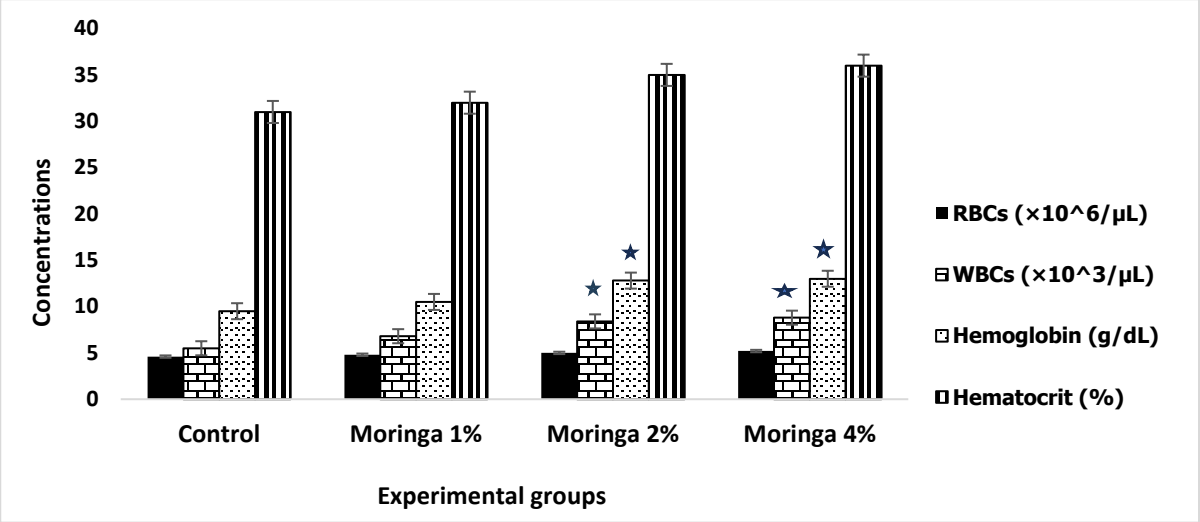

Fig. 5. Hematological indices of rabbits supplemented with moringa powder for 10 weeks. RBCs (red blood cells), WBCs (white blood cells). (★) Significant difference at $P \leq 0.05$ Vs control.

3.3 Cytokine response

A significant elevated levels ($\mathbf{P} \leq 0.05$ **)** of pro-inflammatory cytokines (IL-1, IL-6, TNF-α, and IFN-γ) were observed in the Moringa groups, indicating a robust immune response. Concurrently, a significant increased levels ($\mathbf{P} \leq 0.05$ **)** of the anti-inflammatory cytokine IL-10 suggested a balanced immune mechanism (Figure 6). This dual effect of enhancing immunity while modulating inflammation demonstrates the immunomodulatory properties of *Moringa oleifera*. These findings align with those of Díaz-Prieto et al. [22], who highlighted the role of plant-based supplements in maintaining immune homeostasis.

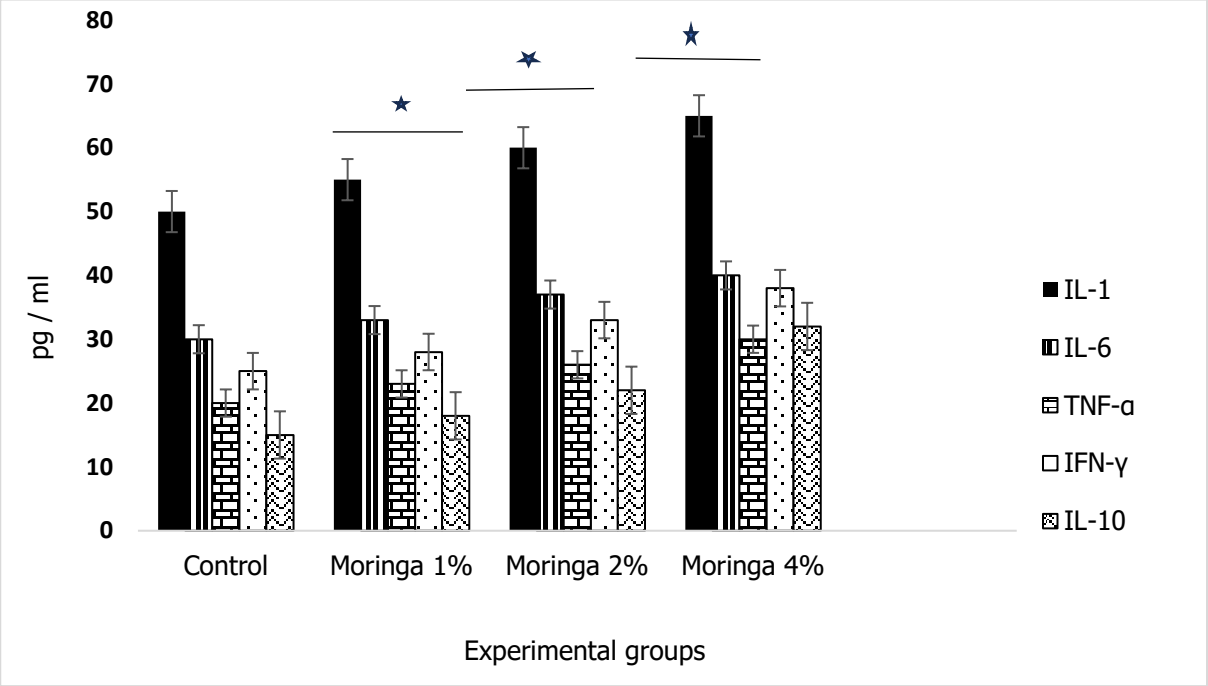

Fig. 6. Cytokine levels of rabbits supplemented with moringa powder for 10 weeks. IL-1 (interleukin-1), IL-6 (interleukin-6), TNF-A (tumor necrosis factor alpha), IFN-ɣ (interferon gamma), IL-10 (interleukin-10).
(★) Significant difference at $P \leq 0.05$ Vs control.

3.4 Antioxidant activity

Rabbits supplemented with Moringa (2% & 4%) exhibited significantly higher activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along with lower malondialdehyde (MDA) levels, indicating reduced oxidative stress and lipid peroxidation (Figure 7). These antioxidant effects are attributed to the bioactive compounds in Moringa, which scavenge free radicals and protect cellular components. Similar antioxidant benefits have been documented by Kashyap et al. [23] and Kushwah et al. [24], who demonstrated the potential of Moringa to enhance antioxidant defenses and improve overall health in animal models.

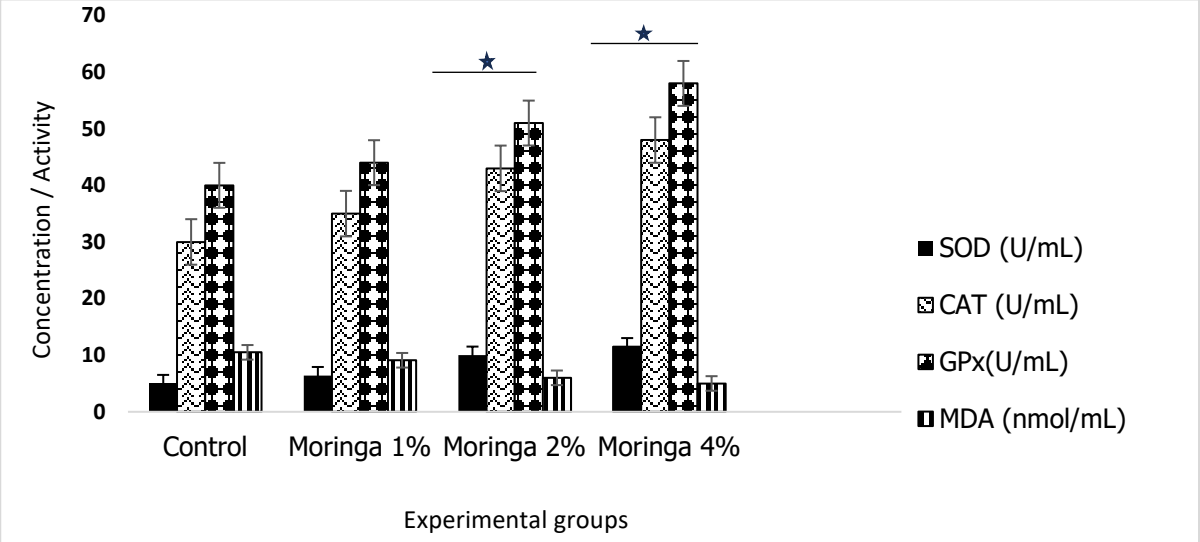

Fig. 7.Antioxidant parameters of rabbits supplemented with moringa powder for 10 weeks. SOD (superoxide dismutase),CAT(catalase),GPX (glutathione peroxidase),MDA (malondialdehyde).(★) Significant difference at $P \leq 0.05$ Vs control

Conclusions

The results of this study demonstrate the potential of *Moringa oleifera* as an effective dietary supplement for enhancing rabbits growth performance, metabolic health, immune responses, and antioxidant defenses. The findings indicate that *Moringa oleifera* could play a significant role in improving overall health and productivity in rabbits farming. Given these positive outcomes, it is recommended that *Moringa oleifera* be considered as a valuable additive to rabbits diets to support better health and performance outcomes.However, further research is warranted to determine the optimal inclusion levels of *Moringa oleifera* and to assess the long-term effects of its supplementation in rabbits.

Abbreviations

IL-1: Interleukin-1
IL-6: Interleukin-6
TNF-α: Tumor Necrosis Factor Alpha
IFN-γ: Interferon Gamma
IL-10: Interleukin-10
SOD: Superoxide Dismutase
CAT: Catalase
GPx: Glutathione Peroxidase
MDA: Malondialdehyde
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
RBC: Red Blood Cells
WBC: White Blood Cells
FCR: Feed Conversion Ratio
Moringa: Moringa oleifera

Conflict of Interest

The author declare that they have no conflict of interest.

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