Assessment of the Influence of Methanolic Extract of *Moringa Oleifera* Leaves on Serum Biochemical Parameters in Japanese Quails

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Abstract

This study delved into the effects of *Moringa Oleifera* on glucose, cholesterol, and diabetic states in Japanese quails. A total of 195 Japanese quails were meticulously chosen for this investigation. The subjects were divided into three dosage groups (1 mg, 2 mg, and 3 mg) along with a group induced with diabetes through a 45 mg/kg alloxan injection, all subjected to comprehensive analysis. The methanolic extract of *Moringa Oleifera* showed a significant increase in serum glucose levels in all additive groups, with the highest increase observed in group C (3mg) compared to other treated and control groups. Conversely, the extract led to a decrease in serum cholesterol levels in all additive groups, with the most notable decrease (112.40) observed in group C (3mg) compared to other treated and control groups. Additionally, the extract demonstrated a decrease in serum glucose levels in diabetic-induced groups, with the most significant decrease recorded in Japanese quails in group C. The comprehensive investigation into the effects of *Moringa oleifera* on glucose, cholesterol, and diabetic quails, backed by rigorous statistical analyses and pertinent literature, strengthens its promise as a dietary supplement for metabolic health. The nuanced responses observed in Japanese quails have contributed valuable insights to the expanding body of evidence supporting the health benefits of *Moringa oleifera*.

Keywords: Blood Serum, Cholesterol, Glucose, *Moringa oleifera*, Japanese quails.

I. INTRODUCTION

Poultry feed has traditionally included conventional synthetic feed additives such growth promoters, antibiotics, antioxidants, antiparasitics, and antifungal agents [1]. Their use, however, poses serious hazards to the public's health because it raises questions about antibiotic resistance in consumers and residues in animal products [2]. As a result, the European Union outlawed the addition of antibiotics to animal feed as growth promoters. This change in regulation has sparked innovation in the manufacturing of animal feed and given rise to substitute additives like phytogenics. The use of herbs and their metabolites, or "bioactive substances," as feed additives has gained popularity [3]. These substances, which include flavonoids, carotenoids, and herbal oils, have shown promise in improving the productivity and health of animals and guaranteeing the safe and wholesome goods that are produced [4]. Their main purpose is to optimize nutrition metabolism by boosting pancreatic efficiency and modifying gut flora. Long-term health advantages are provided by medicinal plants, which are abundant in phytochemicals and bioactive substances such proteins, alkaloids, vitamins, and trace metal ions [5]. When plant-based chemicals are used instead of antibiotics, consumers feel more confident [6]. Herbs have been shown in numerous studies to improve livestock health and metabolic

functions, including feed consumption, utilization, and carcass characteristics, as well as the activity of digestive enzymes [1, 2, 5]. However, studies on the impact of certain additives, such as cocoa husks, savory, oregano, and Nigella sativa L., on broiler development characteristics have yielded conflicting results. Renowned for its anti-inflammatory and antioxidant characteristics, *Moringa Oleifera* has become a viable addition to feed. It has been demonstrated that administering leaf extracts of *Moringa Oleifera* inhibits the growth of pathogenic bacteria while boosting antioxidant activity, boosting immunological response, and improving broiler performance [6, 7]. Its potential as a natural feed supplement is highlighted by the health advantages of moringa, which contain essential vitamins, minerals, and strong antibacterial components. Research suggests that supplementing poultry with *Moringa Oleifera* can enhance feed conversion ratio, strengthen immune system, and lower plasma cholesterol and abdomen fat [8]. Research on the bioactive components of *Moringa Oleifera* leaves and their effect on the antioxidant status of meat is still lacking, despite these encouraging results [5, 7, 8, 9]. Thus, the purpose of this study is to assess the effects of methanolic extract of *Moringa Oleifera* on serum biochemical parameters in Japanese Quails.

II. MATERIALS AND METHODS

This research was conducted at Experimental poultrt farm, Faculty of Veterinary and Animal Sciences (FVAS) in Quaid-e-A zam Campus Gomal University, Dera Ismail Khan.

Preparation of Moringa Oleifera Extraction

The extraction process of Moringa Oleifera was meticulously conducted to ensure optimal results: First, 300 grams of Moringa Oleifera were accurately measured and placed into a beaker. Subsequently, 1000 ml of methanol was carefully added to the beaker, completely submerging the plant material. To maintain the integrity of the process, the beaker was securely sealed, preventing the escape of methanol vapor, and left undisturbed for a week to facilitate thorough extraction. After the designated period, the liquid extract was separated from the solid plant material residue using a filtration process employing filter paper. The resulting filtrate was then transferred into a round bottom flask and subjected to a Rota vapour (rotary evaporator) to eliminate the solvent (methanol) through evaporation, thus concentrating the extract. Throughout this process, the extract gradually transformed into a thicker, more concentrated form, resembling a dense paste. To further enhance the drying process, the concentrated extract was transferred into a wide beaker, allowing for increased surface area exposure. Subsequently, the beaker containing the extract was positioned under a fan or in a well-ventilated area to facilitate the removal of any residual methanol, ensuring complete drying. This meticulous approach resulted in the production of a dry, potent *Moringa Oleifera* extract ready for further analysis and application.

Serum Collection

The collection of serum samples from a cohort of Japanese quails was conducted with meticulous attention to detail. Special care was taken to prevent coagulation by utilizing heparin tubes, a critical step to maintain the biochemical composition and integrity of the serum for subsequent analytical processes. This precaution ensured the reliability and accuracy of the collected samples for further analysis.

Serum Analysis

In order to thoroughly evaluate the impact of the *Moringa Oleifera* extract on the avian subjects, serum levels of glucose and cholesterol were meticulously analyzed at two pivotal time points: prior to and post-extract administration. This comprehensive approach facilitated a nuanced understanding of the extract's potential effects on avian physiology. cholesterol, and glucose utilizing commercial test kits provided by Idexx laboratories in accordance with the manufacturer's authorized technique, as performed by the high-precision Chemistry Analyzer (AMP Piccos II). This state-of-the-art instrument ensured precise measurements by analyzing samples at distinct wavelengths (505 nm for cholesterol and 546 nm for glucose), thereby guaranteeing accurate and reliable results.

Statistical Analysis

The results were tabulated after analyzing the data statistically using SPSS (Statistical Package for Social Sciences) software (version 15.0). One-way ANOVA (Analysis of variance) and Duncan multiple range Tests were applied on the data for the assessment of variance and significant difference.

III. RESULTS

The methanolic extract of *Moringa Oleifera* showed a significant increase in serum glucose levels in all additive groups, with the highest increase observed in group C (3mg) compared to other treated and control groups (Table I). Conversely, the extract led to a decrease in serum cholesterol levels in all additive groups, with the most notable decrease (112.40) observed in group C (3mg) compared to other treated and control groups (Table II). Additionally, the extract

demonstrated a decrease in serum glucose levels in diabetic-induced groups, with the most significant decrease recorded in Japanese quails in group C (Table III).

Table I: Effect of Methanolic extract of *Moringa Oleifera* on serum Glucose level of Japanese quails

S.No.	Day	Methanolic extract of *Moringa Oleifera*			Control
		Group A (1 mg)	Group B (2 mg)	Group C (3 mg)	
1	22nd day	164.23±5.05[a]	221.10±4.90[b]	261.16±5.86[c]	280±40[c]
2	26th day	291.6±4.17[a,b]	316.96±6.81[b]	310.00±7.54[b]	267.00±35.59[a]
3	30th day	328.13±6.61[b]	348.16±6.15[b]	352.06±5.00[b]	278.36±35.00[a]
4	36th day	309.66±8.08[a]	330.33±8.50[b]	339.73±9.60[b]	301.66±12.53[a]
5	40th day	328.46±10.29[b]	343.80±15.06[b]	351.46±15.16[b]	256.33±40.07[a]

Note: The values presented in parentheses represent the mean along with the standard deviation.
If different superscripts appear in the same row alongside values in parentheses, it signifies that the results are significantly distinct from each other

Table II. Effect of Methanolic extract of *Moringa Oleifera* on serum Cholesterol level of Japanese quails

S.No.	Day	Methanolic extract of *Moringa Oleifera*			Control
		Group A (1 mg)	Group B (2 mg)	Group C (3 mg)	
1	22nd day	81.00±6.00[a]	86.00±6.0[b,c]	88.66±3.51[b,c]	95.06±5.00[b]
2	26th day	95.60±4.40[a,b]	102.80±2.89[b]	104.16±5.05[b]	86.00±11.53[a]
3	30th day	122.06±4.60[b]	117.36±5.06[b]	108.06±6.10[b]	88.40±17.56[a]
4	36th day	141.83±4.08[c]	124.13±5.95[b,c]	112.40±6.06[b]	85.16±23.37[a]
5	40th day	141.83±4.08[c]	124.13±5.95[b,c]	112.40±6.06[b]	85.16±23.37[a]

Note: The values presented in parentheses represent the mean along with the standard deviation.
If different superscripts appear in the same row alongside values in parentheses, it signifies that the results are significantly distinct from each other.

Table III. Effect of Methanolic Extract of *Moringa Oleifera* on serum Glucose level of Diabetic (induced) Japanese quails

S.No.	Day	Methanolic Extract of *Moringa Oleifera*			(−) Control	(+) Control
		Group A (1 mg)	Group B (2 mg)	Group C (3 mg)		
1	50th day	285.00±8.54[b]	350.33±12.22[a,c]	329.00±46.77[b,c]	386.66±15.27[a]	290.00±30.00[b]
2	51th day	305.66±5.50[b,c]	357.66±8.08[a,c]	337.00±42.50[c,d]	386.66±15.27[a]	290.00±34.53[b]
3	52nd day	335.66±9.01[c]	381.00±13.45[a]	350.66±28.74[a,c]	386.66±15.27[a]	290.00±32.02[b]
4	53rd day	284.00±8.54[a]	349.33±12.22[b]	328.00±46.77[a,b]	284.00±40.00[a]	351.66±20.00[b]
5	54th day	278.66±4.04[a]	347.00±12.12[b]	324.66±46.19[a,b]	278.66±39.00[a]	345.33±20.50[b]

Note: The values presented in parentheses represent the mean along with the standard deviation.
If different superscripts appear in the same row alongside values in parentheses, it signifies that the results are significantly distinct from each other

IV. DISCUSSION

Certain plants utilized in herbalism, known as medicinal plants, are valued for their therapeutic benefits due to their possession of medicinal properties. These plants contain bioactive components or compounds that contribute to their therapeutic qualities. These bioactive elements can be found in various parts of the plant, including its components or the entire plant, making them valuable for medicinal applications [4]. Medicinal plants often serve as the primary healthcare resource in less developed nations, where access to modern medical facilities and pharmaceutical drugs may be limited [1]. Plants play a crucial role in both plant and human health, being the primary source of diverse medicines. Numerous herbs and plants are recognized for their medicinal and therapeutic properties [2]. The dosage form, such as tablets or capsules, is considered the most efficient and reliable tool for treatment. Many tablets or capsules consumed daily are derived from plant-based substances, including Aspirin, digoxin, paclitaxel, and numerous other drugs [3]. Furthermore, in comparison to the control group, all dietary treatments, including *Moringa Oleifera* leaves, *Moringa Oleifera* seeds, and MSL, significantly reduced both triglycerides and cholesterol levels. However, no dietary intervention had any effect on ALT, albumin, total protein, globulin, or the albumin/globulin ratio (A/G). Additionally, albumin (ALB) and urea (UA) concentrations in laying hens in the *Moringa Oleifera* leaves group were significantly lower ($p < 0.05$) than in the control group [9]. The aim of this study was to assess the impact of balsam apple leaf extract on the blood glucose levels and hematological markers of Japanese quails. A significant decrease in blood glucose levels was observed, which may be attributed to the alpha-glucosidase inhibitory activity of the balsam apple leaf extract. Alpha-glucosidase inhibitors are preferred medications for type 2 diabetes mellitus as they impede carbohydrate digestion, reducing the impact of carbohydrates on blood sugar levels [9]. The current findings are consistent with previous studies [10] and [11], which reported that all doses (2%, 3%, 4%, 5%, and 6%) of drumstick leaf meal significantly increased hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), and total leukocyte count (TLC) in birds compared to the control group ($P < 0.05$). The increase in TLC suggests improved immunity, possibly due to the immunomodulatory properties of moringa leaf meal. However, [3] found no significant differences in hemoglobin levels among treatments for broiler chicks fed 0%, 0.5%, 1.0%, and 5.0% *Moringa Oleifera* leaf meal (MOLM). Similarly, [12, 13] and [14] observed no significant differences in Hb, PCV, or RBCs among treatment groups of broiler chicks fed MOLM. Additionally, [13, 15, 16] reported a significant reduction in the heterophil to lymphocyte (H/L) ratio

with MOLM supplementation.

V. CONCLUSION

The comprehensive investigation into the effects of Moringa oleifera on glucose, cholesterol, and diabetic quails, backed by rigorous statistical analyses and pertinent literature, strengthens its promise as a dietary supplement for metabolic health. The nuanced responses observed in Japanese quails have contributed valuable insights to the expanding body of evidence supporting the health benefits of Moringa oleifera.

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