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Original article

Inorganic Mineral Dynamics in Two Medicinal Plants and Their Influence on Body Weight Gain in Experimental Rabbits

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Abstract
This study compares the macro-mineral composition of *Moringa oleifera* and *Ziziphus spina-christi* leaf extracts and evaluates how their inorganic profiles relate to physiological outcomes in rabbits. The elemental analysis revealed marked differences between the two species. *Moringa oleifera* showed exceptionally high Calcium levels (68302 ± 9689.1 ppm), nearly double those of *Ziziphus spina-christi*, suggesting strong bio-accumulation of divalent cations within its tissues. In contrast, *Ziziphus spina-christi* exhibited significantly higher concentrations of Potassium (11272 ± 130.1 ppm) and Magnesium (7316 ± 747.4 ppm), with low variability in Potassium indicating a stable distribution pattern. Correlation analysis highlighted a strong negative relationship between Calcium and both Magnesium and Potassium, reflecting classical ionic antagonism between Ca^{2+} and other cations involved in metabolic and osmotic regulation. A moderate positive association between Potassium and Magnesium suggests coordinated accumulation in metabolically active tissues. Although the trends were chemically consistent, some correlations did not reach statistical significance due to the limited sample size. The independent samples t-test confirmed a significant difference only in Potassium levels ($p = 0.026$), indicating that *Ziziphus* is a superior source of this electrolyte. Differences in Magnesium and Calcium were not statistically significant, although Calcium showed a marginal trend favoring *Moringa*. The unequal variance detected by the Brown–Forsythe test reflects the contrasting mineral accumulation patterns of the two plants. Physiologically, rabbits treated with *Moringa oleifera* exhibited the greatest increase in final body weight (2113 ± 159.81 g), likely linked to its high Calcium content and its structural role in supporting skeletal growth. *Ziziphus spina-christi* led to moderate weight gain (1815 ± 106.77 g), consistent with its higher Potassium and Magnesium levels, which primarily support metabolic and enzymatic functions rather than structural tissue development. Overall, the findings indicate that the distinct mineral signatures of the two botanical extracts influence both intracellular ionic balance and growth-related physiological responses.
Keywords. Moringa Oleifera, Ziziphus Spina-Christi, Mineral Bioaccumulation, Rabbit Physiology.

Introduction

The integration of medicinal plants into animal nutrition has gained significant momentum due to their rich profile of bioactive compounds and essential inorganic elements [1]. Among these, Moringa oleifera and Ziziphus spina-christi stand out as potent bio-reservoirs of macro and micro-minerals that play pivotal roles in metabolic pathways. In inorganic biochemistry, minerals such as Calcium (Ca), Magnesium (Mg), Potassium (K), and Iron (Fe) are not merely structural components but act as essential cofactors for numerous enzymatic reactions governing growth and homeostasis [2]. The precise quantification of these minerals is crucial for understanding their bioavailability and subsequent impact on animal physiology [3]. Unlike traditional methods, ICP-MS allows for a comprehensive inorganic fingerprinting of plant tissues, which is essential when correlating mineral intake with physiological outcomes [4].

In lagomorphs, particularly rabbits, the dietary intake of minerals directly influences growth dynamics and organometry. For instance, Calcium and Magnesium are vital for skeletal integrity and neuromuscular function, while Potassium regulates osmotic pressure and acid-base balance across visceral organs [5]. Iron, as the central atom in the heme group, is indispensable for oxygen transport and cellular respiration [6]. A deficiency or imbalance in these inorganic constituents can lead to significant alterations in body weight and the relative weight of vital organs such as the liver, kidneys, and heart. Despite the known benefits of *Moringa* and *Ziziphus*, there is a gap in the literature regarding the high-precision ICP-MS profiling of their mineral content in direct correlation with rabbit organometry [7]. This study aims to fill this gap by evaluating how the inorganic matrix of these plants modulates the physical development and internal organ health of rabbits.

Materials and Methods

Plant Material Collection and Preparation

Fresh leaves of *Moringa oleifera* collected from Samno, Sabha, Libya, and *Ziziphus spina-christi* collected from the Green Mountain region, Libya, were obtained in February 2025. The botanical identity of both species was authenticated at the Department of Botany, Omar Al-Mukhtar University. The leaves were gently washed with distilled water, shade-dried at room temperature for two weeks, and ground into a fine powder

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using an electric grinder. The powdered samples were stored in airtight containers at 4°C until further analysis.

Mineral Analysis

Elemental measurements were performed using a Thermo Scientific iCAP TQ ICP-MS to ensure precise mineral quantification. Sample preparation involved a microwave-assisted acid digestion process to achieve complete mineralization. Concentrations of essential minerals including calcium, magnesium, potassium, and iron, as well as trace and heavy metals such as zinc, cadmium, and lead, were determined. The instrument was operated in TQ-O_2 and SQ-KED modes to eliminate spectral interferences, and results were expressed in ppm.

Experimental Animals

Fifteen healthy male New Zealand White rabbits aged six months were used in this study. The animals were housed individually in stainless steel cages under controlled environmental conditions of 22–26°C with a 12-hour light/dark cycle, in compliance with US-EPA (2004) standards for animal care. They were randomly assigned into three experimental groups of five animals each. The control group received a standard basal diet and distilled water. The Moringa group was administered *M. oleifera* extract at a dose of 400 mg/kg body weight daily via oral gavage. The Sidr group received *Z. spina-christi* extract at a dose of 200 mg/kg body weight orally every other day. The experimental period lasted for twelve successive weeks, during which individual body weights were recorded weekly to monitor growth performance. At the end of the study, rabbits were fasted overnight and sacrificed by decapitation.

Statistical Analysis

Data were analyzed using ANOVA with GraphPad Prism 8 or Minitab version 17. Post-hoc comparisons were conducted using Tukey's or Duncan's Multiple Range Test to identify significant differences between groups. A p-value of less than 0.05 was considered statistically significant.

Results

The elemental analysis of the two botanical extracts reveals significant disparities in their macro-mineral architectures, specifically regarding alkaline earth metals (Group 2) and alkali metals (Group 1). *Moringa oleifera* exhibits a remarkably high sequestration of Calcium (68302 ±9689.1), nearly double that found in *Ziziphus spina-christi* (38753 ±9203.0). From an inorganic perspective, this suggests a robust bio-accumulation mechanism for divalent cations within the *Moringa* matrix, potentially stored as calcium oxalates or carbonates within the plant tissue. *Ziziphus spina-christi* demonstrates a superior concentration of Potassium (11272 ±130.1) compared to *Moringa* (9283 ±444.8).

The Magnesium levels are also notably higher in *Ziziphus* (7316 ±747.4) than in *Moringa* (6291 ±457.2). The low standard deviation in the Potassium levels of *Ziziphus* indicates a highly stable and uniform distribution of this electrolyte across the sampled material. The Comparative Distribution highlights that while *Moringa* is a superior source for structural minerals (Calcium), *Ziziphus* is more potent in metabolic and enzymatic co-factors (Magnesium and Potassium). The inverse relationship observed between Calcium and Potassium concentrations across these two species suggests different physiological strategies for maintaining ionic balance and osmotic pressure.

Table1. Descriptive Statistics of Mineral Composition for Moringa oleifera and Ziziphus spina-christi Extracts

Descriptive Statistics	Mean & Std.	Minimum	Maximum	Mean & Std.	Minimum	Maximum
	Moringa oleifera			**Ziziphus spina christi**		
Magnesium	6291± 457.2	5756	6826	7316±747.4	6787	7844
Calcium	68302 ±9689.1	61451	75153	38753±9203.0	32245	45260
Potassium	9283±444.8	8968	9597	11272 ±130.1	11180	11364

The Pearson correlation matrix and the associated heatmap provide critical insights into the geochemical and physiological interactions between the macro-minerals (Potassium, Magnesium, and Calcium) within these plant matrices. The most striking feature of the dataset is the very strong negative correlation between Calcium (Ca^{2+}) and Magnesium (Mg^{2+}). From an inorganic perspective, these two Group 2 alkaline earth metals often exhibit biological antagonism. Because they share similar chemical properties but differ in ionic radii (Mg^{2+}, approx 72 pm vs Ca^{2+}, approx 100 pm, they frequently compete for the same binding sites on transport proteins and cell wall carboxyl groups. The high r-value suggests that in these species, conditions favoring the sequestration of Calcium likely suppress the accumulation of Magnesium. There is a strong negative correlation between Calcium and Potassium (K^+). This illustrates the classical "cation balance" phenomenon in plant physiology, where the uptake of divalent cations like Ca^{2+} often occurs at the expense

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of monovalent cations like K^+. This inverse relationship is fundamental to maintaining the electrochemical potential across plant cell membranes.
In contrast to the relationships with Calcium, Potassium, and Magnesium, which show a moderate positive correlation. This suggests a degree of synergy in their accumulation, potentially indicating that these two minerals are co-transported or localized in similar metabolic tissues, such as the cytoplasm and chloroplasts, where they both play essential roles in enzymatic activation and photosynthesis. While the correlation coefficients (r) for Calcium-Magnesium and Calcium-Potassium are high, the p-values (0.071 and 0.160, respectively) exceed the traditional alpha = 0.05 threshold. This indicates that while the trends are biologically suggestive and chemically consistent, they should be interpreted as strong indicators rather than absolute certainties, likely due to a limited sample size (n) in the descriptive study. The correlation data reveal a competitive inorganic landscape where Calcium acts as an antagonist to both Magnesium and Potassium. This suggests that these plants employ distinct ionic regulation strategies, which is a vital consideration when formulating these extracts for specific therapeutic uses, such as addressing calcium deficiency versus providing electrolyte support."

Table 2. Pearson Correlation Matrix for Potassium, Magnesium, and Calcium Concentrations in Ziziphus spina-christi and Moringa oleifera

Pearson Correlation		Pearson's r	p
Potassium	Magnesium	0.596	.404
Potassium	Calcium	−0.840	.160
Magnesium	Calcium	−0.929	.071

The application of the Independent Samples T-Test and the resulting distribution profiles provide a rigorous basis for distinguishing the inorganic chemical signatures of *Moringa oleifera* and *Ziziphus spina-christi*. The statistical analysis reveals a significant difference in Potassium concentrations between the two species (t = -6.071, p = 0.026). From a chemical standpoint, the negative t-value confirms that *Ziziphus spina-christi* possesses a significantly higher concentration of this monovalent alkali metal compared to *Moringa oleifera*. The significance of this finding suggests that the biological mechanisms for K^+ uptake and storage are uniquely optimized in *Ziziphus spina-christi*, making it a statistically superior source for this specific electrolyte.
Although raw mean values suggested disparities, the t-test indicates that the differences for magnesium (p = 0.306) and calcium (p = 0.089) are not statistically significant at the conventional alpha = 0.05 level. For Calcium, the p-value of 0.089 suggests a "marginal trend" toward higher concentrations in *Moringa oleifera*, but it does not meet the threshold for scientific certainty. The lack of significance for Magnesium suggests that both plants may share similar biochemical requirements or environmental availability for this Group 2 metal.
It is critical to note the significant Brown-Forsythe test result ($p < 0.05$), which indicates a violation of the equal variance assumption. In inorganic analytical chemistry, this "heteroscedasticity" often arises when one plant species has a more diverse or "unstable" mineral accumulation pattern compared to the other, which is reflected in the wider density distribution curves.

Table 3. Independent Samples T-Test Results Comparing Mineral Concentrations Between Moringa oleifera and Ziziphus spina-christi

Minerals	T	Df	P
Potassium	−6.071	2	.026[a]
Magnesium	−1.362	2	.306[a]
Calcium	3.127	2	.089[a]

[a]Brown-Forsythe test is significant ($p < .05$), suggesting a violation of the equal variance assumption

The data presented in Table 4 illustrate a clear disparity in body weight gain and visualized in illustrate a clear disparity in body weight gain between the control and experimental groups. From a biochemical and inorganic perspective, these results can be interpreted as follows: The *Moringa oleifera* group exhibited the most significant increase in body weight (2113 ± 159.81 g) compared to both the control and *Ziziphus* groups. This pronounced growth likely correlates with the high Calcium (Ca^{2+}) content identified in *Moringa* (68302 ±9689.1 ppm), which acts as a critical structural macro-element.In skeletal development, calcium serves as a fundamental building block; the high concentration of this divalent cation likely supported superior bone mineralization and muscle-skeletal framework development, resulting in higher overall mass.
Rabbits treated with *Ziziphus spina-christi* showed a mean weight of 1815±106.77 g, an improvement over the control (1740 ± 19.09 g) but notably lower than the *Moringa* group. While *Ziziphus* is statistically superior in Potassium (K^+) (11272 ± 130.1 ppm), Potassium functions primarily as an intracellular electrolyte for osmotic balance rather than a structural tissue-building block. The higher standard deviation in this group

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(106.77 g) suggests a more varied individual biological response to the *Ziziphus* mineral matrix compared to the highly consistent control group. The comparative analysis suggests that the specific mineral "signature" of the plant extract directly influences physiological outcomes. The high Magnesium (Mg^{2+}) and Potassium (K^{+}) in *Ziziphus* likely prioritize metabolic efficiency and enzymatic cofactor availability. Conversely, the Calcium density in *Moringa* appears more effective for rapid physical mass accumulation in this experimental rabbit model.

Table 4. Comparative Effects of Z. spina-christi and M. oleifera Leaf Extracts on the Final Body Weight of Experimental Rabbits.

Body weight (g)	Mean & Std.
Control	1740 ± 19.09
Ziziphus spina Christi	1815 ± 106.77
Moringa oleifera	2113 ± 159.81

Discussion

The mineralogical characterization of *Moringa oleifera* and *Ziziphus spina-christi* demonstrates that each plant exhibits a distinct macro-mineral profile that aligns with its known physiological functions and ecological adaptations. The exceptionally high Calcium content observed in *Moringa oleifera* suggests a strong bio-accumulation capacity for divalent cations, likely facilitated by the formation of calcium oxalates or carbonates within specialized storage tissues [8]. This pattern agrees with earlier research indicating that *Moringa* species act as effective reservoirs for structural minerals that support cell wall rigidity and mechanical strength [9]. The superior Potassium and Magnesium concentrations in *Ziziphus spina-christi* reflect its metabolic orientation toward maintaining osmotic regulation and supporting enzymatic processes[10]. Potassium is the dominant intracellular cation responsible for stomatal regulation and turgor pressure, while Magnesium acts as the central atom in chlorophyll and an essential activator of numerous metabolic enzymes. The stable and narrow variability in Potassium measurements in *Ziziphus* indicates controlled uptake pathways that maintain ionic homeostasis across tissues, consistent with observations in other xerophytic species [11].

The correlation matrix revealed a striking antagonistic relationship between Calcium and both Potassium and Magnesium. This inverse association aligns with classical cation-competition mechanisms in plant physiology, where high uptake of Ca^{2+} restricts the transport of other cations due to shared transport routes or competing electrochemical gradients. The strong negative correlation between Ca^{2+} and Mg^{2+} is especially expected because these Group 2 elements share similar charge but differ in ionic radius, contributing to competitive exclusion at membrane channels and cell wall binding sites [12]. The moderate positive correlation between Potassium and Magnesium suggests co-localization within metabolically active tissues, where both ions serve as essential partners in ATP-dependent reactions, chloroplast functioning, and protein synthesis. Such synergistic behavior is reported in plants experiencing balanced nutrient availability, where K^{+} supports cytosolic charge balance while Mg^{2+} activates ribosomal and enzymatic processes [13].

The body weight outcomes further reinforce the functional impact of the mineral signatures. Rabbits receiving *Moringa oleifera* demonstrated the greatest weight gain, likely due to its high Calcium density that supports skeletal growth and tissue development. Calcium is essential for bone mineralization and metabolic signaling, and diets enriched with Ca^{2+} have been shown to stimulate growth performance in animal models [14-25]. Although *Ziziphus spina-christi* improved weight relative to the control group, its mineral profile, dominated by Potassium and Magnesium, supports metabolic efficiency rather than structural mass increase [26-40]. Potassium enhances cellular hydration and metabolic turnover, but it does not contribute directly to bone tissue formation, explaining the lower overall weight gain compared to the *Moringa* group [41-49].

Conclusion

The comparative analysis of *Moringa oleifera* and *Ziziphus spina-christi* demonstrates that each plant possesses a distinct mineral signature that directly influences physiological outcomes in rabbits. *Moringa* showed superior Calcium bioaccumulation, supporting greater structural growth, while *Ziziphus* excelled in Potassium and Magnesium, enhancing metabolic balance. These findings highlight the complementary nutritional roles of both plants and their potential applications in targeted therapeutic and dietary formulations.

Conflict of interest. Nil

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