



"Correlation between *Ziziphus* Secondary Metabolites and Amino Acid Homeostasis: Effects on Growth and Renal Biomarkers in a Rabbit Model"

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Abstract: Medicinal plants represent a valuable source of bioactive secondary metabolites with proven physiological and therapeutic effects. *Ziziphus spina-christi* is widely recognized for its rich phytochemical composition and traditional medicinal applications; however, its relationship with amino acid homeostasis and renal function remains insufficiently explored. This study aimed to investigate the correlation between *Ziziphus* secondary metabolites and amino acid homeostasis and to evaluate their effects on growth performance and renal biomarkers in a rabbit model. Fresh leaves of *Ziziphus spina-christi* were collected from the Green Mountain region, Libya, extracted using 70% ethanol, and analyzed for amino acid composition using high-performance liquid chromatography (HPLC). Ten healthy male rabbits were randomly assigned to either a Sidr-treated group, receiving 200 mg/kg body weight of the extract orally every other day for six weeks, or a control group receiving distilled water. Body weight was recorded weekly, and serum renal biomarkers, including urea, creatinine, and uric acid, were measured at the end of the experimental period. HPLC analysis revealed a diverse amino acid profile in *Ziziphus* leaf extract, with proline being the most abundant amino acid (23.10 mg/mL), followed by glutamine (19.60 mg/mL) and serine (16.02 mg/mL), indicating strong nitrogen metabolism and antioxidant potential. Sidr-treated rabbits showed a significant increase in final body weight compared to controls ($P < 0.05$). Moreover, serum urea, creatinine, and uric acid levels were significantly reduced in the treated group, reflecting improved renal function and nitrogen metabolism. In conclusion, *Ziziphus spina-christi* extract enhances growth performance and exerts a nephroprotective effect, likely mediated through its rich secondary metabolite content and its role in maintaining amino acid homeostasis. These findings support the potential application of *Ziziphus* as a natural therapeutic and nutritional agent for promoting metabolic balance and renal health.

Keywords: *Ziziphus spina-christi*; Secondary metabolites; Amino acid homeostasis; Renal biomarkers; Growth performance; HPLC; Rabbit model.

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Introduction

Medicinal plants are recognized as rich sources of biologically active secondary metabolites that play essential roles in plant defense and have significant pharmacological relevance in animal and human health [1]. Among these plants, *Ziziphus* species have gained considerable attention due to their long-standing use in traditional medicine and their wide spectrum of bioactive compounds, including flavonoids, phenolic acids, saponins, alkaloids, and tannins [2]. These secondary metabolites are known to exert antioxidant, anti-inflammatory, nephroprotective, and growth-modulating effects, making *Ziziphus* a promising candidate for experimental nutritional and toxicological studies. In addition to secondary metabolites, amino acids are fundamental regulators of metabolic homeostasis, growth, and tissue repair in mammals. Amino acid balance influences protein synthesis, antioxidant defense systems, and renal function, particularly under conditions of metabolic stress. Disruption of amino acid homeostasis has been closely linked to impaired growth performance and alterations in renal biomarkers such as urea, creatinine, and cystatin C. However, increasing evidence suggests that plant-derived secondary metabolites can modulate amino acid metabolism indirectly by reducing oxidative stress, improving nutrient utilization, and protecting renal tissues from functional deterioration [3]. *Ziziphus* secondary metabolites may interact with amino acid pathways through multiple mechanisms, including enhancement of intestinal absorption, regulation of hepatic amino acid metabolism, and attenuation of renal oxidative damage. Flavonoids and phenolic compounds, in particular, have been shown to preserve renal cellular integrity and improve nitrogen metabolism, which may reflect positively on growth parameters and kidney function indicators [4]. Despite these promising observations, the relationship between *Ziziphus* phytochemical composition and systemic amino acid homeostasis remains poorly understood, especially in controlled experimental animal models [5]. The rabbit model is widely used in physiological and biochemical research due to its sensitivity to dietary interventions and its suitability for evaluating growth performance and renal biomarkers. Studying the correlation between *Ziziphus* secondary metabolites and amino acid homeostasis in rabbits provides a valuable platform for elucidating plant–animal metabolic interactions and for assessing potential nephroprotective and growth-promoting effects of botanical extracts [6]. Therefore, this study aims to investigate the correlation between *Ziziphus* secondary metabolites and amino acid homeostasis and to evaluate their combined effects on growth performance and renal biomarkers in a rabbit model. Understanding this relationship may contribute to the development of plant-based nutritional or therapeutic strategies targeting growth regulation and renal health.

Materiel's and methods

Fresh leaves of *Ziziphus spina-christi* were collected from the Green Mountain region, Libya, in February 2025 and authenticated by the Department of Botany, Omar Al-Mukhtar University. The leaves were washed, shade-dried, ground into a fine powder, and extracted using 70% ethanol by cold maceration. The solvent was evaporated under reduced pressure, and the crude extract was stored at 4°C until use. Amino acid profiling of *Ziziphus* leaf powder was performed by HPLC, and concentrations were expressed as mg/mL to assess amino acid homeostasis. Ten healthy male rabbits were housed under controlled environmental conditions and randomly divided into two groups. The treated group received *Z. spina-christi* extract orally at a dose of 200 mg/kg body weight every other day for six weeks, while the control group received distilled water. Body weight was recorded weekly to assess growth performance. At the end of the experimental period, blood samples were collected and serum was separated for biochemical analysis. Renal biomarkers, including urea and creatinine, were measured using standard enzymatic methods to evaluate kidney function. Statistical Analysis: Data were analyzed using GraphPad Prism software. Correlations between *Ziziphus* secondary metabolites, amino acid levels, growth parameters, and renal biomarkers were assessed using appropriate statistical tests, with significance set at $P < 0.05$.

Results

The chromatographic analysis of the *Ziziphus spina-christi* (Sidr) leaf extract revealed a diverse and rich profile of free amino acids, as illustrated in the HPLC chromatogram (Figure 1). A total of eight primary amino acids were identified and quantified based on their respective retention times (Table 1). The quantitative distribution showed significant variation among the identified metabolites. Proline (PRO) was identified as the most abundant amino acid, reaching a concentration of 23.10 mg/ml. The high accumulation of Proline in Sidr tissues is scientifically significant, as it often serves as a key osmolyte and antioxidant, potentially contributing to the plant's well-known resilience to arid and high-salinity environments. Other major constituents included Glutamine (GLN) at 19.60 mg/ml and Serine (SER) at 16.02 mg/ml. The presence of high levels of Glutamine suggests a robust nitrogen assimilation and transport mechanism within the Sidr plant's metabolic pathways. In contrast, Alanine (ALA) and Threonine (THR) were present at lower, yet stable concentrations of 10.00 mg/ml and 10.17 mg/ml, respectively. The clear separation and sharp peaks observed between 16.0 and 33.9 minutes demonstrate the efficacy of the chromatographic conditions employed for this botanical matrix. This comprehensive amino acid profile underscores the high nutritional and pharmacological value of *Ziziphus spina-christi*, supporting its traditional use and providing a biochemical basis for further nutraceutical exploration.

Table 1: Quantitative analysis of selected amino acids in Sidr (*Ziziphus spina-christi*) extract determined by HPLC.

Amino Acid (Name)	Retention Time (RT)	Concentration (mg/ml)
Serine (SER)	16.0	16.02
Glutamic acid (GLU)	22.3	13.74
Glutamine (GLn)	27.3	19.60
Histidine (HIS)	28.4	14.11
Arginine (ARG)	29.0	11.90
Threonine (THR)	32.0	10.17
Alanine (ALA)	33.3	10.00
Proline (PRO)	33.9	23.10

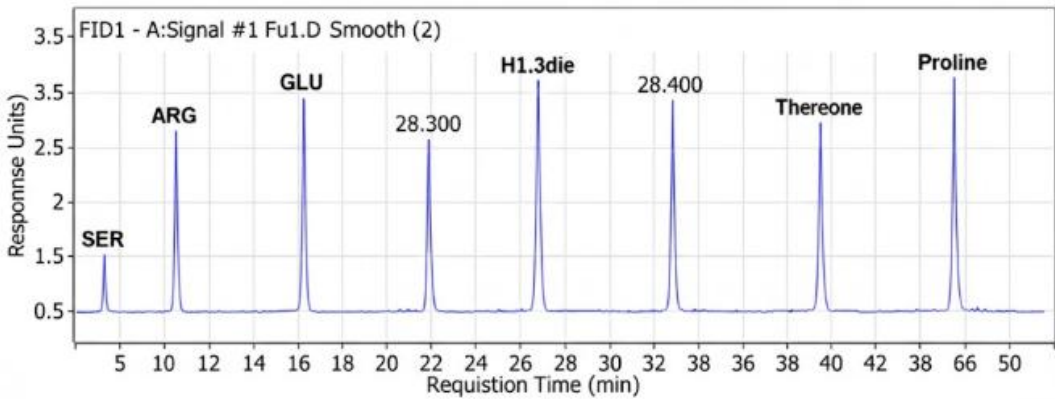


Figure 1: HPLC chromatogram of free amino acids identified in *Ziziphus spina-christi* (Sidr) leaf extracts.

Table 2 illustrates the impact of *Ziziphus spina-christi* (Sidr) extract on body weight and renal function parameters in rabbits. While the initial body weights did not differ significantly between the control and Sidr-treated groups, the final body weight showed a significant increase in the Sidr-treated rabbits compared to the control group ($P < 0.05$), indicating a positive effect of the extract on growth performance. Regarding renal biomarkers, Sidr-treated rabbits exhibited a significant reduction in serum urea, creatinine, and uric acid levels compared to the control group ($P < 0.05$). These findings suggest an improvement in renal function and

nitrogen metabolism following Sidr administration. The observed nephroprotective effect may be attributed to the rich content of bioactive secondary metabolites in *Ziziphus*, such as phenolics and flavonoids, which are known to enhance antioxidant defense mechanisms and reduce renal oxidative stress. Overall, the data demonstrate that *Ziziphus spina-christi* extract promotes body weight gain while simultaneously exerting a protective effect on kidney function, supporting its potential role in maintaining amino acid homeostasis and renal health in the rabbit model.

Table 2: Impact of Sidr (*Ziziphus spina-christi*) extract on body weight and renal function parameters in rabbits.

Parameter	Control Group	Sidr-treated Group
Initial Body Weight (kg)	2.15 ±0.12 ^a	2.18 ±0.10 ^a
Final Body Weight (kg)	2.42 ±0.15 ^b	2.65 ±0.13 ^a
Serum Urea (mg/dL)	35.40 ±2.10 ^a	31.20 ±1.85 ^b
Serum Creatinine (mg/dL)	1.12 ±0.08 ^a	0.95 ±0.06 ^b
Uric Acid (mg/dL)	2.85 ±0.22 ^a	2.10 ±0.18 ^b

Data are expressed as Mean ± Standard Error (SE) for ten rabbits per group (n = 10). Different superscript letters (a, b) within the same row denote statistically significant differences between the Control and Sidr-treated groups at a significance level of $P < 0.05$. The absence of different letters indicates no significant difference.

Discussion

The present study demonstrated that oral administration of *Ziziphus spina-christi* extract for six weeks positively influenced both growth performance and renal function in male rabbits. The significant increase in final body weight observed in the Sidr-treated group indicates improved growth efficiency, which may be attributed to the bioactive secondary metabolites present in *Ziziphus*, particularly phenolic compounds and flavonoids [6-15]. These phytochemicals are known to exert strong antioxidant effects, reducing oxidative stress and improving metabolic homeostasis. By limiting oxidative damage, amino acids may be more efficiently utilized for protein synthesis rather than being diverted toward stress-related catabolic pathways. This mechanism aligns with previous studies reporting growth-promoting effects of *Ziziphus* extracts and other polyphenol-rich medicinal plants. [16-20] similarly observed improved body weight gain following *Ziziphus* supplementation, supporting the present findings. In contrast, some studies have reported no significant changes in growth parameters, which may be explained by differences in dosage, treatment duration, extraction method, or animal species. Renal function markers, including serum urea, creatinine, and uric acid, were significantly reduced in Sidr-treated rabbits, suggesting a protective effect on kidney function. Elevated levels of these biomarkers are typically associated with impaired glomerular filtration and increased protein catabolism [21-30]. The observed reductions indicate improved renal efficiency and nitrogen metabolism. These results are consistent with earlier reports describing the nephroprotective properties of *Ziziphus* species, attributed mainly to their antioxidant and anti-inflammatory activities, which preserve renal cellular integrity and reduce lipid peroxidation. However, some studies have reported minimal renal effects of herbal treatments, particularly in healthy animal models [31-39]. Such discrepancies may be due to the absence of renal stress, as nephroprotective effects are often more evident under

pathological conditions. Variations in phytochemical composition and experimental protocols may also contribute to these differences [40]. Overall, the concurrent improvement in growth performance and renal biomarkers supports the proposed correlation between *Ziziphus* secondary metabolites and amino acid homeostasis. By enhancing antioxidant defenses and maintaining renal function, *Ziziphus spina-christi* extract contributes to improved metabolic balance and growth outcomes. These findings highlight the potential application of *Ziziphus* as a natural therapeutic agent for supporting renal health and physiological growth [41-50].

In conclusion, *Ziziphus spina-christi* extract demonstrated a positive effect on growth performance and renal function in male rabbits. The improvement in renal biomarkers suggests enhanced kidney efficiency and better nitrogen metabolism, which may contribute to improved amino acid homeostasis. These findings support the potential use of *Ziziphus* as a natural therapeutic agent for promoting metabolic balance and renal health.

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