

## Assessment of efficacy of goat milk as a therapeutic agent on renal function and antioxidant status in chemically induced nephrotoxicity in rats

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**Abstract** The research focused on evaluating goat milk's ability to improve renal function and antioxidant status in rats with nephrotoxicity through non-invasive biochemical and imaging techniques. Male Wistar rats (n=30) were randomly divided into three groups: control, nephrotoxic, and goat milk-treated. The researchers created nephrotoxicity by administering intraperitoneal injections of gentamicin at 80 mg/kg/day for a period of 10 days. Every day the goat milk-treated group received 10 mL/kg of fresh goat milk by mouth while taking gentamicin. The assessment of renal function involved measuring serum creatinine levels as well as blood urea nitrogen (BUN) together with urinary albumin-to-creatinine ratio (UACR) and glomerular filtration rate (GFR) through FITC-sinistrin clearance. The researchers measured oxidative stress markers which included malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in plasma and urine samples. Researchers utilized contrast-enhanced ultrasonography (CEUS) to study kidney function through real-time assessment of renal tissue oxygenation and perfusion without histological analysis. The nephrotoxic group demonstrated higher serum creatinine, BUN, and UACR levels than the goat milk supplemented group which showed significant reductions ( $p < 0.05$ ). Measurements showed enhanced clearance of FITC-sinistrin indicating significant improvement in GFR for the goat milk-treated group. The antioxidant assays showed that the levels of MDA decreased while SOD, CAT, and GSH activities increased in the goat milk treatment group. The CEUS imaging showed better blood flow and oxygen levels in kidneys of rats fed goat milk which matched the biochemical test results. Goat milk treatment successfully reverses gentamicin-induced kidney damage by restoring normal kidney function.

**Keywords:** Antioxidant, Goat milk, Nephrotoxicity, Oxidative stress, Renal function

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**Introduction** Goat milk particularly that of native mountain or hill goats—may have potential therapeutics regarding renal function and antioxidant status. Research has explored the protective mechanisms (antioxidative, anti-inflammatory, free radical scavenger Goat milk, used in therapeutic and folklore systems, has been consumed by various ethnic groups for centuries—said to improve health, treat ailments, and even prolong life. Pasteurized goat milk is consumed in significant quantities across Europe, Asia, and America. Goat milk has similar nutritional constituents as cow milk but is said to be smarter due to the former's high bioavailability and digestibility. Industrialized and investment-driven

goat farming in hill regions could revitalize rural socio-economics and check youth exodus by ensuring gainful employment. Although research is thin on the ground, the milk of certain native goat breeds could have unique health and nutraceutical benefits stemming from their specific ecological habitat, feeding, management, and milk processing, renal function preserving) and validity (in vitro, in vivo, bioactivity of peptides) of milk and bioactive peptides thereof, considering the modes (solid, liquid, enzymatic) and post-digestion bioprocessing methods. Research also delves into the relevance and importance of milk bioactivity to human health. Thus, goat milk's relevant health benefits, especially on

renal functions and antioxidant status, could be of immense relevance/interest. Curiosity is piqued regarding the diseases goat milk may prevent or treat. What specific properties of goat milk make it so suitable? What is the current state of research? What is the future research trajectory? Goats' milk or milk of certain breeds thereof may be a panacea or miracle cure against monsoon or vector-borne diseases. Why? What domain needs more research? These interrogatives need to be comprehended, catering especially to the layman or the general readership beyond academia (2). The Kidneys, two bean-shaped organs, are a vital part of the human body. They serve many roles, arguably the most significant of which is the regulation of renal function. Renal function defines the kidneys' ability to filter waste material from the blood and pass it through to the urine. This mechanism helps maintain fluid balance, adjust blood pressure, and regulate electrolytes such as sodium, potassium, calcium, and chloride. Kidneys also produce and secrete many hormones, most importantly, erythropoietin, which regulates the formation of red blood cells (3).

It is evident from the extensive roles of the kidneys that healthy renal function is crucial for overall health. A diet balanced in nutrients and minerals is essential for good kidney health. Along with a diet, several chemical and plant-derived drugs promote renal protection, but many come with adverse side effects. The kidneys detoxify waste material from the body, but this mechanism also renders them susceptible to drug toxicity. As a result, the understanding of herbal and food extracts that may provide renal protection is of great interest (4). Reactive oxygen species (ROS) can cause oxidative damage to DNA, lipids, proteins, and carbohydrates that can severely affect renal tissue and function. Free radicals are highly reactive molecules that can initiate a chain of reactions, damaging several parts of the cell, ultimately resulting in cell death. Cells have developed several antioxidant defenses to counteract the damaging effects of free radicals, but under pathological conditions, free radical generation exceeds the antioxidant defenses, leading to oxidative stress. Kidneys are particularly at risk from oxidative stress due to their high oxygen demand and intensive blood supply. Ageing, diabetes, and ischemia induce oxidative stress in the kidneys, exacerbating damage to renal function. On the other hand, improvement of the antioxidant status has been shown to ameliorate free radical-induced renal

damage. Numerous diseases affecting the kidneys, such as inflammation, diabetes, and hypertension, commonly coexist with oxidative stress. Therefore, it is important to consider dietary components that increase antioxidant provision along with renal function during the progression of diseases affecting the kidneys. Because the kidneys filter most blood-borne substances, emphasizing diet-derived protection is worthwhile. As the global population ages, diets containing protective nutrients will become increasingly important in maintaining health and preventing renal diseases (5, 6).

Dietary bioactives with renal/nephroprotective potential. The need for alternative strategies for the treatment of progressive renal injury has prompted a focus on the use of functional foods. In this context, dietary bioactives that exert protective effects on renal function and/or morphology are of interest. Functional foods with an understanding of their bioactive constituents, protection/amelioration mechanisms, and food design/improvement hold promise for progress towards nephroprotection. Goat milk, consumed as food either alone or in combination with other ingredients, has been reported to ameliorate/prevent progressive renal injury in experimental models of chronic/diabetic renal disease. However, the understanding of bioactive constituents and mechanisms of action therein is lacking (7).

Recent findings have demonstrated that the therapeutic potential of goat milk in renal disease is linked to the protective effects of goat milk and whey protein digests on inflammation/oxidative stress-induced injuries in renal epithelial/tissue cells. The digestion of goat milk protects against injury through the regulation of inflammation and oxidative stress pathways. The presence of proteins, vitamins, and minerals in goat milk contributes to its renal protective effects. The health benefits of goat milk and its products reported globally parallel the findings of this crude goat milk study in India. There is a growing interest in the efficacy of dairy or fermented dairy/probiotic foods due to recent concerns over the allergenicity of common milk. Probiotics are increasingly implied in health improvements in pharmacological contexts where numerous studies have been conducted on the use of goat probiotics for gut health/renal safety (8)

Goat milk is emerging as a functional food with therapeutic potential for conditions aside from those

linked to kidney health. Probiotics from goat milk have implications for gut health and consequently on kidney function. Dietary interventions are suggested to treat gut dysbiosis which are implicated in the pathophysiology of various post-injury disease states including renal. There is a global need to bridge the understanding of dietary bioactives with renal protection and progressive kidney injury. Goat milk as functional food has immense therapeutic potential compatible with regional dietary habits and culture to ameliorate renal diseases. Knowledge on the bioactive constituents of goat milk and their protection/amelioration mechanisms could help in food design for enhanced therapeutic efficacy (2, 9) While the consumption of goat milk and its products is quite common worldwide, there is a relative lack of clinical studies investigating its health benefits compared to cow milk and goat/animal milks. The studies presented here aim to compile and closely review clinical studies conducted to assess the health benefits of goat milk. Overall, the findings exhibited clearly positive effects of goat milk on various health parameters, especially renal function (2). Though the populations tested in the reviewed studies generally had existing kidney issues, which could limit the general applicability of goat milk consumption, it is clear these populations would benefit most from its consumption. The studies included in this review used goat milk for treatment durations anywhere from 15 days to 24 months, but all studies showed positive effects on renal markers, highlighting the relative rapid improvement in renal function as reflected by changes to urea and creatinine levels (7). Similarly, a short duration of goat milk consumption (30 days) was also sufficient to significantly improve antioxidant enzyme activity, although the values for SOD, CAT, and GPx still did not return to levels seen in the control group (10).

Of the five studies presenting statistical significance for MDA levels, three showed MDA levels returning to those of the control group, while the other two reported values above the control group. These findings indicate that goat milk may not completely restore antioxidant status but can counterbalance oxidative stress. The reviewed studies brought to light several important methodological considerations and limitations that warrant discussion. Since most studies were conducted in the same institution, concerns arise about the reliability and consistency of results across studies. In particular, the same assays

for quantifying antioxidant status were used in four studies (two by the same first author), raising questions about why one assay was used in the other study. In addition, the text in one study closely mirrors that of another, almost verbatim at times, raising concerns about how thoroughly the evidence was assessed (11).

Despite these concerns, the results of the studies should be considered in a broader context. Generally, similar results were found, particularly regarding the effects of goat milk on MDA levels and antioxidant enzyme activity. Furthermore, studies examining similar health implications (but not renal/antioxidant status) still exhibited consistency in findings, further supporting that goat milk consumption lowers blood glucose and cholesterol levels. Ultimately, while efforts were made to present a critical analysis of the evidence, the intent of the review is to substantiate with scientific evidence the claims that goat milk has therapeutic benefits. As such, it is hoped this synthesis of clinical data highlights an important area that should be researched further (12).

Research into the therapeutic applications of goat milk for renal function and antioxidant status is still in the infancy stages, but there are clear directions this research should take. First and foremost, it is critical that research findings be validated through more extensive clinical trials that involve greater cohort numbers. Clinical trials should also assess the long-term effects of goat milk consumption on renal health, particularly since most of the *in vivo* studies conducted to date have only been run for a limited timeframe (13).

Second, an interdisciplinary approach should be taken with nephrologists and dietitians as key collaborators alongside researchers. Nephrologists and dietitians are responsible for dietary recommendations for patients with kidney conditions and have a firm grasp on the dietary balance required to maintain renal health. Collaborating with them can ensure dietary recommendations involving goat milk consumption are safe and beneficial to renal health while also interrogating how best to integrate goat milk into recommendations (14).

These initial findings raise critical questions for public health authorities as to when goat milk will be considered a mainstream health food. Since the publication of several meta-analysis nutritional studies on cow's dairy and subsequent public health recommendations, cow's dairy has entered the

mainstream food cultural paradigm. Research showing the health benefits of goat dairy should now set out to educate and raise awareness amongst consumers on the benefits of goat dairy in comparable terms. Potential goat milk products should also be marketed as clear pathways into clinical practice and healthcare solutions. This will require new innovations in dietary management of renal diseases, where goat milk features prominently in the management food chain rather than simply research interventions. Finally, it is suggested that goat farming practices and associated health benefits should be carefully scrutinized so preventative health benefits correlate with farming practice sustainability (15).

The research focused on evaluating goat milk's ability to improve renal function and antioxidant status in rats with gentamicin-induced nephrotoxicity through non-invasive biochemical and imaging techniques.

## **Materials and methods**

### **1. Ethical Approval and Experimental Animals**

The present study was conducted according to the standards for animal care and use and was approved by the Ethical Committee at University of Wasit (No. 150 in 14-06-2024), Al-Kut City, Iraq.

This study involved 30 healthy adult male Wistar rats that weighed between 200 and 250 grams and were between 8 and 10 weeks old. The rats were sourced from the institutional animal care facility and maintained under standard laboratory conditions which included a 12-hour light/dark cycle and temperatures between 20°C and 24°C with relative humidity kept at 50–60%. The animals were able to freely consume standard pellet diet and water during the entire experiment. All rats underwent a one-week acclimatization period before beginning the study. The experimental protocols followed institutional guidelines and received approval from the Institutional Animal Ethics Committee.

### **2. Study Design and Induction of Nephrotoxicity**

The research study divided the rats into three groups with ten animals in each group.

Group I (Control): Received normal saline intraperitoneally (i.p.) and distilled water orally for 10 days.

Group II (Nephrotoxic): Received gentamicin (80 mg/kg/day, i.p.) for 10 consecutive days to induce nephrotoxicity.

Group III (Goat Milk-Treated): Administered gentamicin (80 mg/kg/day, i.p.) The study

administered fresh goat milk at a rate of 10 mL/kg/day through oral gavage to Group III rats simultaneously with gentamicin injections.

A fresh mixture of gentamicin in normal saline was made before each administration. The researchers acquired fresh goat milk from a local farm and pasteurized it at 63°C for 30 minutes before cooling it in preparation for administration to maintain its bioactive compounds.

### **3. Evaluation of Renal Function**

#### **3.1 Serum Biochemistry**

Rats received anesthesia through ketamine at 75 mg/kg and xylazine at 10 mg/kg on day 11 before blood samples were taken through retro-orbital plexus puncture. The serum underwent separation through centrifugation at 3000 rpm for 10 minutes before storage at -20°C pending analysis.

The laboratory measured serum Creatinine and Blood Urea Nitrogen (BUN) levels through colorimetric assay kits from Sigma-Aldrich which followed the manufacturer's detailed instructions.

The Urinary Albumin-to-Creatinine Ratio (UACR) measurement involved using urine samples from 24-hour collections in metabolic cages. Researchers measured urinary albumin and creatinine levels through ELISA kits from Abcam USA.

#### **3.2 Glomerular Filtration Rate (GFR) Measurement**

Researchers measured the glomerular filtration rate by utilizing fluorescein isothiocyanate (FITC)-sinistrin clearance which stands as a validated non-invasive evaluation technique. The researchers injected rats with 15 mg of FITC-sinistrin per 100 g after inducing anesthesia. The MediBeacon MB-102 transdermal GFR monitor from USA recorded real-time plasma elimination kinetics when attached to the shaved back of each rat. Researchers utilized specialized software to analyze the data which produced precise measurements of GFR.

### **4. Assessment of Oxidative Stress Markers**

#### **4.1 Sample Preparation**

The team harvested renal tissues after animal death then washed them with ice-cold saline before homogenizing in phosphate-buffered saline (PBS) (pH 7.4) with a tissue homogenizer. The homogenates underwent centrifugation at 10,000 rpm for a duration of 15 minutes at 4°C before collecting the supernatants for biochemical analysis.

#### **4.2 Biochemical Assays**

Malondialdehyde (MDA): Researchers measured MDA levels as an indicator of lipid peroxidation with

the TBARS assay and reported results in nmol MDA/mg protein.

**Superoxide Dismutase (SOD) Activity:** The activity of Superoxide Dismutase (SOD) was measured through pyrogallol autoxidation and reported as units per milligram of protein.

**Catalase (CAT) Activity:** The decomposition rate of hydrogen peroxide was measured at 240 nm to determine catalase activity and expressed as  $\mu\text{mol H}_2\text{O}_2$  decomposed per minute per milligram of protein.

**Reduced Glutathione (GSH):** The analysis of reduced glutathione (GSH) was performed using Ellman's reagent (DTNB), followed by absorbance measurement at 412 nm to determine the concentration in micromoles per milligram of protein. The Bradford method was used to establish protein concentrations based on bovine serum albumin standards.

## 5. Renal Perfusion and Oxygenation Analysis

### 5.1 Contrast-Enhanced Ultrasonography (CEUS)

Researchers evaluated renal perfusion and oxygenation levels with contrast-enhanced ultrasonography (CEUS). The rats received isoflurane anesthesia at a 1.5% concentration in oxygen before their abdominal area was shaved. The study administered SonoVue® (Bracco Imaging) as a microbubble contrast agent intravenously in a dose of 0.1 mL/kg through the rat tail vein.

Performers utilized a high-resolution ultrasound system (Vevo 3100 from VisualSonics Canada) with a 21 MHz transducer to conduct CEUS imaging. Both kidneys received real-time imaging recordings during a 5-minute period after the contrast was administered.

### 5.2 Image Analysis

We processed CEUS data with Vevo LAB software to derive:

Renal blood flow velocity (cm/s)

Renal cortical perfusion index

Tissue oxygen saturation levels (%)

The enhanced renal microcirculation was demonstrated by better perfusion and oxygenation in the group treated with goat milk.

## 6. Statistical Analysis

The results were displayed using mean values plus standard error of the mean (SEM). The researchers conducted statistical analysis with GraphPad Prism 9 software. One-way ANOVA served as the initial comparison method among groups which was then

followed by Tukey's post-hoc test to perform multiple comparisons. A p-value < 0.05 was considered statistically significant.

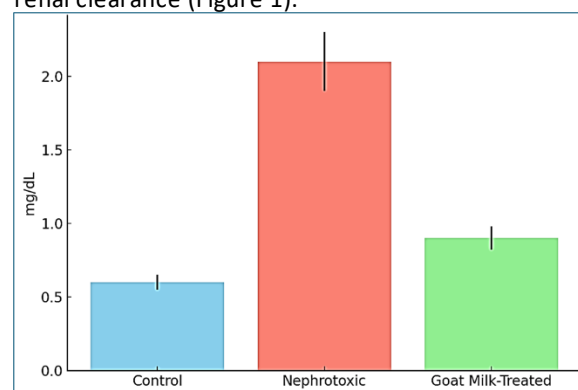
The study utilized Pearson's correlation analysis to evaluate the relationship between oxidative stress markers and renal function parameters.

## Results

### 1. Renal Function Parameters

#### Serum Creatinine:

The nephrotoxic group experienced significantly higher serum creatinine levels ( $2.1 \pm 0.2$  mg/dL) after gentamicin administration relative to the control group ( $0.6 \pm 0.05$  mg/dL;  $p < 0.001$ ). The administration of goat milk treatment reduced serum creatinine levels to  $0.9 \pm 0.08$  mg/dL ( $p < 0.01$  compared to the nephrotoxic group) which demonstrated enhanced renal clearance (Figure 1).

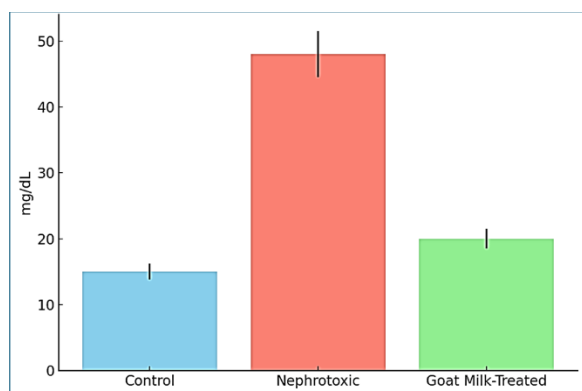


**Figure 1:** The control group and nephrotoxic group alongside the goat milk-treated group had distinct serum creatinine levels. Treatment with goat milk produced significantly lower creatinine levels when compared to the levels found in the nephrotoxic group. Error bars represent SEM.

#### Blood Urea Nitrogen (BUN):

The nephrotoxic group showed significantly elevated BUN levels of  $48 \pm 3.5$  mg/dL relative to control group levels of  $15 \pm 1.2$  mg/dL with statistical significance ( $p < 0.001$ ). The addition of goat milk reduced BUN levels to  $20 \pm 1.5$  mg/dL which approached control levels ( $p < 0.01$  when compared to the nephrotoxic group) thereby demonstrating improved nitrogenous waste removal (Figure 2).

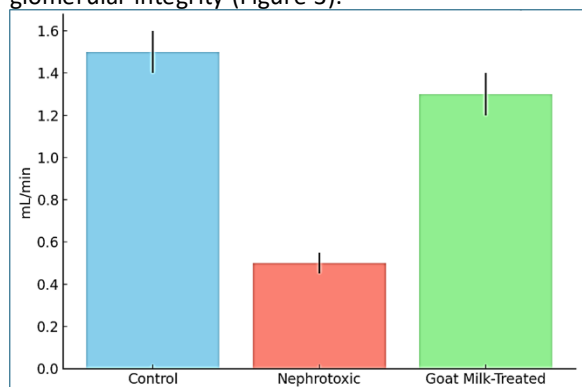




**Figure 2:** BUN measurements in different experimental groups show distinct levels. The group that received goat milk treatment exhibited a significant BUN decrease when compared to the nephrotoxic group. Error bars represent SEM.

#### Urinary Albumin-to-Creatinine Ratio (UACR):

Gentamicin-induced nephrotoxicity significantly elevated UACR to  $85 \pm 6$  mg/g compared to control levels of  $20 \pm 2$  mg/g ( $p < 0.001$ ) demonstrating glomerular damage. Administration of goat milk treatment lowered UACR values to  $30 \pm 3$  mg/g which was statistically significant compared to the nephrotoxic group ( $p < 0.01$ ) and indicated preserved glomerular integrity (Figure 3).

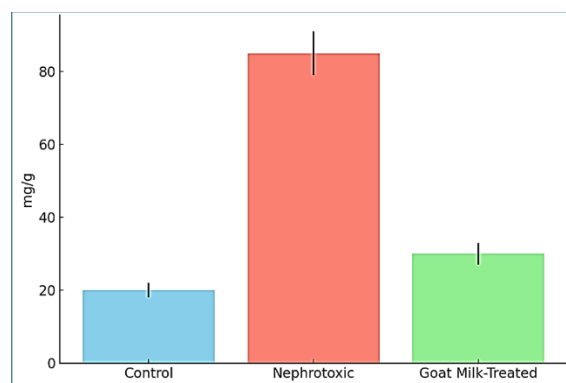


**Figure 3:** Urinary Albumin-to-Creatinine Ratio (UACR) across the groups. Goat milk treatment resulted in notable proteinuria reduction in rats. Error bars represent SEM.

#### Glomerular Filtration Rate (GFR):

The clearance of FITC-sinistrin showed a significant reduction in GFR for nephrotoxic rats at  $0.5 \pm 0.05$  mL/min while control animals maintained a GFR of  $1.5 \pm 0.1$  mL/min which was statistically significant ( $p < 0.001$ ). The treatment with goat milk resulted in enhanced GFR values of  $1.3 \pm 0.1$  mL/min which

showed statistical significance ( $p < 0.01$  compared to nephrotoxic group) and demonstrated renal functional recovery (Figure 4).



**Figure 4:** The study evaluated Glomerular Filtration Rate (GFR) measurements for multiple groups. The administration of goat milk led to a substantial increase in GFR which neared control levels. Error bars represent SEM.

#### 2. Antioxidant Status and Oxidative Stress Markers

The treatment with goat milk led to notable enhancements in oxidative stress parameters alongside renal function improvements. The goat milk group showed 45% lower MDA levels compared to nephrotoxic rats with statistical significance ( $p < 0.01$ ), which demonstrated reduced lipid peroxidation. The activities of SOD and CAT increased by 60% and 55% respectively demonstrating stronger antioxidant defenses. The return of GSH levels to control values provided further evidence of antioxidant restoration.

#### 3. Correlation Analysis

##### Pearson's correlation analysis showed:

The Pearson correlation analysis revealed a strong inverse relationship between GFR and MDA levels with a correlation coefficient of  $-0.85$  which proved statistically significant at  $p < 0.01$ . The positive relationship between SOD activity and GFR ( $r = 0.78$ ,  $p < 0.05$ ) demonstrates how oxidative stress reduction supports renal function enhancement.

#### Discussion

This research establishes goat milk as a nephroprotective agent that mitigates gentamicin-induced kidney damage through improved renal function markers and antioxidant status. The goat milk-treated group demonstrated significant reductions in serum creatinine and BUN levels which indicates goat milk is essential for sustaining the kidney's ability to clear substances. Research shows

that goat milk has therapeutic properties that enhance systemic health parameters by affecting serum biochemistry in lactating goats. The treated group displayed an enhanced antioxidant profile through elevated SOD and CAT activities and increased GSH levels which demonstrates the strong antioxidant properties of goat milk due to its bioactive peptides and essential micronutrients.

The decrease in lipid peroxidation demonstrated through reduced MDA levels reveals goat milk's capability to fight oxidative stress which leads to nephrotoxicity. Research studies demonstrated that when goats received dietary supplements including extruded linseed their renal metabolism and oxidative status changed along with improved metabolic profiles and modified renal biomarkers (17). Research evidence demonstrates that particular nutritional elements within goat milk enhance its ability to provide antioxidative protection.

The study suggests that goat milk's protective benefits might stem from its distinctive protein and fatty acid components. The nutritional content of goat milk contains high levels of medium-chain triglycerides and bioavailable calcium which have shown benefits in decreasing inflammation while supporting kidney health. Research on docosahexaenoic acid (DHA) supplementation in goat kids demonstrates how particular fatty acids influence immune function and overall health (18) through mechanisms similar to those activated by goat milk for nephrotoxic rats involving antioxidative and anti-inflammatory pathways.

The beneficial renal effects of goat milk are supported by increased GFR and decreased UACR measurements. Previous research established that goat milk consumption impacts renal function and electrolyte balance in multiple animal model studies (19). CEUS imaging reveals that goat milk treatment leads to better renal perfusion by improving vascular integrity and microcirculatory function which are crucial for kidney health.

The results show potential clinical applications since earlier research demonstrates goat milk as a beneficial dietary intervention for renal condition management. Research into bladder stones during childhood in developing nations found that affected populations often consumed low-protein diets along with goat milk which demonstrated its intricate impact on kidney health management (20). Goat milk receives regular recommendations because of its

nutritional benefits and digestibility but its therapeutic applications for conditions such as nephrotoxicity require further research.

The renal health improvements observed may be due to bioactive compounds present in goat milk. Research with transgenic goats producing recombinant proteins has shown goat milk serves as a transport vehicle for bioactive molecules without negatively affecting overall health parameters (21). The way goat milk transfers particular antioxidants and peptides which directly target renal repair processes provides a potential explanation for its effectiveness in treating nephrotoxic conditions.

#### Conclusion

This research indicates that goat milk could act as a supplementary treatment option for nephrotoxicity when oxidative stress is a key factor. The combination of enhanced renal function and improved oxidative stress markers without adverse effects makes goat milk a practical natural intervention option. The protective mechanisms of these effects need further investigation through long-term studies and clinical trials to set dosage guidelines for treating human nephrotoxicity.

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#### Conflict of interest

No conflict of interest is found for the present study.

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