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EFFECT OF GOAT'S MILK ON KIDNEY FUNCTION IN ALBINO RATS EXPOSED TO CADMIUM CHLORIDE AND LEAD POISONING

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ABSTRACT

The study aimed to know the effect of goat milk as a functional food to get rid of chemical toxins that laboratory animals are exposed to, the compound $CdCl_2$, and the element Pd, by examining creatinine, urea, and histological .This study was the use of 36 white male albino rats, which were randomly divided into six equal groups and the experience lasted six weeks, the control group (C) and the rest of the groups were injected with the compound $CdCl_2$ or the element Pd and dosed with goat milk in specific quantities to know the effect of milk on the levels of creatinine and urea in the kidneys, as well as their histological .The results were that the animals exposed to $CdCl_2$ and Pb poisoning, where Creatinine reached 0.4412, 0.4057 mg/dl, respectively, compared to the control treatment, which reached 0.2059 mg/dl, while the urea level reached 65.32, 66.68 mg/dl, respectively, compared to the control treatment, which reached 39.72 mg/dl—the protective role of goat milk in improving kidney function.

INTRODUCTION

Heavy metals that accumulate in the human body cause a lot of damage as they affect many major organs such as the liver, kidneys, heart, brain, etc. (Muzhda and Yahya, 2023). They cause oxidative stress in cells, which leads to an increase in the number of reactive oxygen species and a decrease in the level of antioxidants, as heavy metals interfere with natural biological mechanisms, leading to disruption of natural reactions and leads to cell death. Because these minerals are not biodegradable, they remain for a longer period and pose long-term health risks (Renu *et al*., 2021). They also inhibit some metabolic pathways and thus lead to a dysfunction of the immune system (Islamy *et al*., 2024). Therefore, many international organizations have set standards regarding the presence of minerals in the environment, food, and drinking water (Balali-Mood *et al.*, 2021). Goat milk has nutritional and functional properties and health-promoting benefits due to its content of biologically active components, especially peptides derived from its proteins, oligosaccharides, short and medium chain fatty acids, and phospholipids, which contribute to the treatment of some pathophysiological disorders (Siddiqui *et al*, 2023; Fahad, 2023). Goat milk also contains taurine, which affects detoxification and accumulation of fatty acids (Hidayet and Mustafa, 2021; Muntaha *et al*., 2023).

MATERIALS AND METHODS

Raw Materials used in the Study

Goat Milk

Local goat milk (Iraqi) was obtained from a farm in the Najaf desert in Najaf Governorate/Iraq. The milk was pasteurized by slow pasteurization at 62.8 °C for 30 minutes (Nayik et al., 2021), and the experimental animals were given 3.5 ml for 6 weeks. The milk was pasteurized and cooled before oral administration through a special tube. (Amr, *et al*., 2023)

Heavy metals

Two common heavy metals were selected in this study, namely cadmium chloride $CdCl_2$ and lead Pb. The required amount of the mentioned elements was weighed using a sensitive balance and then dissolved in deionized water (Hiba,2011). The required dose of $CdCl_2$ was prepared according to the method of (AL-Dleamy and Jawad,2022), and the dose of lead was determined according to the method of (Ahmed *et al*., 2013). Doses are fixed amounts per treatment.

Experimental Animals

This study included 36 white male albino rats. The average weight of the rats was (200-250) g. The animals were placed in controlled conditions in terms of proper ventilation and at a temperature ranging from (20- 25) ˚C, while the lighting was 12 hours of light/12 hours of darkness. The animals were randomly divided into six groups with six rats per group and were placed in plastic cages with a metal clip. The floor of the cages was covered with sawdust, with care taken to clean them and monitor the health status of the rats. All animals were provided with water and feed. The animals were given food, feed, and tap water freely throughout the experiment and according to the animals' needs. The experiment continued for 7 weeks, including a week of animal adaptation.

Experimental Treatments

The experimental animals were randomly divided into six groups with six rats per group:

1) Control group (C): fed feed and provided with tap water.
2) The second group (T1) was given pasteurized goat's milk at a dose of 3.5 ml daily for 6 weeks in addition to feed and tap water.
3) The third group (T2) was injected with 0.2 ml of cadmium chloride at a concentration of 1 mg/kg intraperitoneally in two doses every week, one dose in addition to feed and water.
4) The fourth group (T3) was injected with 0.2 ml of lead at a concentration of 25 mg/kg body weight intraperitoneally for 7 days and daily from the beginning of the experiment, in addition to feed and water.
5) The fifth group (T4) was injected with 0.2 ml of cadmium chloride at a concentration of 1 mg/kg body weight, injected intraperitoneally in two doses every week, one dose, and was given pasteurized goat milk at a dose of 3.5 ml for 6 weeks via a special tube.
6) The sixth group (T5). The lead element was injected in an amount of 0.2 ml at a concentration of 25 mg/kg of body weight into the peritoneum for 7 days on

a daily basis, and pasteurized goat milk was given in a dose of 3.5 ml for 6 weeks: animal sacrifice and sample collection.

At the end of the experiment, after 6 weeks, all animals were sacrificed. Rats were deprived of food for 8 hours and were anesthetized with Ketamine and Xylazine by intramuscular injection. The abdominal cavity was opened from the lower abdomen to the pharynx to obtain the organs used in the study (kidneys). The organs were washed immediately after the killing process and placed in a special box containing a number indicating the group to which they belonged. The box contained formalin at a concentration of 10% for preservation purposes, with the formalin in the box being replaced twice every 12 hours.

Estimating Creatinine in the Blood

Creatinine was estimated in the blood serum using the analysis kit prepared by the French company Biolabo, according to the method (Burtis and Ashwood,1999).

Estimating Urea in the Blood

The concentration of urea in the blood serum was estimated according to (Tietz,1995) using the analysis kit prepared by the German company Hannover.

Histological Tests Method

The method of Hilal (2020) was followed to make and prepare the histological sections of the kidneys for all animals for the purpose of studying them.

Statistical Analysis

The experimental data were analyzed using a completely randomized design (CRD). Rand completely used six treatments, and each treatment was applied to six laboratory animals. The means were compared using Duncan's Multiple Range test (Al-Rawi *et al*., 2000) at a probability level (0.05≥P). The data were analyzed using the statistical analysis program.

RESULTS AND DISCUSSION

Effect of Goat Milk, $CdCl_2$, and Pb on Kidney Functions: Creatinine and Urea

The results in Table (1) indicate a significant increase in Creatinine levels for treatments 2T, 3T that were poisoned with $CdCl_2$ and Pb, reaching 0.4412, 0.4057 mg/dl, respectively, compared to the control treatment, which reached 0.2059 mg/dl. A significant difference is noted for treatments T5, T4, which were poisoned with $CdCl_2$ and Pb and dosed with goat milk, reaching 0.3743, 0.3857 mg/dl compared to the control treatment and treatment T2. However, it is not significant with treatment T3. As noted in Table (1), there was a significant increase in Urea levels for treatments T2 and T3 that were poisoned with $CdCl_2$ and Pb, reaching 65.32 mg/dl and 66.68 mg/dl, respectively, compared to the control treatment, which reached 39.72 mg/dl. Significant differences were noted between treatments T5 and T4, which reached 55.65 and 58.50 mg/dl, respectively, compared to treatments that were poisoned with the same metals, T3 and T2. Still, they were not significant compared to the control treatment. The results show a decrease in creatinine and urea levels in the blood of rats that were poisoned with heavy metals and were given goat milk in treatments T4 and T5, which may be due to the direct effect of milk components calcium, magnesium, potassium, phosphorus and protein on kidney function, as regular consumption of dairy products protects against inflammation, oxidative stress and kidney endothelial dysfunction. Given other studies, it is noted that increased oxidative stress and inflammation are risk factors for kidney dysfunction (Jaffer *et al.*, 2023; Gopinath *et al.*, 2016).

Alharbi *et al.*, (2022) indicated a decrease in creatinine and urea levels in rats fed fermented camel and cow milk compared to the group fed a diet rich in cholesterol. Dadupanthi, & Bhargava (2021) found increased creatinine and urea levels in the blood of Swiss white mice exposed to chronic doses of cadmium. Helal *et al.* (2020) found that using a mixture of lead and cadmium chloride led to an increase in kidney levels.

Khamphaya *et al.*, (2022) found that urea levels increased significantly in animals exposed to lead acetate compared to the control group, while creatinine levels did not show significant differences, and that urea changes were more responsive than creatinine changes to the early stages of lead-induced kidney dysfunction. Khalil-Manesh et al. (1992) indicated that exposure to lead may cause nephropathy due to its harmful effect on kidney function and disease. Reddy *et al.* (2014) stated that lead poisoning leads to a change in the balance between oxidants and antioxidants through the generation of Reactive oxygen species (ROS). As a result of oxidative stress, protein degradation increases, leading to increased concentrations of ammonia and urea in the serum. (Khalid *et al.*, 2017).

Table (1): Effect of goat milk, $CdCl_2$, and Pb on levels of kidney function, Creatinine, and Urea in the blood of experimental animals the experience lasted six weeks.

*symbol	Urea (mg/dl)	Creatinine (mg/dl)
C	39.72 a	0.2059 a
T1	43.79 a	0.2410 a
T2	66.68 c	0.4412 c
T3	65.32 c	0.4057 bc
T4	55.65 ab	0.3857 b
T5	58.50 ab	0.3743 b

C= control (tap water), T1= goat's milk only, T2 = 0.2 ml of CdCl2, T3= 0.2 ml of lead
T4= 0.2 ml of CdCl2 + pasteurized goat milk, T5=0.2 ml of lead + + pasteurized goat milk
* Different letters within the same column indicate a significant difference between the averages.

4-2- Histological test results

Histological sections of the kidneys of experimental rats were stained using hematoxylin and eosin stains for the cortex and medulla regions of the kidneys of rats. The histological sections were photographed using a light microscope at a magnification of 100X. As shown in Figure (1). Histological tests of kidney sections in the control treatment C showed that the tissue composition of the cortex and medulla regions was normal, as the renal tubules (proximal and distal) appeared normal with a normal glomerulus surrounded by Bowman's capsule. Histological tests of kidney sections in the treatment group that received local goat milk T1 showed that the histological composition of the cortex and medulla regions was normal for this group. Rai *et al.* (2022) Goat milk has antimicrobial, anti-allergic, anti-inflammatory, and antioxidant properties that can affect immune and inflammatory processes and provide nutrients to the body.

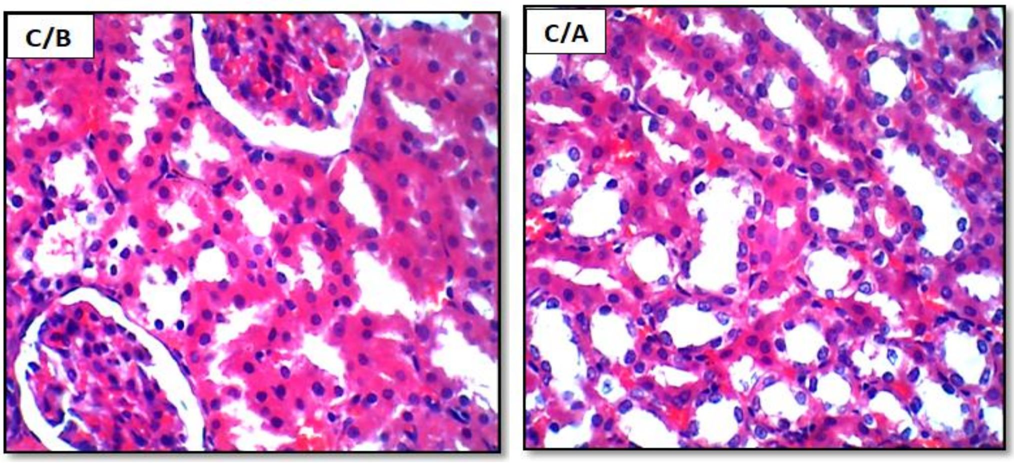
The low sugars found in goat milk and vitamins are useful in enhancing immunity and help maintain the physical and chemical properties of the body. Histological tests of the group of rats that were poisoned with cadmium chloride T2 showed necrosis of the proximal and distal renal tubules (black arrow) that formed spaces in the affected area with hemorrhage (yellow arrow) in the cortex area. Severe hemorrhage (black arrow) and proliferation of inflammatory cells (yellow arrow) were also observed in the medulla area. Mitochondria and NADPH oxidase are damaged by cadmium, which accumulates in the proximal tubular site of the nephrons and are therefore the main sources of reactive oxygen species. Therefore, the underlying mechanism of cadmium-induced renal toxicity is increased oxidative stress and associated damage to DNA, proteins, and lipids, which ultimately leads to cell death, kidney injury, and decreased renal function—cadmium and redox imbalance (Yan & Allen, 2021; Yousief *et al*., 2023). Hussen (2012) reported that cadmium exposure causes morphological and pathological changes and degeneration of tubular epithelial cells in the kidney. Genchi *et al*. (2020) reported that cadmium induces oxidative stress and the production of ROS, namely superoxide radicals, hydrogen peroxide, and hydroxyl radicals. Oxidative stress leads to the oxidation and damage of important biomolecules.

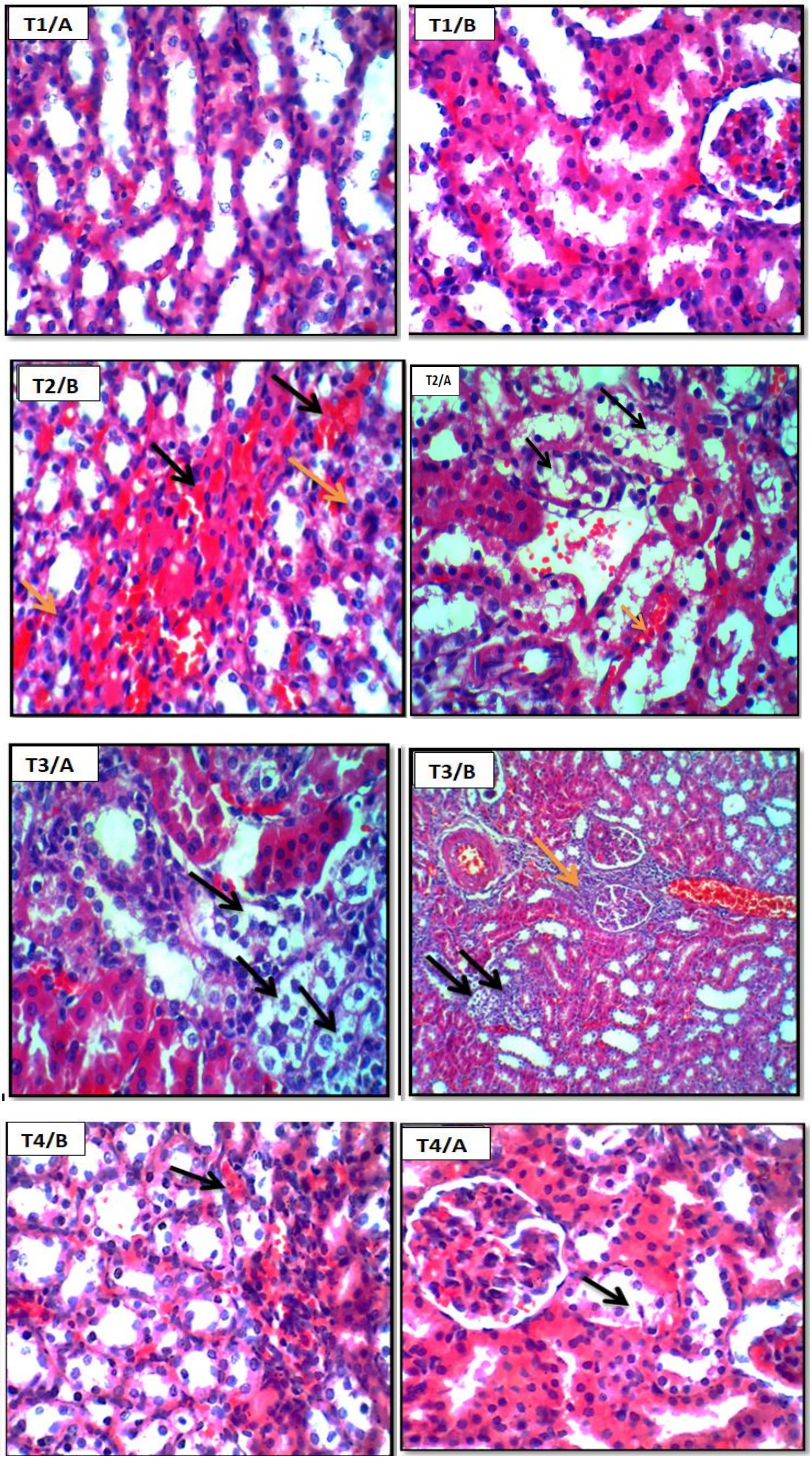
Histological tests of the T3 lead-poisoned rat group showed severe necrosis in the proximal and distal renal tubules and the proliferation of severe inflammatory cells (black arrow) between the renal tubules and around the glomerulus in the cortex area (Ilham, 2009). Acute inflammatory cells were also observed in clusters formed with hemorrhage (yellow arrow) in the renal medulla. Lentini *et al*. (2019) indicated that the kidney is a target organ in heavy metal poisoning due to its ability to reabsorb and concentrate divalent metals. Carocci *et al*. (2016) reported that lead binds to proteins in the tubular cells proximal to the glomeruli, and these lead-protein complexes are found as intracellular impurities characteristic of acute lead nephrotoxicity. Lead accumulates in the mitochondria of the kidney, leading to changes in both structure and function. Free radicals can also damage renal cells, disrupting their brush border epithelial linings and rendering the cells impermeable to urea and creatinine (Yuan *et al.,* 2014).

Histological sections of the liver of male rats exposed to cadmium chloride poisoning and fed T4 goat milk showed mild destruction of the epithelial cells (black arrow) lining the proximal tubules and individual renal tubules in the cortex. In the medulla, normal tissue cells with slight hemorrhage were observed. Shaikh *et al*. (1999) reported that oxidative stress has an important role in chronic hepatotoxicity and renal toxicity caused by cadmium, and that antioxidants protect the body from cadmium toxicity. Alharbi *et al*. (2022) reported that the decrease in creatinine and urea may be due to the ability of lactic acid and probiotic bacteria to control the change in lipid profile, leading to improvement of renal damage. Goat milk contains major and trace minerals, including Ca, Na, Mg, P, K, Mn, and Fe, which have significant health benefits. Goat milk contains a high percentage of zinc and acts as an antioxidant and eliminating reactive oxygen species. It also contains selenium and has an important effect as a cofactor for the work of glutathione peroxidase, which is an antioxidant enzyme that gets rid of harmful free radicals in the body and helps activate macrophages (Panta *et al*., 2023).

Histological sections of male rats that were poisoned with lead and were given T5 goat milk, where a slight spread of focal inflammatory cells (black arrow) and slight destruction of the epithelial cells lining the individual renal tubules in the cortex area were observed. Slight destruction of the epithelial cells (black arrow) of the loop of Henle's tubules with slight bleeding in the medulla area was also observed. (Raja, 2011; Nasser *et al*., 2021).

Rai *et al.* (2022) indicated that goat milk is useful for the formation of bile salts because it contains a higher concentration of the amino acid taurine. The potassium in goat milk helps maintain kidney function, and chloride helps maintain osmotic pressure (Kucukler *et al*., 2021). Also stated that calcium, Potassium, and copper significantly alleviate the kidney toxicity caused by lead acetate, PbAc. Goat milk contains a large amount of free amino acids, mainly found in goat milk, such as glycine, glutamic acid, and taurine found in goat milk have a significant effect on organs and detoxification, and accumulation of fatty acids (Chauhan *et al.*, 2021). Amin *et al*. (2020) explained that natural products containing flavonoids, vitamins, and other biologically active components reduce the harmful effects of lead toxicity due to their ability to reduce oxidative stress. These natural products have an important effect in reducing lead toxicity by suppressing oxidizing agents and enhancing antioxidant levels within damaged cells.




T1/A
T1/B
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T2/A
T3/A
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T4/B
T4/A

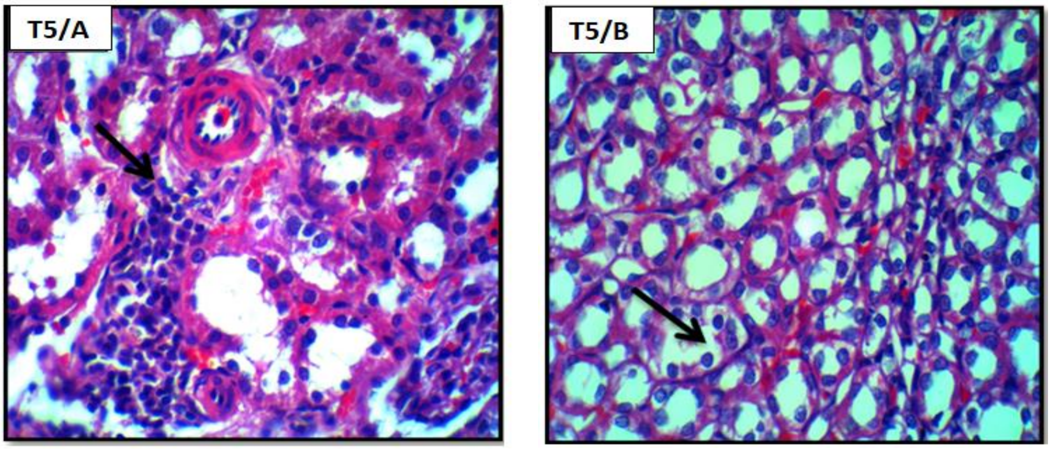

Figure (1): Histological sections the experience lasted six weeks

(A)in the cortex and (B) in the medulla of the kidneys of male rats stained with hematoxylin and eosin (C) The control group was fed a normal diet. (T1) was fed a diet + goat milk dose. (T2) was fed a diet + CdCl2. (T3) was fed a diet + Pb. (T4) was fed a diet + CdCl2 + goat milk. (T5) was fed a diet + Pb + goat milk.

CONCLUSIONS

The study highlights the detrimental effects of cadmium chloride ($CdCl_2$) and lead (Pb) exposure on kidney function, as indicated by increased creatinine and urea levels. Treatments with goat milk (T4 and T5) reduced these biomarkers, suggesting a protective effect against nephrotoxicity. Histological analysis revealed severe renal damage in metal-exposed rats, while rats receiving goat milk showed milder damage, indicating partial protection. The bioactive components of goat milk, such as calcium, magnesium, potassium, phosphorus, and antioxidants like zinc and selenium, may help reduce oxidative stress and inflammation. These findings support the potential of goat milk as a natural dietary intervention for mitigating heavy metal-induced kidney dysfunction. Future research should further explore its molecular mechanisms and therapeutic potential in humans exposed to environmental toxins.

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CONFLICT OF INTEREST

The researcher affirms that there were no conflicts of interest related to the publication of this study.

تأثير حليب الماعز على وظائف الكلى في الفئران البيضاء المعرضة للتسمم بكلوريد الكادميوم والرصاص

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الخلاصة

هدفت الدراسة الى معرفة مدى تأثير حليب الماعز كغذاء وظيفي للتخلص من السموم الكيميائية المعرضة لها حيوانات التجارب مركب CdCl2 وعنصر Pd عن طريق فحص الكرياتين واليوريا والفحص النسيجي. تضمنت هذه الدراسة استعمال 36 جرذ من ذكور Rats male Albino بيضاء اللون وقسمت عشوائياً الى 6 مجاميع متساوية، مجموعة السيطرة (C) وبقية المجاميع حقنت بمركب CdCl2 او عنصر Pd وجرعت بحليب الماعز بكميات محددة لمعرفة تأثير الحليب على مستويات Creatinine و Urea في الكلى وكذلك التشريح النسيجي لها. وأظهرت النتائج ان الحيوانات المعرضة للتسمم CdCl2 و Pb اذ بلغت الكرياتينين (0.4412، 0.4057) mg/dl على التوالي مقارنة مع معاملة السيطرة التي بلغت 0.2059 mg/dl. اما مستوى اليوريا بلغ (65.32, 66.68) mg/dl على التوالي مقارنة بمعاملة السيطرة التي بلغت 39.72 mg/dl دور حليب الماعز الوقائي في تحسين وظائف الكلى من خلال تقليل السمية الكلوية وتقليل الالتهابات التي سببها التسمم بالمعادن الثقيلة.

الكلمات المفتاحية: حليب الماعز، كلوريد الكادميوم، الرصاص، الكرياتين، الكلى.

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