RESEARCH PAPER

PRECLINICAL INVESTIGATION OF LUTEOLIN AGAINST LEAD ACETATE-INDUCED NEPHROTOXICITY IN MALE WISTAR RAT

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Abstract:

The aim of this study was to evaluate the protective effect of flavonoid luteolin against lead acetate, which induces nephrotoxicity in male Wistar rats. Lead acetate is well know heavy toxic metal worldwide has increased occupational and ecosystem exposure. The heavy metal damage affects kidney function through oxidative stress, inflammation, and mitochondrial dysfunction. EDTA (ethylene-diamine-tetraacetic acid) was used against the lead acetate damage to the kidney function. EDTA reduces the lead burden by chelating and improves the renal morphology. Lead exposure markedly increases the serum creatinine and blood urea nitrogen levels, which increases the renal damage. The luteolin treatment significantly alters the renal biomarker by attenuating oxidative stress.

Keyword

Nephrotoxicity, Nephrotic effects, Lead acetate, EDTA (ethylene-diamine-tetraacetic acid), Flavonoid, Luteolin, Oxidative stress, inflammation

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INTRODUCTION

Lead is a widely pervasive material with a long history of human use, and industrial manufacturing relies heavily on this metal, which, unfortunately, pumps a dangerous amount of lead acetate into the environment because lead cannot biodegrade and remains in nature indefinitely. Increased lead levels in both ecosystems and living organisms. This is causing long-term resistance and severe health risks from industrial and environmental exposure. Lead acetate is a highly poisonous metal affecting almost every organ of the human body and the ecosystem, and of all organs, the nervous system is the most affected and targeted by lead acetate. Chronic exposure to lead accumulates in organs such as the kidney, liver, and brain. The toxicity of lead affects both adults and children [1]. EDTA (ethlenediaminetraacetic acid) is a strong metal that is used in heavy metal toxicity, such as lead poisoning therapy. EDTA is a stable complex with a lead ion, which binds to the Pb ion and increases urinary extraction from the urinary system[2]. Luteolin is the flavonoid-containing compound (3,4,5,7-tetrahydroxyflavone) found in naturally occurring compounds from flavonoids to flavone, and the polyphenolic chemical present in fruits and vegetables, like celery, green paper, chamomile, onion, and carrot. [3] Several clinical studies have demonstrated that flavonoids have an inflammatory protective effect against nephrotoxicity in patients with kidney disease.[4] By neutralizing reactive oxidative stress, luteolin minimizes lipid peroxidation within tissues and also modulates the signaling pathway NF-Kb and MAPK, which is involved in inflammation, and the studies show that luteolin protects the renal tissue against oxidative stress-induced damage. It enhances the antioxidant enzymes like SOD, CAT, and glutathione, which act as nephroprotective phytochemicals. [6,7,8]

MATERIAL AND METHOD:

CHEMICAL REAGENT:

Lead acetate (60mg/kg) was to induce the nephrotoxicity, EDTA(50mg/kg) serve as the standard treatment and luteolin (100 mg/kg, 200 mg/kg, p.o) its was investigated for its kidney protactive effect.

Animal and the experimental ethics protocol

For the experiment, male wistar rate with body weights of about 200 to 250 gm 2 to 3 months old, were used for the study and were obtained. The animals were kept at a fixed temperature. Animals

were housed under controlled conditions (21 ± 1°C, 75 ± 5% humidity, 12 h light/dark cycle). Animals were allowed to reach feed with the standard pellet diet and provided water from time to time. All procedures follow the animal ethical committee and CPCSEA [9]

Chemical experimental and sampling protocol:

After an acclimation period of 7 days in this experimental model, where animal divided in to the 5 group, (n=6) four animals were used in the studies. [10]

Group 1, (Normal control group), Normal group dose not receive any treatment.

Group 2, (Disease inducing group) Disease control group, received only lead acetate 60mg/ kg once a day with oral route of administration by oral gavage.

Group 3 (Standard group) EDTA, and the lead acetate dose was 50mg/kg (lead acetate 60mg/kg Pb),

Group 4 (Treatment) Luteolin oral suspension 100mg/kg once a day, or group 5 received a dose through oral gavage after 28 days of treatment.

Group 5 (high dose treatment) Luteoline oral suspension 200 mg/kg received once a day. [11]

Bichemical estimations:

After 28 days all animals were sacrificed by the process of cervical dislocation, thee blood was obtain from of the abdominal aorta using the syringe and serum is saparated and for the histopathology bilateral kidney collected carefully wash in normal saline solution and then transfer into the 10% formalin solution contener [12]

Kidney function parameters:

markers of renal damage, such as urea and creatinine, were evaluated using commercial kits following the manufacturer's protocol.

Biochemical analysis: After 28 days all animals were sacrificed by the process of cervical dislocation, thee blood was obtain from of the abdominal aorta using the syringe and serum is saparated and serum was separated for the estimation of creatinine, urea, organic phosphate, and blood nitrogen [13] [14]

Hitopathology:

histopathology bilateral kidney collected carefully wash in normal saline solution and then transfer into the 10% formalin solution contener [15]

RESULT:

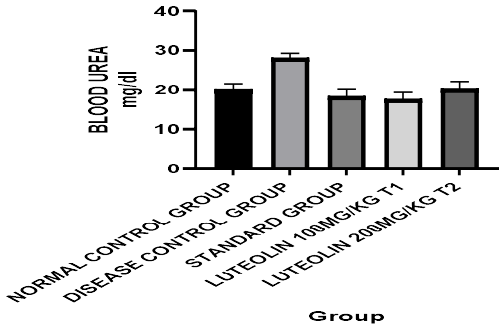
Statistical evaluation:

The data were expressed as the mean ± SEM (n=6). The statistical analysis was carried out by using the one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. A p- value > 0.005 was considered statistically significant, with $p < 0.001$ and $p < 0.001$, this is indicating a higher level of significance.

Table 1. Effect of luteolin on blood urea levels in lead acetate-induced nephrotoxicity (mg/dl)

S. No	Group Name	Blood Urea	S. no	Tukey's multiple comparison tests	Mean Difference	95.00 % CI of diff.	Significant?	Adjusted P Value
1	Normal Control	19.98 ± 1.514	**1**	Normal vs. Disease	-7.950	-10.87 to -5.027	Yes	<.001
2	Disease Control (Lead Acetate)	27.93 ± 1.341	**2**	Disease vs. Treatment luteoline 100mg/kg	10.42	7.494 to 13.34	Yes	<.001
3	Treatment group (*luteolin 100mg/kg)*	17.52 ± 1.887	**3**	Disease vs. Treatment Luteolin 200mg/kg	7.813	4.748 to 10.88	Yes	<.001
4	Treatment group Luteoline 20	20.12 ± 1.914	**4**	Disease vs. Standard (60mg/kg/50 mg/kg)	9.667	6.744 to 12.59	Yes	<.001

	0mg/kg							
5	Standard Group EDTA 50 mg/kg +	18.27 ± 1.895	**5**	Treatment 100 mg/kg vs Treatment 200mg/ kg	-2.603	-5.669 to 0.46622	No	.124
			6	Treatment with 200mg/kg vs EDTA 50mg/kg	-1.853	-4.9191 to 1.212	No	.407

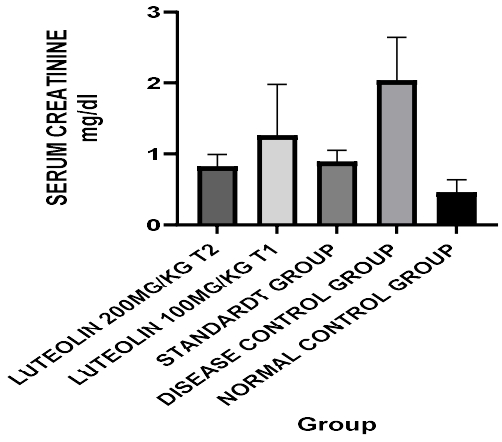


(Graph 1.) Effect of luteolin against lead acetate induced in blood urea.

Table 2. Effect of luteolin on serum creatinine levels in lead acetate induced nephrotoxicity (mg/dl)

S. No	**Group Name**	**Serum creatinine**	**S. no**	**Tukey's multiple comparison tests**	**Mean Difference**	**95.00 % CI**	**Significant?**	**Adjusted PV**								
1	Normal Control	**0.4500 ± 0.1871**	**1**	Normal vs. Disease	-5.582	-2.337 to -0.82666	Yes	<.001								
2	Disease Control (Lead Acetate)	**2.032 ± 0.6089**	**2**	Disease vs. Treatment luteoline 100mg/kg	0.7773	0.022255 to 1.5332	Yes	0.41								
3	Treatment group (*luteolin 100mg/kg)*	**1.254 ± 0.7264**	**3**	Disease vs. Treatment Luteolin 200mg/kg	1.213	0.458331 to 1.9688	Yes	.001								
4	Treatment group Luteoline	**0.8183 ± 0.1750**	**4**	Disease vs. Standard (60mg/kg/ 50mg/kg)	1.147	0.391602 to 1.9022	Yes	<.001								

	200mg/kg							
5	Standard Group EDTA 50mg/kg	**0.8850 ± 0.1676**	**5**	Treatment 100mg/kg vs Treatment 200mg/ kg	0.4360	--0.31911to1.1911	No	.454
			6	Treatment with 200mg/kg vs EDTA 50mg/kg	0.066667	-0.68841to0.82177	No	.999

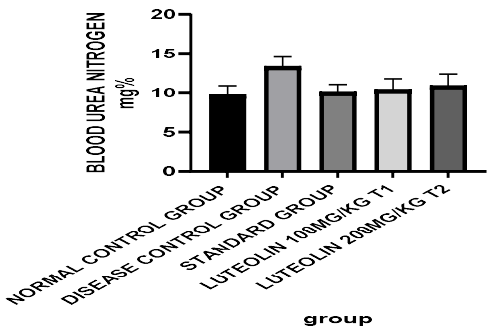


(Graph 2.) Effect of luteolin against lead acetate induced in serum creatainine.

Table 3. Effect of luteolin on blood urea nitrogen levels in lead acetate induced nephrotoxicity (mg%)

S.No	**Group Name**	**Blood Ureanitrogen**	**S.no**	**Tukey's multiple comparison tests**	**Mean Difference**	**95.00% CI of diff.**	**Significant?**	**Adjusted P Value**
1	Normal Control	**9.700 ± 1.152**	**1**	Normal vs. Disease	-3.600	-5.830 to -1.370	Yes	<.001
2	Disease Control (Lead Acetate)	**13.30 ± 1.337**	**2**	Disease vs. Treatment luteoline 100mg/kg	2.983	0.75315.480	Yes	.002
3	Treatment group (*luteolin 100mg/kg)*	**10.32 ± 1.440**	**3**	Disease vs. Treatment Luteolin 200mg/kg	2.483	0.25314.714	Yes	.024
4	Treatment group	**10.82 ±**	**4**	Disease vs. Standard (60mg/kg/50	3.250	1.0205.	Yes	.002

	Luteoline 200mg/kg	**1.5570**		mg/kg)		480		
5	Standard Group EDTA 50 mg/kg	**10.05 ± 0.9975**	**5**	Treatment 100 mg/kg vs Treatment 200mg/ kg	-0.5000	-2.7300 to 1.7300	No	963
			6	Treatment with 200 mg/kg vs EDTA 50mg/kg	-0.7667	-2.9977 to 1.4644	No	.849

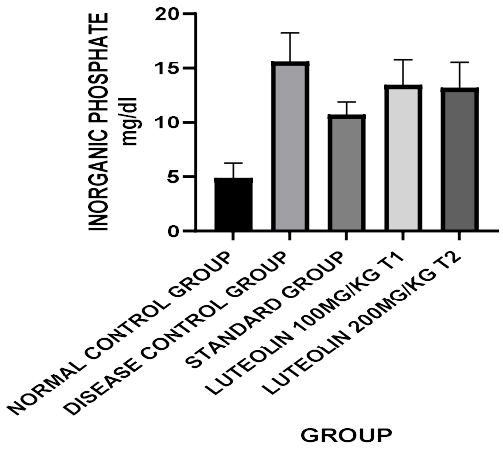


(Graph 3.) Effect of luteolin against lead acetate induced in blood urea nitrogen.

Table 4. Effect of luteolin on inorganic phosphate levels in lead acetate induced nephrotoxicity (mg/dl)

S.No	**Group Name**	**Inorganic phosphate**	**S.no**	**Tukey's multiple comparison tests**	**Mean Differ ence**	**95.00 % CI of diff.**	**Significant?**	**Adjusted P Value**
1	Normal Control	**4.833 ± 1.428**	**1**	Normal vs. Disease	-10.72	-14.27 to -7.160	Yes	<.0001
2	Disease Control (Lead Acetate)	**15.55 ± 2.689**	**2**	Disease vs. Treatment luteoline 100mg/kg	2.150	-1.407 to 5.707	No	.409
3	Treatment group (*luteolin 100mg/kg)*	**13.40 ± 2.373**	**3**	Disease vs. Treatment Luteolin 200mg/kg	2.400	-1.157 to 5.957	No	.304
4	Treatment group Luteoline 200mg/kg	**13.15 ± 2.377**	**4**	Disease vs. Standard (60mg/kg/50mg/kg)	4.877	1.320 tbo 8.434	Yes	.004

5	Standard Group EDTA 50 mg/kg	**10.67 ± 1.205**	**5**	Treatment 100 mg/kg vs Treatment 200mg/ kg	0.2500	-3.307 to 3.807	No	>.999
			6	Treatment with 200 mg/kg vs EDTA 50mg/kg	-2.477	-6.034 to 1.080	No	.275



(Graph 3.) Effect of luteolin against lead acetate induced in inorganic phosphate.

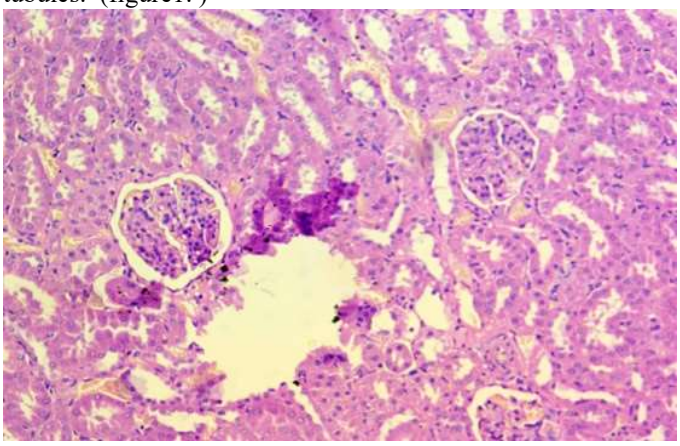
HISTOPATHOLOGY:

Histopathology analysis of the renal tissue:

For the histopathological examination, the renal tissue sample was collected and fixed in the 10% formalin solution. The fixed tissue was processed under the light microscope. The section of the kidney was stained for further microscopic examination, the slide was placed under a light microscope, and the morphological change in the renal tissue was observed.

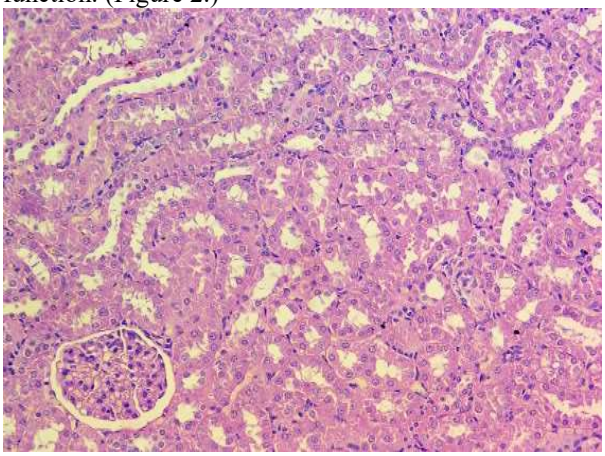
Histopathology of the normal group:

Histopathological examination of the control group normal renal architecture with intact glomeruli and tubules. (figure1.)



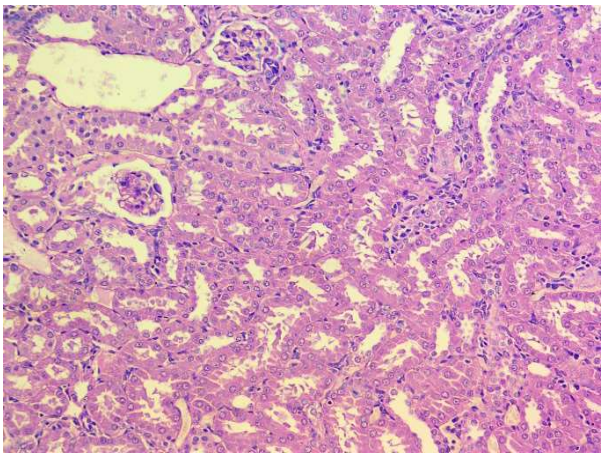
Histopathological examination of the disease group.

The disease group showed severe impairment of renal function. (Figure 2.)

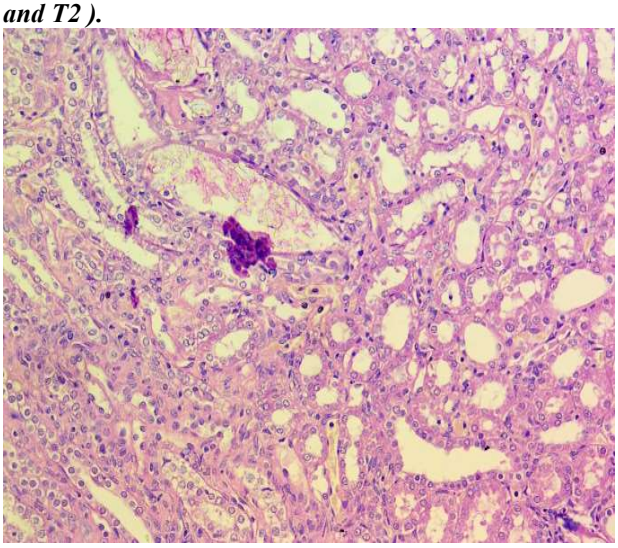


Histopathological examination of the standard group.

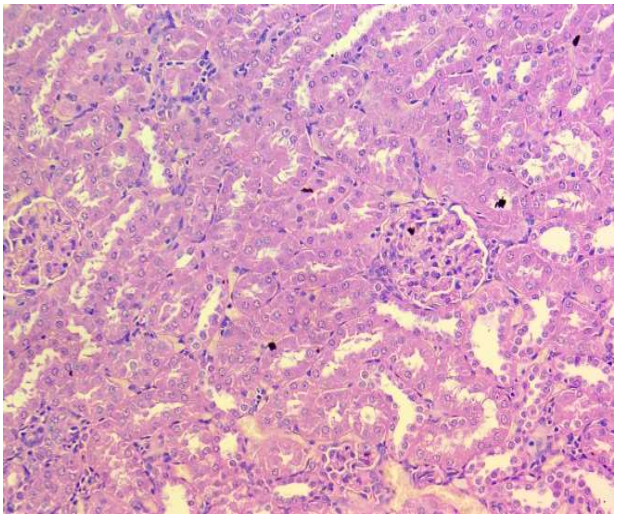
Treatment with the standard group EDTA has significantly restored the biochemical parameter towards the normal value. (figure 3.)



Histopathological examination of the luteoline (T1 and T2).



Similarly, treatment with luteolin results in a reduction of the elevated kidney function marker. As compared with the T1 and T2, its show that the lower serum creatinine as well as the blood urea level its indicated protact, which leads to renal damage, is induced by the lead acetate. Among the treatments, T2 shows better normalization of the serum creatinine inorganic phosphate level than T1. Its findings show that the luteolin passes the protective effect against the lead acetate induce the nephrotoxicity. Possibly through the antioxidant and anti-inflammatory properties. (figure 4, 5)



Test group, T1 Test group, T2

DISCUSSION:

This study was conducted to investigation of protective effect of luteolin and EDTA against the lead acetate-induced nephrotoxicity in the male Wistar rat. Lead acetate is a heavy, toxic metal that can accumulate in the kidneys and cause severe kidney damage through oxidative stress, inflammation, cellular injury, and apoptosis in the renal tubular damage and impaired kidney function. In the lead treatment group, which increased the level of serum creatinine, urea, and blood nitrogen, the phosphorus level in the blood also increased. These changes are due to oxidative stress, inflammation, and damage to the kidney cells by lead exposure. Treatment with luteolin significantly improves renal function parameters, and luteolin is a natural flavonoid known for its activities, which help in neutralizing the free radicals, reducing oxidative stress, and protecting kidney tissues from further injury. It reduces lipid peroxidation and enhances endogenous antioxidant defenses, thereby protect ranal damage, and the finding suggestive the luteolin can effectively reduce the lead-induced renal damage. Similarly, EDTA treatment demonstrated significant nephroprotective activity against lead acetate, which damages the kidney tissues. EDTA functions as a potent chelating agent by forming the chelate complex with the lead ion. There by the increing the excretion and reducing the renal lead burden and supporting the recovery of normal renal function. The overall study presents an investigation that revealed that administration of oral luteolin and EDTA suspension improves renal function in the lead acetate-exposed rats. The lead intoxication resulted in significant kidney impairment as evidenced by artring the biological and morphological parameters and renal tissue damage. Luteolin demonstrated protective effects by enhancing

antioxidant defense mechanisms and reducing inflammatory responses associated with lead toxicity. EDTA, through its metal-chelating properties, facilitated the removal of accumulated lead and minimized its harmful effects on renal tissues. The improvement observed in both treatment groups indicates their effectiveness in mitigating lead-induced renal injury. These findings support the potential use of luteolin and EDTA as promising nephroprotective agents against heavy metal-induced kidney damage.[15, 16]

CONCLUSION:

The findings of the animal experiment suggest that luteolin provides significant protection against the mechanism of oxygen-derived free radicals that lead to nephrotoxicity induced by lead acetate in male Wistar rats. Treatment improves renal function and decreases the severity of the kidney injury. This result shows that the luteoline and EDTA have the potential to reduce the nephrotoxicity.

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