A R DIGITECH
International Journal Of Engineering, Education And Technology (ARDIJEET)
www.ardigitech.in ISSN 2320-883X,VOLUME 11 ISSUE 01 25/01/2023

EFFECT OF *CITRUS LIMON* SEED EXTRACT AGAINST NEPHROTOXICITY IN RATS

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ABSTRACT

In lead induced nephrotoxicity model, the Albino Wister rats were randomly divided into four equal groups each containing six animals: first group is treated with vehicle control, second group is treated with lead acetate, dose of 500mg/kg through intraperitoneally served as toxic control, third group is treated with *Citrus limon* seed extract, dose of 400mg/kg by oral route, fourth group is treated with lead acetate dose of 500mg/kg through interperitoneally and *Citrus limon* seed extract at dose 400mg/kg through oral route. Second and fourth group will be treated with lead acetate (500mg/kg p. o) only on 1st day. Fourth group will be pre-treated with *Citrus limon* seed extract for 12 days at dose (400mg/kg p. o). Twenty-four hours after the last treatment, different biochemical analysis was performed like urine biomarkers, serum biomarkers, antioxidants and then subjected to histopathological studies. *Citrus limon* seed extract (CLSE) in presence of lead (Pb) was responsible for significant reduction in serum biomarkers: Urea, Albumin, Creatinine, Aspartate transaminase (AST), Alanine transaminase (ALT) compared to Pb toxic control. CLSE in presence of Pb was responsible for significant decrease in urine biomarkers: Total Protein, Sodium, Potassium compared to Pb toxic control. There was an increase in urine volume and decrease in kidney weight in CLSE in presence Pb compared to toxic control Pb. CLSE treatment in presence of Pb was also responsible for significant increase in antioxidants such as superoxide dismutase (SOD), Glutathione (GSH) and Catalase activity in kidney tissue homogenate compared to Pb toxic control. In mercury induced nephrotoxicity model, the Albino Wister rats were randomly divided into four groups each containing six animals: first group is treated with vehicle control, second group is treated with Mercury chloride (HgCl2), dose of 5mg/kg through intraperitoneally served as toxic control. Third group is treated with high dose of *Citrus limon* seed extract (CLSE), dose of 400mg/kg through oral route. Fourth group is treated with mercury chloride (5mg/kg i. p) with *Citrus limon* seed extract at dose (400mg/kg p. o). Second and fourth group will be treated with HgCl2 (5mg/kg, i. p) only for three days. The animals of fourth group will be pre-treated with CLSE for 12 days at dose (400mg/kg p. o). After twenty-four hours of last treatment, different biochemical analysis was carried out like urine, serum biomarkers and antioxidants and later subjected to histopathological observation. *Citrus limon* seed extract (CLSE) in presence of HgCl2 was responsible for significant decrease in serum: Albumin, Urea, Aspartate transaminase (AST), Alanine transaminase (ALT) and creatinine was observed compared to toxic control HgCl2. CLSE in presence of HgCl2 was responsible for significant reduction in Urine biomarkers: Total protein, Sodium, Potassium. *Citrus limon* seed extract in presence of HgCl2 was also responsible for significant increase in antioxidants such as superoxide dismutase (SOD), Glutathione (GSH) and Catalase. There was increase in urine volume and decrease in kidney weight. Results were further supported by histopathological studies. Thus, investigational finding conclude that *Citrus limon* seed extract possesses potential benefits in treating animals with lead and mercury induced nephrotoxicity.

Keywords: *Citrus limon,* Nephrotoxicity, Lead Acetate, Mercury Chloride.

1. Introduction

Nephrotoxicity is the most common kidney problems and occurs when body is exposed to toxic chemicals or drugs[1]. Nephrotoxicity is defined as rapid deterioration in kidney function due to toxic effect of medication and chemicals. There are various forms and some drugs may affect renal functions is more than one way. Different mechanisms lead to nephrotoxicity like renal tabular toxicity, inflammation, glomerular damage, crystal nephropathy and thrombotic microangiopathy[2]. It has been shown that nephrotoxicity caused by many toxic drug treatments is associated with increased oxidative stress, which might be the major contributing factor towards renal injury[3].

Kidney is an essential organ required for the body to perform several important functions such as maintenance of homeostasis, regulation of extracellular environment like detoxification and excretion of toxic metabolites and drugs. Therefore, the kidney can be considered as major target organ for exogenous toxicants. Nephrotoxicity is a kidney-specific in which excretion does not go smoothly owing to toxic chemical or drugs. Average 20% of nephrotoxicity is induced by drugs, in elderly medication increases the incidence of nephrotoxicity up to 66% as the average life span increases[4]. Due to increased industrialization and population, there is release of varieties of chemicals including heavy metals into the atmosphere leading to slow toxicity of the most vital organs in human body[5].

Heavy metals are defined as metallic elements that have a high density compared to water[6]. Their toxicity depends on different factors such as dose, route of exposure, and chemical species as well as the age, gender, genetics of exposed individuals. Because of their high degree toxicity, arsenic, cadmium, chromium, lead and mercury rank in the priority metals that are public health significance[7]. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. And human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications[8]. Heavy metals are naturally occurring elements that are found throughout the earth's crust, most environmental contamination and human exposure result from the anthropogenic activities such as mining, industrial production and use and domestic and agriculture use of metals and metal-containing compounds [9 10 11 12].

Heavy metals in plasma exist in an ionized form, which is toxic and leads to acute toxicity, when a metal is conjugated with metallothionein and are delivered to the liver and possible causing the kidney chronic damage[13].

Lead is an extremely toxic heavy metal. Lead is a blue grey and highly toxic divalent metal that occurs naturally in the earth's crust. Lead is soft and malleable, and also has relatively low melting point. Due to its continuous use and non-biodegradable nature, it gets accumulated in the environment leading to various hazards [14 15]. There are many different uses of lead. It may be used as a pure-metals, alloyed with other metals, or as chemical compounds[16]. Kidney is one of targeted site of lead toxicity for being major route of excretion from body and facilities damage via oxidative stress and lipid peroxidation. Lead through endocytosis and erythrophagocytosis it locates in different tissues and organs including kidney, liver where it causes oxidative damage on cells, tissues .and cellular organelles. All among the cells proximal tubules are more susceptible to lead induced cellular damage followed by apoptosis. Lead is highly toxic in nature affecting almost every organ in our body leading to nephrotoxicity, neurotoxicity and hepatotoxicity and cardiotoxicity [17 18 19].

Mercury is the metallic element that is liquid at standard conditions for temperature and pressure. Mercury is a highly toxic metal where in any form is poisonous leading to neurologic, gastrointestinal and renal organ toxicities. Mercury and its compound have been used in medicines although they are much less common today than they once were, now that the toxic effects of mercury and its compound are more widely understood [20 21]. Mercury has many therapeutics uses including various medications, ointments, dental fillings, contact lens, cosmetics, paints as well as in different instruments like thermometer and sphygmomanometers[22]. Mercury has greater affinity to bind with thiol containing enzymes, it inactivates the enzymes with thiol group through irreversible oxidation which leads to depletion of total thiol content and oxidative stress

With 10% of the population is affected in by chronic kidney disease and millions of people die because they could not afford the modern treatment. It is estimated that mortality rate has been increased due to kidney species especially in developing countries such as China and India etc

Herbal medicines have been widely used to treat disease for thousands of years and has greatly contributed to the health of human beings. The nephrotoxicity of herbal medicines has attracted worldwide attention. More than 100 kinds of herbal medicines induce renal toxicity, including some animal, herbal medicine, herbs and minerals[26].Herbal medicine is the use of plants and plants extract to treat disease. Many modern drugs were originally extracted from plant sources, even if they are now made synthetically[27]. Allopathic medicines are effective in treating the disease but fall back due to their various adverse effects and cost is more when compared to natural medicines. Hence drugs of natural origin are the best choice for the treatment of nephrotoxicity. The drug species have showed their significant role in lowering the toxicity of kidney. Many other drugs having a prominent role in reducing nephrotoxicity are lemon, apple, ginger, spinach, strawberry, onion etc

Citrus limon (L.) burm. f, belonging to family, *Citrus limon* has many physiological functions Phyto-constitutes such as flavonoids, citrus acid, ascorbic acid, minerals, coumarins, cymene etc. the fruit is utilized in fresh and in cooking and beverages and it also has an application in preservative due to its antioxidant property[29].

Till now there is no reported study which witnessed the effect of *Citrus limon* seed extract against lead and mercury induced nephrotoxicity. Hence the present study was designed to demonstrate the effect of citrus limon seed extract against lead and mercury induced nephrotoxicity by using rat as an experimental animal

Specific objectives:

1. To collect and authenticate *Citrus limon* seed extract against lead and mercury induced nephrotoxicity
2. To carryout extraction of *Citrus limon* seed using suitable solvent and extraction procedure.
3. To explore the nephroprotective activity in *Citrus limon* seed extract on urine biomarkers such as Urine Volume, Total Proteins, Sodium, Potassium
4. To study the effect of *Citrus limon* seed extract on Serum biomarkers such as Serum Albumin, Serum Creatinine. Serum Urea, Serum ALT, Serum AST.
5. To explore the nephroprotective role of *Citrus limon* seed extract on various antioxidant parameters such as GSH, Catalase, Superoxide Dismutase and Kidney weight.
6. To study the histopathological analysis of kidney.

Methodology

Animals

Rats of either sex weighing 175-250g were housed in standard polypropylene cages and maintained under controlled room temperature (25º±5ºC) and humidity (55±5%) in a well-ventilated animal house under 12:12h light and dark cycle. All the rats were provided with commercially available pellet diet, water ad Librium. Prior to each study, the animals were made to fast for 12:14h but had free access to water. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

Preparation of seed extract:

The seed will be washed and dry at ambient temperature in the darkness until used. The seeds will finely ground 3g of this ground materials is extracted by stirring with 30ml of pure methanol for 30min. The extract is then kept for 24 h at 4ºC, filter through a Whatman No. 4 filter paper to obtain a 25ml final volume, under evaporate under vacuum to dryness and store at 4ºC until analyzed.[54]

Phytochemical estimation of the extract:[64 65]

Extract of *Citrus Limon* seed were subjected to qualitative analysis to investigate the presence of various phytochemicals constituents such as carbohydrates, glycosides, proteins, tannins saponins and flavonoids.

EXPERIMENTAL MODEL

Lead induced nephrotoxicity in rats:

The present study was designed for 12 days. The experimental rats will be randomly divided into four groups, six animals in each as follows.

- **Group -I**-Vehicle control
- **Group-II**-Lead acetate (500mg/kg i.p) served as toxic control.
- **Group-III**-High dose of citrus limon seed extract (400mg/kg, p.o)
- **Group-IV**-Lead acetate (500.g/kg i.p) +citrus limon seed extract (400mg/kg, p.o)

The animals of group II and IV will be treated with lead acetate (500mg/kg p.o) only on first day. The animals of group IV will be pretreated with Citrus Limon seed extract for 12 days at dose (400mg/kg, p. o). Twenty-four hours after last treatment different biochemical analysis were performed on collected serum and urine. Then after sacrificing the rats' kidney were removed and weighed. Half of the kidney samples the kidney tissue homogenate (KTH) were prepared with sucrose solution and subjected for antioxidant studies. Remaining kidney samples were subjected for histological examination.

The different parameters estimated were

1. Serum: Albumin, Creatinine, Urea, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).
2. Urine: Urine volume, $Na^{+,}$ K^{+} ions, and total proteins.
3. Kidney homogenate: Glutathione Peroxidase, Catalase and super oxide dismutase.
4. Physical parameters: Kidney weight
5. Histological analysis.
6. **Mercury induced nephrotoxicity in rats:**

The present study was designed for 12 days. The experimental rats will be randomly divided into four groups, six animals in each as follows

- **Group-I**- Vehicle control
- **Group-II**-$HgCl_2$(5mg/kg/day i.p)
- **Group-III**-High dose of Citrus limon seed extract (400mg/kg, p.o).
- **Group-IV**-$HgCl_2$ (5mg/kg/day, i.p for 3 days) + Citrus limon seed extract for 12 days at dose(400mg/kg/p.o)

The animal of group II and IV will be treated with $HgCl_2$ (5mg/kg/day, i.p) only for three days. The animals of group IV will be pretreated with *Citrus limon* seed extract for 12 days at dose (400mg/kg. p.o). Twenty-four hours after last treated different biochemical analysis were performed on collected serum and urine. Then after sacrificing the rat's kidney were removed and weighed. Half of the kidney samples the kidney tissue homogenate (KTH) were prepared with sucrose solution and subjected for antioxidant studies; remaining kidney were subjected for histological examination.

The different parameters estimated were

1. Serum: Albumin. Creatinine, Urea. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)
2. Urine: Urine volume, Na^+. K^+ ions and total proteins
3. Kidney homogenate: Glutathione Peroxidase, Catalase and Super oxide dismutase.
4. Physical parameters: Kidney weight.
5. Histological analysis.

Table1: Effect of CLSE on serum biomarkers in lead induced nephrotoxicity

Treatment	**Albumin (mg/dl)**	**Creatinine (mg/dl)**	**Urea (mg/dl)**	**ALT (U/L)**	**AST (U/L)**
Normal control	8.19±0.03	0.26±0.06	22.68±0.04	52.32±0.03	65.06±0.67
Toxic control 500mg/kg (LA)	21.72±0.05***	2.49±0.04***	67.84±0.03***	261.19±1.53***	255.94±0.54***
CLSE(400mg/kg)	12.60±0.26$^{###}$	0.80±0.02$^{###}$	23.80±0.02$^{###}$	63.28±2.53$^{###}$	97.69±0.54$^{###}$
(CLSE +LA)	13.70±0.12$^{***###}$	1.54±0.17$^{***###}$	40.51±0.03$^{***###}$	95.41±2.53$^{***###}$	127.67±1.53$^{***###}$

n=5, values are expressed in MEAN±SEM, one-way ANOVA followed by Tukey-Kramer multiple comparison test. **P<0.01, ***P<0.001 when compared to control and #P<0.05, ##P<0.01, ###P<0.001 compared to normal.

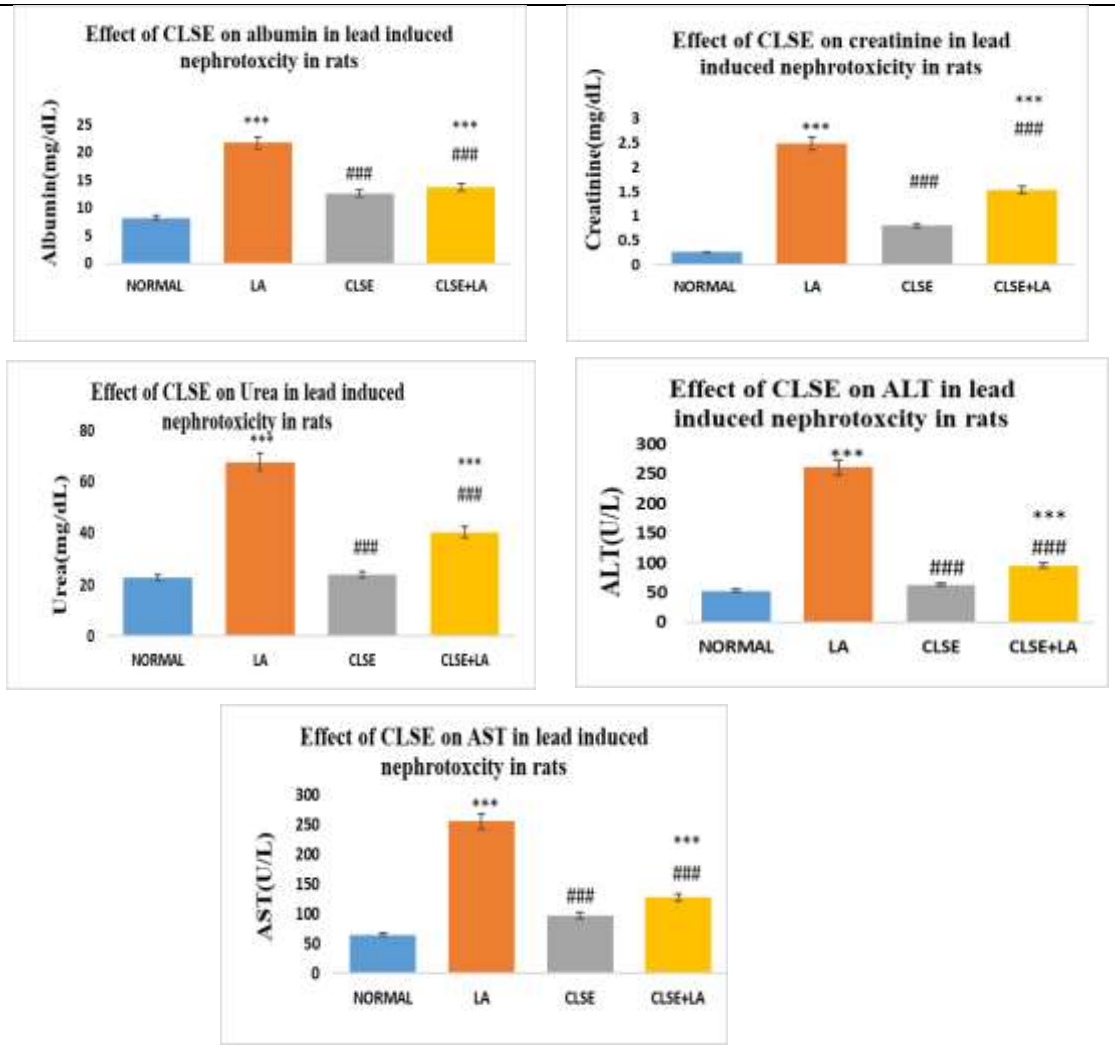


Figure 1. Effect of CLSE on serum biomarkers in lead induced nephrotoxicity

3. Effect on antioxidants

Effect on GSH

In this experiment model, the toxic control reveled a moderately significant ($p<0.01$) decrease in GSH levels when compared to the normal control. Prior treatment of (LSE+LA) showed extremely significant ($p<0.05$) increase in GSH level compared to that of toxic control.

Effect of Catalase

Toxic control revealed an extremely significant ($p<0.001$) decrease in catalase activity when compared to the normal control. On the pre-treatment of (CLSE+LA) showed significant ($p<0.05$) increase in catalase activity when compared to toxic control.

Effect of SOD

In this experiment model, the toxic control showed an extremely significant ($p<0.001$) decrease in SOD levels when compared with normal control. Prior treatment with (CLSE+LA) showed a extremely significant ($p<0.05$) increase in SOD level compared to that of toxic control.

Effect on kidney weight

There was an extremely significant ($p<0.001$) increase in kidney weight in toxic control when compared to that of normal control. The pre-treatment with (CLSE+LA) showed extremely significant ($p<0.001$) decrease in kidney weight compared to toxic control.

Table 2: Effect of CLSE on antioxidants in lead induced nephrotoxicity

Treatment	GSH (U/Mg)	CATALASE (U/Mg)	SOD (U/Mg)	Kidney weight(g)
Normal control	4.41±0.03	42.48±0.03	9.3±0.03	1.36±0.08
LA (500mg/kg)	2.22±0.05***	22.09±0.21***	3.84±0.03***	2.64±0.09***
CLSE (400mg/kg)	4.13±0.07###	39.32±0.03###	8.20±0.06###	1.71±0.11###
(CLSE+LA)	3.71±0.03***###	32.27±0.05***###	7.98±0.04***###	2.08±0.02***##

n=5, values are expressed in MEAN±SEM, one-way ANOVA followed by Tukey-Kramer multiple comparison test. **P<0.01, ***P<0.001 when compared to control and #P<0.05, ##P<0.01, ###P<0.001 compared to normal.

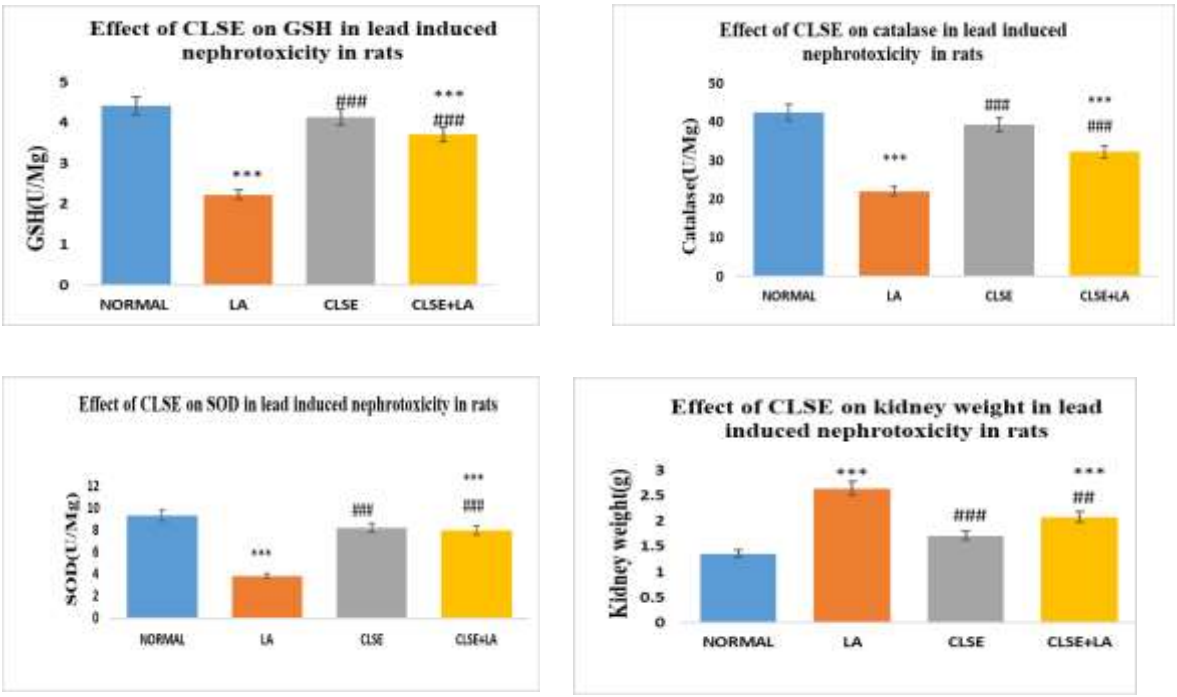
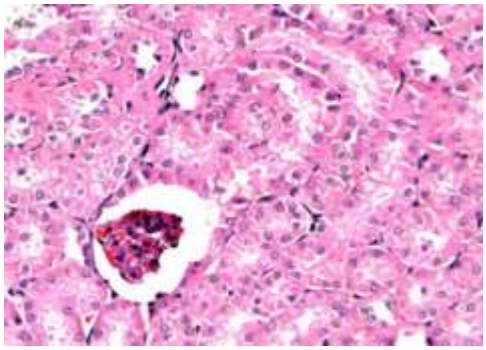


Figure 2 Effect of CLSE on antioxidants in lead induced nephrotoxicity

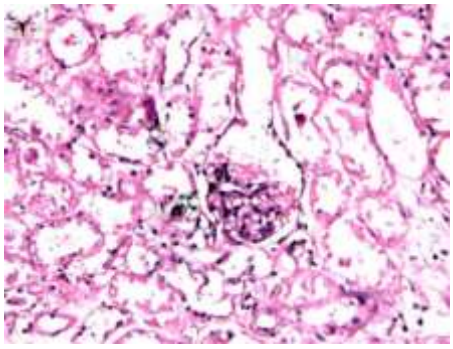
Histopathological observation:

The section of kidney rats of different groups was stained by hematoxylin and eosin.

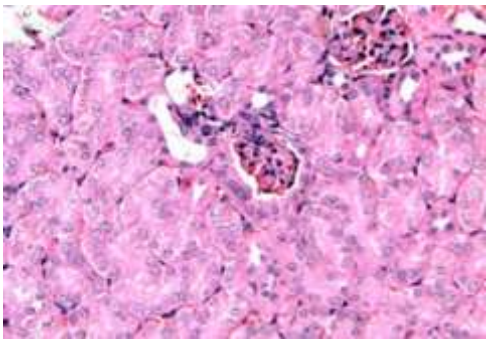
Normal rat kidney showing normal architecture of renal corpuscles with their glomeruli and renal tubules. When lead acetate is treated it shows shrinkage, fragmentation of glomeruli and renal tubules showing degeneration and necrosis. When CLSE is treated normal renal corpuscles with their glomeruli as well as renal tubules. When CLSE + lead acetate is treated it shows normal histological structure of renal tubules and glomerulus.



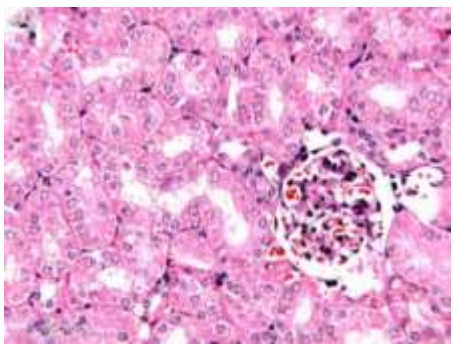
(a) Normal group



(b) Lead acetate group



(c) CLSE (400mg/kg)



(d) CLSE+LA (500mg/kg)

3. Histopathological slides of kidney in Lead induced nephrotoxicity.

Mercury induced nephrotoxicity

Effect on urine biomarkers

Effect on urine volume

$HgCl_2$ toxic control reported extremely significant ($p<0.001$) decrease in urine volume compared to that of normal control. Prior treatment of CLSE+ $HgCl_2$ showed moderately increase ($p<0.001$) in urine volume when compared to that of toxic control.

Effect on Total protein

In experimental model, toxic control demonstrated extremely significant ($p<0.001$) increase in protein level compared to that of normal control. The pre-treatment with CLSE+$HgCl_2$ showed extremely significant ($p<0.001$) decrease in protein level when compared to that of toxic control.

Effect on sodium

There was extremely significant ($p<0.001$) increase in sodium level in toxic control compared to that of normal control. CLSE+$HgCl_2$ showed extremely significant ($p<0.001$) decrease in sodium level when compared with toxic control.

Effect on potassium

Toxic control demonstrated extremely significant increase ($p<0.001$) in potassium level when compared to normal control. The pre-treatment with CLSE+$HgCl_2$ showed extremely significant reduction ($p<0.001$) in potassium level compared to that of toxic control.

Table. 3 Effect of CLSE on urine biomarkers in mercury induced nephrotoxicity

Treatment	Urine volume (ml)	Total protein (mg/dl)	Sodium (mEq/l)	Potassium (mEq/l)
Normal control	15.44±0.14	21.66±0.09	132.97±0.11	3.51±0.05
HgCl2(5mg/kg)	5.50±0.10***	37.42±0.10***	197.67±1.70***	6.7±0.05***
CLSE (400mg/kg)	12.77±0.07###	23.35±0.05###	148.59±0.10###	3.90±0.07###
CLSE+HgCl2	8.075±0.01***###	32.76±0.07***###	177.13±1.17***###	4.94±0.02***###

n=5, values are expressed in MEAN±SEM, one-way ANOVA followed by Tukey-Kramer multiple comparison test. **P<0.01, ***P<0.001 when compared to control and #P<0.05, ##P<0.01, ###P<0.001 compared to normal.

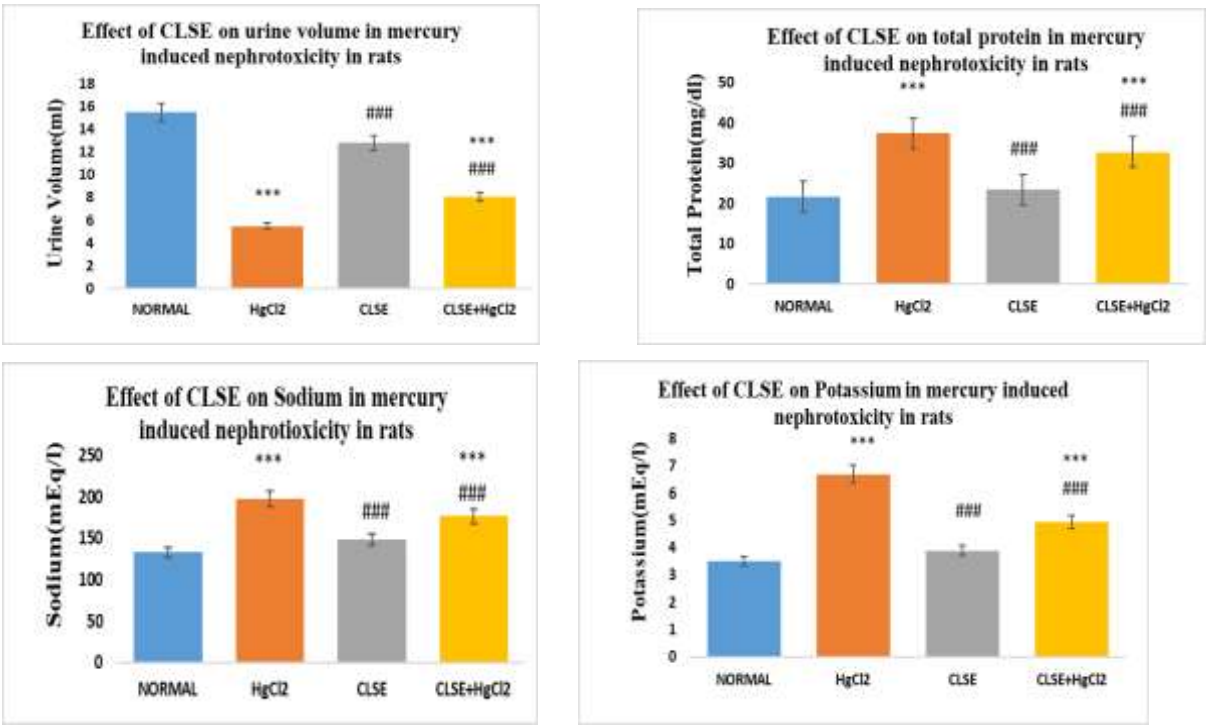


Figure 4. Effect of CLSE on urine biomarkers in mercury induced nephrotoxicity

5.4.2 Effect on serum biomarkers

Effect on Albumin

There was extremely significant increase (p<0.001) in Albumin level in toxic control when compared to normal control. On pre-treatment with CLSE+HgCl2 it reported as extremely significant (p<0.001) decrease in albumin level compared to that of toxic control.

Effect on creatinine

HgCl2 showed that there extremely significant (p<0.001) increase in creatinine level compared to that of normal control. The pre-treatment with CLSE+HgCl2 reported mild significant (p<0.05) decrease in creatinine level compared to that of toxic control.

Effect on urea

Toxic control demonstrated extremely significant (p<0.001) increase in urea level when compared to normal control. On treatment with CLSE +HgCl2 it is reported as extremely significant (p<0.001) decrease in urea level compared to that of toxic level.

Effect on ALT

There was extremely significant (p<0.001) increase in ALT level compared in toxic control to that of normal control. The pre-treatment CLSE+HgCl2 showed extremely significant (p<0.001) decrease in ALT level compared to that of toxic control.

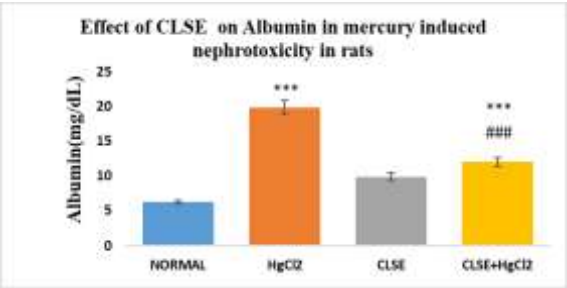
Effect on AST

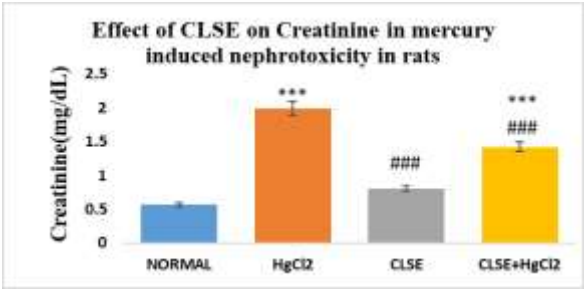
Toxic control showed extremely significant (p<0.001) increase in AST level compared to that of normal control. In the pre-treatment of CLSE+HgCl2 is reported as extremely significant (p<0.001) reduction in AST level compared to that of toxic control.

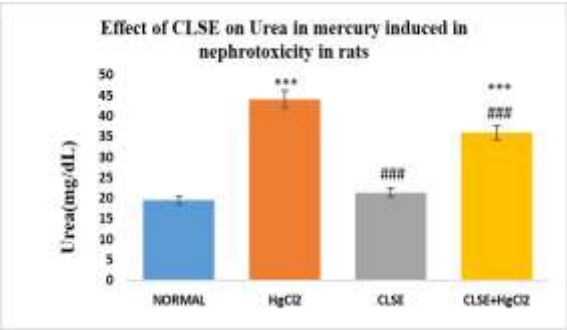
Table 4: Effect of CLSE on serum biomarkers in mercury induced nephrotoxicity

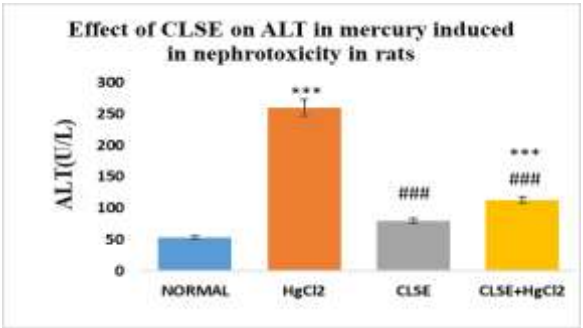
Treatment	Albumin (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	ALT (U/L)	AST (U/L)
Normal control	6.23±0.07	0.565±0.03	19.57±0.06	53.25±0.49	73.98±0.12
HgCl2(5mg/kg)	19.82±0.15***	1.99±0.04***	44.02±0.18***	259.2±0.50***	241.06±0.98***
CLSE (400mg/kg)	9.88±0.16###	0.80±0.05###	21.4±0.13###	79.23±0.22###	90.03±0.14###
CLSE+HgCl2	12.01±0.20***###	1.42±0.03***###	35.94±0.67***###	112.77±0.93***###	123.7±0.42***###

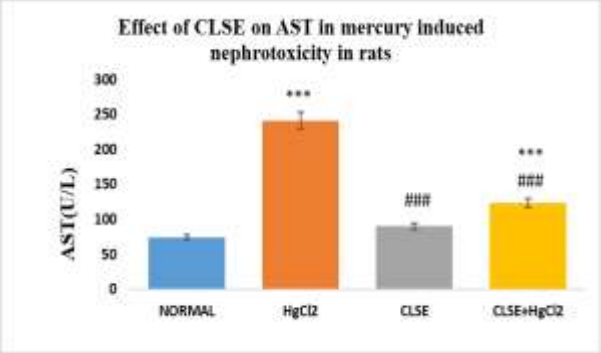
n=5, values are expressed in MEAN±SEM, one-way ANOVA followed by Tukey-Kramer multiple comparison test. **P<0.01, ***P<0.001 when compared to control and #P<0.05, ##P<0.01, ###P<0.001 compared to normal.











Discussion

The aim of the present study was to explore the effect of *Citrus limon* seed extract (CLSE) against lead and mercury induced nephrotoxicity in rats.

Observed results suggested that *Citrus limon* seed extract (400mg/kg, p.o) showed beneficial results. *Citrus limon* seed extract indicated better results against lead and mercury induced nephrotoxicity in rats.

Citrus limon (L.) burm. f, belonging to family Rutaceae. *Citrus limon* has many physiological functions phyto-constitutes such as flavonoids, citrus acid, ascorbic acid, minerals, coumarins, cymene etc. The fruits are utilized for cooking and beverages and it has an application in preservative due to its antioxidant property[29].

Lead (Pb) is an extremely toxic heavy metal. Due to its continuous use and non-biodegradable nature, it gets accumulated in the environment leading to various hazards[14 15]. Lead is highly toxic in nature affecting almost every organ in our body leading to nephrotoxicity, neurotoxicity and hepatotoxicity and cardiotoxicity[17 18 19].

In Lead induced nephrotoxicity model toxicity induced by treating experimental animals with dose of 500mg/kg intraperitoneally, hypothetically Pb^{2+} competes with Ca^{2+}, dysregulates the calcium homeostasis, as a result Ca^{2+} release from mitochondria is stimulated, which initiates the opening of the mitochondria transitional pore in turn mitochondria damages reactive species generation and oxidative stress leading to altered lipid metabolism. Among the cells, proximal tubules are more susceptible to Pb- induced cellular damage followed by apoptosis[39].

In this present study, it has been demonstrated that significant increase in urine biomarker level such as total protein, sodium, potassium level. It was also revealed significant decrease in urine volume was seen in the lead intoxicated rat.

Treatment with *Citrus limon* seed extract reversed the elevated levels of all the urine biomarkers such as total protein, sodium, potassium decreased urine volume to the near normal levels in the model.

It has also shown that significant increase in serum biomarkers level such as albumin, Creatinine, urea, AST and ALT. It was also revealed significant decrease in antioxidants like GSH, SOD, catalase.

Treatment with *Citrus limon* reversed the elevated levels of all the serum biomarkers such as albumin, urea, AST, ALT, and creatinine, decreased antioxidant enzyme to the near normal level in this model. The histopathological parameters of lead induced nephrotoxicity were normalized by the treatment with *Citrus limon.*

Mercury is also a toxic heavy metal where in any form is poisonous leading to neurologic, gastrointestinal and renal organ toxicities.

In mercury induced nephrotoxicity, toxicity induced by treating the experimental animals with dose of 5mg/kg by intraperitoneal route. Hg deposition is closely related ROS generation, mRNA expression of MT, apoptosis and proximal damage. It inactivates the enzyme with thiol group through irreversible oxidation. Results in

depletion of total thiol content and oxidative stress. Inactivation of sulfhydryl protein also affects the cellular integrity interrupting membrane potential and volume of cells as well organelles. Absence of detoxifying protein or reduced selenothiol containing antioxidant activity also facilitates the proximal tubules damage. Mercury reduces the function of tight junction protein in kidney and perturbs cellular permeability. Hg decreases transepithelial electrical resistance (TER). Facilitates phosphorylation of tight junction protein, occuldin via a protein kinase A(PKA) dependent mechanism.[39]

Observed results suggested that *Citrus limon* seed extract (400mg/kg p.o) showed beneficial results citrus limon indicated better results against mercury induced nephrotoxicity in rats.

HgCl2 group has showed significant increase in urine biomarkers level such as total protein, sodium, potassium level. It was also revealed a significant decrease in urine volume.

In this model it is reported that significant increase in serum biomarkers level creatinine, AST, ALT, urea and decrease in albumin level is seen.

Treatment with *Citrus limon* reversed elevated levels of all the serum biomarkers such as Creatinine, urea, AST, ALT and decreased level of albumin, decreased antioxidants ezyme to the near normal levels in this model.

Citrus limon seed extract is antioxidant. *Citrus limon* may be recommended as a nephroprotective agent to attenuate toxicity of some various drugs like Lead and Mercury that are highly toxic of inducing nephrotoxicity.

CONCLUSION

With the findings of the present study, it can be concluded that *Citrus limon* seed extract has demonstrated significant nephroprotective effect against Lead and Mercury induced nephrotoxicity in rats.

Treatment with *Citrus limon* seed extract reversed the Pb and HgCl2 induced elevated levels of all the urine biomarkers such as total protein, sodium and potassium, decreased urine volume near to the normal levels.

It is worth mentioning that *Citrus limon* seed extract efficiently trims down the elevated levels of serum biomarkers such as AST, AST and urea. It has also restored antioxidant parameters without producing any adverse effect. The results from the present study and histological analysis indicate the administration of *Citrus limon* seed extract has protective effects against Lead and Mercury induced renal necrosis state.

SUMMARY

The present study was designed to explore the nephroprotective activity of *Citrus limon* seed extract against the pathological condition if renal system induced by different experimental models in rats.

The *Citrus limon* seed was procured from local vendor in vegetable market in Mangalore. Then it is isolated and dried, extracted for treatment.

In this study the kidney is damaged by various toxic agents like lead (Pb) and mercury (HgCl2).

The study uses lead and mercury to induce nephrotoxicity in Albino Wister rats.

In lead induced nephrotoxicity model, experimental rats were divided in four groups each containing six animals. Group I were treated with saline served as normal control. Group II and Group IV were treated with Lead acetate dose of 500mg/kg through intraperitoneally only on 1st day to induce nephrotoxicity. Group IV was treated with CLSE (400mg/kg, p.o) for 12 days.

24hr after the last treatment different biochemical analysis were performed on collected serum and urine. Then after sacrificing the rat's kidney were removed and weighed. Half of the kidney samples kidney tissues homogenate were prepared with sucrose solution and subjected to antioxidants studies. Remaining kidney samples were subjected for histopathology studies.

Treatment with *Citrus limon* seed extract showed elevated levels of all the urine biomarkers such as total protein, sodium, potassium, decreased urine volume to the near normal level in the model.

Reported result suggest that, when compared to normal control the Lead toxic group showed a significant increase in serum biomarkers. *Citrus limon* seed extract along with Lead acetate was responsible for the significant reduction in serum biomarkers compared to lead alone treated groups.

It is worth mentioning that *Citrus limon* trims down serum biomarkers level such as AST, ALT creatinine and urea. The results from the present study and histological analysis indicate the administration of *Citrus limon* seed extract has protective effects against Lead induced renal necrosis state.

In Mercury induced nephrotoxicity experimental models, rats were divided into four groups each containing six animals. Group I were treated with saline served as normal control. Group II and Group IV were treated with HgCL2 for 3 days at dose (5mg/kg, i.p). Group IV were pre-treated with CLSE at dose (400mg/kg, p.o) for 12 days.

24hr after last treatment different biochemical analysis were performed on collected serum and urine.

Treatment with *Citrus limon* seed extract with HgCl2 shows significant increase in urine biomarkers such as total protein, sodium, potassium and decrease in urine volume compared to normal.

It is worth mentioning that *Citrus limon* seed extract trims down serum biomarkers such as ALT, AST, urea and creatinine. The results from the present study and histological analysis indicate the administration of citrus limon seed extract has protective effects against mercury induced renal necrosis.

The enhanced activity of *Citrus limon* seed extract could be due its antioxidant property.

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