NEPHROPROTECTIVE EFFECT OF CELASTRUS PANICULATUS SEED EXTRACT AGAINST LEAD ACETATE INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT:

Background: Understanding of chemical induced nephrotoxicity is essential to prevent kidney disease including acute and chronic kidney disease. Lead acetate is a toxic substance, and it is clinically proven to cause renal toxicity. Nephrotoxicity caused by lead acetate is a complex phenomenon, the important characteristics are increase in serum urea and creatinine levels with significant change in the body weight. Many Indian herbs show ameliorating effects against heavy metal such as lead, mercury, and arsenic induced kidney damage. Use of these herbs against nephrotoxicity has gained momentum in recent times. Hence this study aimed to assess the effect of Celastrus paniculatus seeds against lead acetate induced nephrotoxicity in Wistar rats.

Materials and Methods: The animals were divided into five groups, each group with six animals and were treated as follows: Group I, control; distilled water 2mL/kg b.w/day/orally, Group. II, Lead acetate (PbA) 30mg/kg b.w/day/orally; Group.III, PbA + N-acetylcysteine (NAC) 200mg/kg b.w/day/orally; Group. IV & V, PbA + Celastrus paniculatus in two different doses of 400mg and 800mg/kg b.w/day/orally for 28 days were administrated.

Result: Lead acetate significantly decreases the serum urea and creatinine levels. Celastrus paniculatus seeds showed protective effects at 400 and 800mg/kg b.w by significantly reducing the levels of serum urea and creatinine as compared to the lead acetate administrated animals. The data were analyzed with SIGMA PLOT 13 using SYSTAT software and it was expressed as mean± SE and the values with P < 0.05 were considered statistically significant.

Conclusion: To conclude, the results suggest that ethanolic extract Celastrus paniculatus seed exhibits nephroprotective effect against lead acetate induced renal damage and in future, its mechanism of action are validated.

Keywords: Celastrus paniculatus, Kidney, Lead acetate, N-acetylcysteine, and Nephrotoxicity.
INTRODUCTION:

The kidney is a vital organ that is responsible for the maintenance of homeostasis by a sequence of events, the complex process that involves filtration, secretion, and absorption. It excretes toxic substances from the body along with the regular metabolic substances [1]. Therefore, the kidney becomes a major target for the toxicity relative to other organs in the body. Nephrotoxicity due to exposure to a toxin is the most common kidney injury. Among the toxins, lead is the important environmental pollutant which is used in gasoline, fuel, solid waste combustion, industrial processes, paints, ceramics, and dishware [2]. More than 1,000-fold increased in environment level of lead due to the consistent accumulation of lead during the industrial revolution, and particularly between the years 1950 and 2000 because of the use of leaded gasoline [3]. The presence of the predominant number of lead-binding proteins in the kidney leads to early and abundant accumulation of the absorbed lead. These lead-binding proteins act as receptors of lead and also regulate its function [4]. The absorbed lead reduces the levels of antioxidant markers which protects the cells from oxidative stress markers such as hydrogen peroxide, hydroxyl radicals, and lipid peroxidation [5].

Herbal plants have always been used as the major constituent of medicine in the traditional system. In India, among the alternative medicines, herbs are popular because it helps the people to cure illness by synchronizing with the body's self-defense. Literature review shows that many herbs and its extracts have been studied for their nephroprotective property which is because of the presence of flavonoids and other phenolic compounds [6]. Co-administration of herbs containing these bioactive compounds found to possess nephroprotective activity along with various toxic agents may ameliorate its adverse effects.

*Celastrus paniculatus* (CP) described as the ‘Tree of life’ in Ayurveda, it belongs to the Celastraceaefamily that was in use to treat neurological disorders and to improve learning and memory [7]. Previous studies reported that the various extractsof CP seed possess analgesic, anti-inflammatory, sedative, and anticonvulsant properties and it increased the intelligence quotient (IQ) of mentally retarded children [8-11]. But so far no study is available regarding the nephroprotective activity of *Celastrus paniculatus* seeds. Therefore this study was done to investigate the nephroprotective activity by *Celastrus paniculatus* seeds against lead acetate (PbA) induced nephrotoxicity in Wistar albino rats and compared with N Acetyl Cysteine (NAC). In this study, the protective effect of ethanolic extract of CP seeds against lead-induced renal damage by analyzing serum urea and creatinine levels in the rat was studied so that it can be used as a complementary therapy.

MATERIALS AND METHODS:

Chemicals:

The chemicals used in the study were molecular and analytic grade, procured from SISCO research laboratories Pvt Ltd and DK enterprises, India.

Plant material preparation and extraction:

CP seeds were procured from botanist with proper authentication. The seeds were subjected to shadow drying and made into a coarse powder for extraction preparation. The seed sample with a measured amount of 500g powder was soaked in 1L of 90% ethanol. After 72 h, the preparation was filtered and two more separate repeated extractions were done with fresh solvent. The filtrate was combined and evaporated to dryness to get a
viscous residue, the crude extract was then freeze-dried and stored at -4°C until further use and named as the ethanolic extract *Celastrus paniculatus* (EECPL). The extract was dissolved in distilled water for oral administration. Earlier reports revealed the extract is not toxic up to a dose of 5000 mg/kg b.w. in rats [12].

**Animals:**

The study was conducted from September 2019 to February 2020 after getting proper approval from the Institutional animal ethical committee at Saveetha Institute of Medical And Technical Sciences (IAEC Approval No: SU/CLAR/RD/002/2019, dated: 09.08.2019). Female Wistar rats, with a mean weight of 180 ± 20g, four to five months old were obtained from Biogen animal facility, Bangalore. Animals were acclimatized for 10 days before the commencement of experiments and fed with a standard pellet diet and water ad libitum. The experiment was done in accordance with the CPCSEA guidelines.

**Experimental animal care and design:**

The experiment was carried out for 28 days with 30 Wistar rats, animals were divided into five groups, six animals in each group.

- **Group 1:** (Normal Control)  2mL saline /kg b.w/day/orally
- **Group 2:** (Negative Control)  30mg lead acetate /kg b.w/day/orally
- **Group 3:** (Positive Control)  200mg NAC + 30 mg lead acetate /kg b.w/day/orally
- **Group 4:** (Experimental Group I)  400mg EECPL + 30 mg lead acetate /kg b.w/day/orally
- **Group 5:** (Experimental Group II)  800mg EECPL + 30 mg lead acetate /kg b.w/day/orally

After the experiment, the animals were anesthetized using 1% isoflurane in a desiccator chamber for collection of 2mL of blood from retroorbital plexus, all the animals were euthanized to harvest the tissues by dissection. Serum urea and creatinine were determined by the method of Geyer and Dabich (1971), method of Slot (1965) respectively [13,14].

**Data analysis:**

The values were analyzed with SIGMA PLOT 13 using SYSTAT software, USA. The results were expressed as mean±SE and the values with P < 0.05 were considered statistically significant.

**RESULTS AND DISCUSSION:**

**Effects of lead acetate and EECPL on serum urea and creatinine:**

Lead acetate (30mg/kg) when ingested for twenty-eight consecutive days lead to noticeable nephrotoxicity (Table 1), showing a significant increase in serum urea (49 ± 0.5mg/dl), and serum creatinine (1.5 ± 0.1mg/dl) as compared to normal control animals. The co-administration of EECPL with lead acetate significantly alleviated the adverse effects of the lead acetate on serum urea and creatinine compared with the lead acetate group. The ethanolic extract *Celastrus paniculatus* seedshowed protective effects at 400 and 800mg/kg b.w by significantly reducing the levels of serum urea and creatinine as compared to the lead acetate administrated group in a dose-independent manner. There was a significant nephroprotective effect at 800mg/kg
b.w of the *Celastrus paniculatus* with the P valve (*p*<0.05) as compared to the lead acetate and NAC administrated group.

Table 1: Protective effect of *Celastrus paniculatus* on levels of serum urea and creatinine in lead acetate induced toxicity. The values are expressed in mean ± SEM, n=6.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.3± 0.8</td>
<td>0.6 ± 0.03</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>49± 0.5</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Lead acetate + NAC</td>
<td>43± 2.9</td>
<td>0.96 ± 0.1</td>
</tr>
<tr>
<td>Lead acetate + EECPS 400</td>
<td>42.5± 2.0</td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td>Lead acetate + EECPS 800</td>
<td>35± 1.5</td>
<td>0.7± 0.04</td>
</tr>
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Effect of Lead Acetate and EECPL on Body and Organ Weights:

The bodyweight of all the animals was measured at the commencement of the study. Bodyweight and organ (Kidney) weight were measured and the kidney weight to final bodyweight of the animal ratio was calculated after the study. The following formula was used to calculate the percentage of body weight of the animal.

\[
\text{Percentage} = \frac{\text{Final body weight}}{\text{Initial Body weight}} \times 100
\]

Similarly, the percentage of organ weight was calculated with the following formula.

\[
\text{Percentage} = \frac{\text{Organ weight}}{\text{Final Body weight}} \times 100
\]

The percentage of body weight and kidney weights of the different groups was presented (Figure 1). Both lead acetate and lead acetate + NAC group lost weight significantly when compared with control (*p*<0.05) while EECPL group experienced significant weight gain particularly in the EECPL 800mg/kg b.w group as compared with control (Figure 1). There are no significant changes in the kidney weights of the animal across the groups. There was no difference in body weight among all groups at the commencement of the study. Both final body weight and percentage of body weight were significantly decreased in the lead acetate administrated group when compared with the control group. In lead intoxicated animals, EECPL attenuated the decrease in final body weight and the percentage of body weight. The results obtained showed that there was an insignificant difference in kidney weight among all groups. In contrast, a significant increase in kidney weight to final body weight ratio was noted in the lead acetate administrated group in comparison with the control and experimental groups. The gross appearance of the kidney from lead acetate administrated group showed pale appearance, when compared with other groups (Figure 2).
Figure 1: Effect of ethanolic extract of *Celastrus paniculatus* seed 400 mg/kg (EECPL-400) and 800 mg/kg (EECPL-800) compared with n-acetylcysteine (NAC 180 mg/kg) in lead acetate toxicity (PbA, 30 mg/kg) on body weight (%) and kidney weight (%).

Values are mean ± SE (n = 6 each).

The ‘F’ and ‘P’ values are by one way ANOVA with Student Newman Keul’s multiple comparison test.

aSignificantly different from control group.

bSignificantly different from PbA group.

cSignificantly different from NAC group.
Discussion:
Lead acetate is a major environmental pollutant, causing disturbance in the structural and functional integrity of organs, especially the kidney [15-16]. Lead is a major cause of concern among occupational-related health hazards. Absorbed blood lead is then deposited into the tissues, later excreted through the kidney [17].

Figure 2: Showing the renal tissue administered with an ethanolic extract of *Celastrus paniculatus* seed 400 mg/kg (EECPL-400) and 800 mg/kg (EECPL-800) compared with n-acetylcysteine (NAC 180 mg/kg) in lead acetate toxicity (PbA, 30 mg/kg).
The lead acetate administrated animals showed significantly increased serum urea, creatinine which is an indication of impaired kidney function due to toxicity. More specific and sensitive indicator of renal injury is serum urea and creatinine and considered as the best way for evaluation of extending of renal injury in preclinical studies [18]. The results obtained from this study showed increased serum urea and creatinine in nephrotoxic animals that were administrated with lead acetate in comparison to the control animals, these results were similar to Hussein et al., Saka et al. and Aissi et al. [19-21]. Lead acetate administered at 10 mg/kg body weight daily for 40 days in calves, increased the levels of serum urea and creatinine which showed renal damage due to lead toxicity [22]. The glomerular and tubular lesions are a reflection of nephropathy with a clear sign of increased levels of blood urea [23].

Recent studies showed that plants and their products are used for research to understand their therapeutic potential with almost no sideeffects; this study investigated the nephroprotective effect of Celastrus paniculatus. The results indicated that EECPL has a nephroprotective effect against lead acetate induced toxicity, where the co-administration EECPL 400 and 800 mg/kg b.w. showed amelioration of levels of serum urea and creatinine. The nephroprotective property of CP may be due to its ability to control the formation of free radical species in the cells of the kidney intoxicated with lead acetate, thereby preventing renal tissues from cellular damage and induction of oxidative stress. The standard drug, NAC was used due to its ability to normalize the intracellular sulfhydryl, thereby it involves detoxification particularly heavy metal-containing toxins and its metabolites [24].

The results from this study also showed that the administration of lead acetate elicits in a weight reduction of animals. The reduction of body weight occurred due to several factors including the disturbance in digestion, absorption, and metabolism of nutrients from the feed that are essential to maintain proper health, other factors for the reduction were due to altered basal metabolic rate and impaired appetite [25-27]. The observation of body weight reduction in lead-induced toxicity was already reported by some other researchers such as Szymezak et al. (1983) Kamzumman, (2006), and Haque (2005) [28-30]. A significant increase in kidney weight to body weight ratio was observed in lead acetate administrated animals which were due to the deposition of lead in renal tissue. Co-administration of EECPL attenuated both weight reduction and kidney weight to body weight ratio occurred due to the administration of lead acetate. The protective effect of EECPL on appetite, particularly on absorption and deposition of lead in the kidney and thereby ameliorate the adverse effect of lead on liver weight to body weight ratio.

CONCLUSION:
Administration of lead acetate in rats caused nephrotoxicity. Celastrus paniculatus has been proven to be effective in attenuating lead acetate induced nephrotoxicity by its ability to maintain the general health of the animals. This study validated that lead acetate adversely affects the serum renal markers such as urea and creatinine. The adverse effects of lead acetate were greatly reduced when it is co-administrated with Celastrus paniculatus seed extract, suggesting its nephroprotective potential. However, further validation of the bioactive compounds of Celastrus paniculatus seed extract is needed to know the mechanism by which the phytochemicals exert nephroprotective effects.

Ethical clearance: Institutional Human ethical committee were obtained from Saveetha Institute of Medical And Technical Sciences (IAEC Approval No: SU/CLAR/RD/002/2019, dated: 09.08.2019).
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