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Original Research Article

Study of the renoprotective effect of Citrus limon juice and *Emblica officinalis* extract on renal toxicity induced by carbon tetrachloride in Wistar rats

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ABSTRACT

Background: The renoprotective effects of Citrus limon juice and *Emblica officinalis* are not considered till now. Both of them have a strong antioxidant effect.
Methods: Seven groups with six rats in each group. I: Normal control (1 ml distilled water/oral route), Group II: disease control (1 ml distilled water/oral route), group III: Acetylcystine (950 mg/kg), Group IV: Citrus limon (6 ml/kg/oral route), group V: *Emblica officinalis* (700 mg/kg/oral), Group VI: Citrus limon with *Emblica officinalis* (6 ml/kg + 700 mg/kg/oral route) Group VII-Citrus limon with *Emblica officinalis* and acetylcystine (6 ml/kg + 700 mg/kg + 950 mg/kg oral). Only a single dose of CCl_4 was given by intraperitoneal injection of 1.5 ml/kg of 20% carbon tetrachloride dissolved in olive oil for induction of renal injury. One-way ANOVA and paired t-tests were used to analyze data.
Results: The BUN of animals in standard control (Group III) and Citrus limon juice with *Emblica officinalis* alone and in combination (Groups IV, V, and VI) and in combination with the standard drug and Citrus limon juice with *Emblica officinalis* test groups was significantly lower than the disease control group (Group II) ($p<0.05$). Effect on serum creatinine: As seen, serum creatinine was significantly reduced in the positive control (Group III) as compared to the disease control (Group II 4.3) ($p<0.001$).
Conclusions: The present study demonstrated the renoprotective effect of citrus limon juice and *Emblica officinalis* in Wistar rats.

Keywords: Lemon juice, *Emblica officinalis*, Renoprotective

INTRODUCTION

Reactive oxygen species are forms of activated oxygen i.e. free radicals. A disproportionately large number of free radicals and the absence of their scavenging systems in cells lead to oxidative stress resulting increase the risk of several human diseases, like liver injury, carcinomas, and inflammation.[1] The liver is main organ that maintain lipid homeostasis, also susceptible to free radicals injury and damage. The chemical carbon tetrachloride (CCl_4) used to develop experimental animal models of liver failure (caused by free radical production) that mimic human hepatic injury. CCl_4 produces liver injury along with it we also observe toxic effects of it on a kidney, testis, and brain, and the renal injury of CCl_4 is also associated with free radical production.[2-5]

To prevent the damage caused by free radicals, the antioxidant system presents to neutralize it, such as

catalase, superoxide dismutase, and peroxidase.[6] In addition to these natural antioxidants, the natural free radical scavenger also decreases free radical mediated injuries and diseases. The use of antioxidants in the prevention and cure of different diseases is intensifying, and so there is considerable interest in the study of antioxidant activities of molecules, such as citrus limon and *Emblica officinalis*.[6–8]Antioxidants appear to acts by increasing the levels of endogenous antioxidant enzymes and along decreasing lipid peroxidation.[9, 10]

Citrus lemon (*Citrus limon*) is rich a source of vitamin C, flavonoids, and carotenoids.[11] along flavonoids like Eriocitrin and hesperidin. The Eriocitrin is more potent antioxidant than other citrus flavonoids.[12] So we use the lemon as a rich source of antioxidants in the present study.

Emblica officinalis (*Euphorbiaceae*) is commonly known as amla in Marathi and Hindi. In animal studies already shown potent antioxidant, analgesic, antipyretic, adaptogenic, immunomodulatory and antiulcerogenic activities of the fruit of *E. officinalis*.[13,14] As amla has antioxidant properties, we included it in our study to see the nephroprotective activity.

METHODS

Citrus limon

The fresh citrus limon was properly identified and purchased. The citrus limon was authenticated by the botanist in Aurangabad. Juice was collected and stored in a jar.

E. officinalis

E. officinalis was purchased from the market and authenticated by the botanist in Aurangabad.

Preparation of extract E. officinalis

The *E. officinalis* was shed dried and made fine powder by using a mixer. *E. officinalis* powder (5.0 g) was extracted with a mixture of 25 ml of distilled water and 75 ml of ethanol; i.e., a hydroalcoholic was prepared by using a percolator. The extract was dried in fan air and stored in a cool and dry place.

Chemicals

Acetylcysteine (granules) and carbon tetrachloride were used.

Animals

For this study we used Albino Wistar rats of either sex weighing around 150-250 gm. Animals were housed in ventilated animal rooms having a free supply of standard laboratory diet ad libitum and allowed free access to drinking water. The animals were also kept in a 12:12-hour light/dark cycle.

The experimental rats were handled in strict aseptic condition. Prior to initiation of the study, permission of the Institutional Animal Ethics Committee. This study was carried out in Mahatma Gandhi Mission Hospital and Medical College, Chh. Sambhaji Nagar, from 15 January 2022 to 30 March 2022. All guidelines given by the Committee for the Purpose of Control and Supervision on Experiments in Animals (CPSCEA) were strictly followed.

Experimental induction of carbon tetrachloride nephrotoxicity

Carbon tetrachloride-induced acute renal injury was initiated by intraperitoneal injection of 1.5 ml/kg of 20% carbon tetrachloride dissolved in olive oil, as described by Lu et al.[15] CCl_4 was injected intraperitoneally in Wistar rats to produce nephrotoxicity. Blood was collected by retro-orbital plexus and sent for estimation of BUN and serum creatinine levels. and the above test was done before giving the standard and test drugs and after giving the standard and test drugs at the end of the study, i.e., after 6 weeks.

The animals were then randomly divided into seven experimental groups, with six animals in each group, as shown below.

Table 1: Groups of animal and drugs given to each group.

Groups	Drugs	Drug dose
Group I	Normal control	1 ml distilled water/oral route
Group II	CCl_4	1 ml distilled water/oral route
Group III	Acetylcysteine	950 mg/kg
Group IV	Citrus limon	6 ml/kg/oral route for six weeks.
Group V	*E. officinalis*	700 mg/kg/oral route for six weeks
Group VI	*C. limon* + *E. officinalis*	6 ml/kg + 700 mg/kg/oral route for 6 weeks.
Group VII	*C. limon* + *E. officinalis* + Acetylcystine	6 ml/kg + 700 mg/kg + 950 mg/kg oral route for 6 weeks.

Only a single dose of CCl_4 was given to animals for induction of renal injury.

The dose of *C. limon*, *E. officinalis* and acetylcysteine was selected as per its use in previous literature.[16-18]

On the day of commencement of study (day 0), the body weight of the animal was measured; blood was collected by retro-orbital plexus (around 2 ml) and sent for estimation of BUN and serum creatinine levels. The animals were divided into seven experimental groups as shown in (Table 1).

Results were expressed as mean ± standard deviation (SD). The paired data in each group following normal distribution (parametric) was compared using a paired t test. Between the groups, the data that were normally distributed were compared using a one-way ANOVA test.

RESULTS

Baseline measurement as shown in Table 2, the baseline values for body weight, BUN, and Sr. creatinine were compare in all the groups ($p>0.05$). Effect on various parameters on day 42 effect on body weight: As seen in (Table 2), the body weight of rats in all the study groups was comparable ($p>0.05$) on day 42, and there was no statistical difference.

Table 2: Baseline values for body weight, BUN, and Sr. creatinine were compare in all the groups.

Group, (n=6)	Body weight (gm)	BUN (mg/dl)	Sr. creatinine (mg/dl)
Group I	250±5.4	15.4±3.2	0.5±0.12
Group II	234±3	16±2.1	0.68±0.34
Group III	247±4.7	14±3.4	0.43±0.4
Group IV	215±4	16±4.4	0.46±0.25
Group V	269±6	15±3.7	0.6±0.5
Group VI	247±7	14±4	0.58±0.68
Group VII	240±2	15±3.2	0.47±0.76

Baseline values of body weight, BUN and serum creatinine. Values are expressed as mean±SD (n=6). Not significant using the one-way ANOVA test ($p>0.05$). BUN: Blood urea nitrogen Group I: Normal control, Group II: Disease control, Group III: Acetylcystine, Group IV: Citrus limon, Group V: *Emblica officinalis*, Group VI: Citrus limon + *Emblica officinalis*, Group VII: Citrus limon + *Emblica officinalis* + Acetylcystine.

Effect on blood urea

As seen in (Table 3) the BUN of animals in standard control (Group III) and all test groups, it was significantly lower than the disease control group (Group II) ($p<0.05$).

Effect on serum creatinine

Serum, creatinine was significantly reduced in the positive control (Group III) and in the test drugs as compared to the disease control (Group II) ($p<0.001$), but it was not significantly different as compared to the standard drug (Group III).

Table 3: Values of body weight, BUN and serum on day 42. BUN: blood, urea nitrogen.

Group, (n=6)	Body weight (gm)	BUN (mg/dl)	Sr. Creatinine (mg/dl)
Group I	246±4.1	16±1.6	0.43±1.4
Group II	225±4.4	47.22±3.2	4.32±2.6
Group III	245±3	29±1.2	1.5±0.2
Group IV	222±2.5	31±1.5	1.9±1.5
Group V	255±4.5	30±2.1	1.5±2.2
Group VI	251±3.4	26±1	1.3±2
Group VII	238±4	22±1.2	1.2±1.6

Values of body weight, BUN and serum on day 42. BUN: Blood urea nitrogen Group I: Normal control, Group II: Disease control, Group III: Acetylcystine, Group IV: *C. limon*, Group V: *E. officinalis*, Group VI: *C. limon* + *E. officinalis*, Group VII: *C. limon* + *E. officinalis* + Acetylcystine. Values are expressed as mean±SD (n=6). $p<0.05$ vs. group 2 disease Control, and also significant vs. group 3 positive control, One-Way ANOVA.

DISCUSSION

Acute kidney injury is defined as a sudden decline in kidney function, which includes both structural damage and deranged of kidney function test.[19] The acute kidney injury prevalence is rising in developed countries.[20] There are no pharmacologic agents that exist that can be used for prevention or treatment of acute kidney injury.[21] Some herbs and nutraceutical agents like ginger, garlic juice, pomegranate seed oil, *Boerhavia diffusa, Tribulus terresteris, Echinacea pallida*, etc.[22] Lemon, also called citrus limon, has citrate, vitamin C, vitamin E, and flavonoids such as eriocitrin, hesperetin, and limonoids.[23,24] Vitamin E may prevent calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation).[25] In this study we also use *E. officinalis*, commonly called Amla, which contains high levels of vitamin C, tannins, polyphenols (gallic acid and ellagic acid), minerals, fibers, proteins, and lots of amino acids such like glutamic acid, proline, aspartic acid, alanine, cystine, and lysine.[26] Recently, several hydrolysable tannins, flavonoids, and alkaloids.[27] Two such natural plant products are *C. limon* and *E. officinalis* seed, which were found to be beneficial in the chronic pre-clinical models of kidney injury. However, there are no studies evaluating the role of these two plants in preventing nephrotoxicity. Thus, the present study was planned to evaluate the role of these in preventing the development of nephrotoxicity.

The acute kidney injury model of carbon tetrachloride-induced nephrotoxicity was chosen, which causes kidney damage by oxidative mechanisms.

It was found that a 6 ml/kg/day oral dose of *C. limon* juice and a 700 mg/kg/day oral dose of *E. officinalis* showed a nephroprotective effect in carbon tetrachloride-induced nephrotoxicity by preventing a rise in serum creatinine and

BUN. As it compared to the disease control of standard control (acetylcysteine granules given in the dose of 950 mg/kg/oral route), i.e., Groups II and III, which is statistically highly significant.

Although a similar study is not available, "Lemon juice has protective activity in a rat urolithiasis model" by Touhami et al shows that preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation).[28] From this study we can say that the nephroprotective activity of *C. limon* juice is due to its strong antioxidant effect.

Similarly, no study is available for *E. officinalis* as a nephroprotective activity. The study done by Rao, "Amla (*E. officinalis* Gaertn.) extract inhibits lipopolysaccharide-induced procoagulant and pro-inflammatory factors in cultured vascular endothelial cells."[29]

As there is no study for the *C. limon* juice and *E. officinalis* as a renoprotective effect. But in our study, *C. limon* juice decreases the BUN and creatinine levels significantly as compared to disease control and that of the standard drug (as shown in Table 2, $p<0.05$). The same effect occurs in the *E. officinalis* and *C. limon* juice (as shown in Table 2, $p<0.05$). But in group VI we had given *C. limon* juice and *E. officinalis*, both in this group; BUN and creatinine levels decreased significantly as compared to disease control (Table 2, $p<0.001$), but when we compared to standard drug group III Acetylcystine, there was a decrease in BUN and creatinine, but it was not statistically significant. In group VII we combined *C. limon* juice and *E. officinalis* along with acetylcystine; the result was highly significant as compared to disease as well as standard control ($p<0.001$).

From the above result (Table 2), there is a decrease in kidney injury parameters BUN and creatinine levels, which were increased by inducing renal toxicity by carbon tetrachloride in Wistar rats. The probable mechanism of action of *C. limon* juice and *E. officinalis* is the presence of lots of antioxidants, which prevent free radical injury. The main contain of citrate, vitamin C, vitamin E, and flavonoids such as eriocitrin, hesperetin, limonoids.[23,24] *Emblica officinalis*, also called Amla in Hindi, rich in vitamin C, tannins, polyphenols (gallic acid and ellagic acid), minerals, fibers, proteins, and amino acids such as glutamic acid, proline, aspartic acid, alanine, cystine, and lysine.[26] We already know that vitamins C and E have powerful antioxidant properties. The renoprotective effect of *C. limon* juice and *E. officinalis* was attributed to its antioxidant and free radical scavenging properties.

The beneficial effect of *C. limon* juice and *E. officinalis* in the present study could be attributed to the antioxidative potential.

C. limon juice and *E. officinalis* can have therapeutic application in patients (e.g., chronic kidney disease, diabetes mellitus, heart failure, cancer, on medications like NSAIDs, amphotericin, etc.) who are at risk of acute kidney injury. Thus, future studies can be planned to investigate the effect of *C. limon* juice and *E. officinalis* in populations who are at risk of acute kidney injury.

CONCLUSION

The present study demonstrated the nephroprotective effect of *C. limon* juice and *E. officinalis* extract in the model of carbon tetrachloride-induced nephrotoxicity in Wistar rats with acute kidney injury. This nephroprotective effect of these is attributed to its antioxidant potential. The findings of this study should assist researchers in the development of *C. limon* juice and *E. officinalis* nephroprotective agents in humans, especially in patients who are at risk of acute kidney injury.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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