Ameliorative effect of *Cubeba Officinalis* dried fruits against Tacrolimus induced nephrotoxicity in Wistar albino rats

Suman S*, Hayagreeva Dinakar Y, Suhas reddy P V, Sai Sudha Yadav B, Venkateshwar Reddy V

Department of pharmacology, Creative Educational Society’s College of Pharmacy, Kurnool, Andhra Pradesh, India

**Article History:**
Received on: 12.06.2019
Revised on: 20.09.2019
Accepted on: 25.09.2019

**Keywords:**
Cubeba Officinalis dried fruits, Ethanolic extract, Nephrotoxicity, Tacrolimus, Anti-oxidant activity

**ABSTRACT**

*Cubeba Officinalis* is traditionally effective in the treatment of various kidney ailments, and the main adverse effect of tacrolimus is nephrotoxicity. There is no documented evidence about the ameliorative potential of *Cubeba Officinalis* in tacrolimus induced nephrotoxicity. The main endeavor of the study was to determine the nephroprotective activity of ethanolic extract of *Cubeba Officinalis* dried fruits against tacrolimus induced nephrotoxicity in Wistar albino rats. The *Cubeba Officinalis* dried fruits were collected from the local market, and Male albino rats weighing 200-250 g were used for the study. The dose of 200 mg/kg as lower dose and the higher dose of 400mg/kg of test drug (EECO) was used, and silymarin is used as the standard at the dose of 20 mg/kg. The animals were divided into five groups, six animals each, which is started prior to oral administration of tacrolimus and continued with the fourteen days tacrolimus treatment. After the whole period of study, the rats were sacrificed, and histopathological studies and biochemical estimations were carried out. The BUN values were decreased from 33.60±3.84 in nephrotoxic rats to 28.27±2.48 (200mg/kg) and 20.70±0.81 (400mg/kg), creatinine levels from 1.645±0.21 to 0.926±0.19 (200mg/kg) and 0.638±0.07 (400mg/kg), uric acid levels from 1.822±0.249 to 1.092±0.306 (200mg/kg) and 0.806±0.181 (400mg/kg) sodium, potassium and chloride levels from 1.607±0.091, 2.548±0.293 and 259.8±6.42 to 1.302±0.169, 1.023±0.174 and 134.7±9.138 (200mg/kg of EECO) and 0.586±0.092, 0.831±0.174 and 130.2±2.29 (400mg/kg of EECO). The Ethanolic extract of *cubeba officinalis* was found to be effective in treating the nephrotoxicity in tacrolimus induced nephrotoxicity.

**INTRODUCTION**

Nephrotoxicity is a condition in which there is an obstruction to the excretion that occurs when the kidneys are exposed to toxic substances or chemicals and heavy metals (*Soumya et al.*, 2011; *Marcinčáková et al.*, 2018). Nephrotoxicity is the most common disorder, which is fatal. In recent years drug-induced nephrotoxicity is increasing in hospitalized patients who take potent drugs for a prolonged period of time (*Swathi et al.*, 2011). In elderly adults, the prevalence of drug-induced nephrotoxicity is more than 66%. The most prevalent nephrotoxic drugs are analgesics (aspirin, paracetamol), antihistamines (doxylamine), antide-
pressants (amitriptyline), antibiotics (gentamycin), immunosuppressant’s (Tacrolimus), etc. (Naughton et al., 2008).

Tacrolimus is obtained from the fungus Streptomyces tsukubaensis. It is a potent immunosuppressive drug and a macrolide antibiotic whose main adverse effects include nephrotoxicity, neurotoxicity, glucose intolerance, and hypertension. There are two main reasons for tacrolimus induced nephrotoxicity, one being the impaired metabolism and secretion of nitric oxide, prostaglandins, etc. and other being the variation the genes like ACE, TGF-β, CYP2C8 that are crucial for the above processes (Gijsen et al., 2012).

It is well established that many medicinal plants possess potent curable activity against nephrotoxicity. Possessing Antioxidant activity by the medicinal plants is the key factor in the curing of such ailments (Swathi et al., 2011). Cubeba Oficinalis, also known as Piper cubeba or Tailed pepper (English), tokamiriyalu (Telugu), kabab chini (Hindi), etc. Cubeba Oficinalis is a fortified woody stem climber. The heart shape of the leaf is 5-6 inches long and dark green colored. The flowers are tiny and unisexual in clusters. The fruits are little round and resemble pepper, strong flavored. The flowers and fruits are seen in the winter season. The fruits are gathered before they are ripe and carefully dried. The dried pericarp is wrinkled, and the color varies between grayish brown and black. The seed is hard, white, and oily. The plant is seen and cultivated in South India, especially in Kerala and Karnataka.

The traditional system of medicine like Unani and Siddha showed that the plant Cubeba officinalis is effective in treating various genitor urinary tract infections and in gravel and stones from kidney and bladder. It is also known to show positive effects in kidney disorders and hence is the plant of choice in the current study (Aiswarya et al., 2018).

**MATERIALS AND METHODS**

**Collection of Plant material**

The Cubeba Officinalis dried ripped fruits were collected from the local markets of Kurnool (Sirigiri Venkappa Ayurvedic suppliers) and authenticated by CSIR-NISCAIR Ref. number: NISCAIR/RHMD/Consult/2017/3091-40. The dried crude drug was pulverized to get a coarse powder.

**Solvent extraction**

To appreciate the bioactive principle there in the plant, crude drug of the Cubeba Officinalis was sieved (0.2mm), then the powder was crammed in a thimble and is subjected to extraction by using Soxhlet apparatus, ethanol as a solvent. The sample was extracted in the ratio of (1:3) i.e., for one part of the powder material to three parts of solvent.

**Experimental animals**

Male albino rats weighing 150-180g were used for the study. The infection in the untreated animal room ought to be 22°C (+ 3°C). Though the comparative humidity would be at the smallest 30% and, if possible, not exceed 70% other than for the period of room cleaning, the aim should be 50-60%. The animals are haphazardly assigned, clear to license individual documents, and retained in their cages for at least five days before treatment to enable adaptation to the conditions of the study laboratory. The protocol was permitted by the Institutional Animal Ethics Committee (IAEC), Number: IAEC /CESCOP /AUG-18-01.

**Chemicals**

Tacrolimus pure drug was obtained from Hetero labs, Hyderabad. The BUN, uric acid, and creatinine kits are obtained from Agappe ltd, and the electrolyte kit is obtained from excel ltd India. All other chemicals of analytical grade were used.

**Qualitative evaluation of extract**

Different qualitative studies were performed to determine the existence of different phytoconstituents using reported techniques. Alkaloids were tested by dragendorff, Wagner, and Mayer tests, cardenolides were tested by kedde, legal, and Raymond tests while phenolic and flavonoids were tested by ferric chloride, Shinoda, and lead acetate tests. Salkowski test was used for the detection of triterpenoids and steroids, flavonoids. Terpenoids and steroids were detected by vanillinsulphuric acid and Liebermann buchard tests (Kalra et al., 2017).

**Acute toxicity studies**

To determine the lethal dose using Wistar albino mice in the forced environment, the purified and completely dried ethanolic extract of Cubeba Officinalis dried fruits were subjected to the acute toxicity studies. Acute toxicity studies have been conducted in accordance with OECD 423 guidelines. The EECCO (2000 mg/kg, bd. wt) was administered by oral route to a group of rats using an oral feeding needle (22gauge). After treatment to rats were monitored for 14 days.

**Experimental design**

Male albino rats weighing 150-180 g were used for the study. The dose of is lower 200mg/kg, a higher dose of 400 mg/kg was used. The animals were
Table 1: Effect of EECO on serum BUN, Creatinine, and uric acid in tacrolimus (3 mg/kg bd.wt.) induced nephrotoxicity in Wistar albino rats.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>15.09±0.67</td>
<td>0.435±0.09</td>
<td>0.556±0.11</td>
</tr>
<tr>
<td>2</td>
<td>Disease Control</td>
<td>33.60±3.84</td>
<td>1.64±0.21</td>
<td>1.822±0.25</td>
</tr>
<tr>
<td>3</td>
<td>Standard (Silymarin 100 mg/kg, BW, p.o.)</td>
<td>22.91±1.51</td>
<td>0.614±0.12</td>
<td>0.7±0.072</td>
</tr>
<tr>
<td>4</td>
<td>EECO (200 mg/kg, BW, p.o.)</td>
<td>28.27±2.48</td>
<td>0.926±0.19</td>
<td>1.092±0.30</td>
</tr>
<tr>
<td>5</td>
<td>EECO (400 mg/kg BW, p.o)</td>
<td>20.70±0.81</td>
<td>0.63±0.07</td>
<td>0.806±0.18</td>
</tr>
</tbody>
</table>

Table 2: Effect of EECO on serum Sodium, Potassium, and Chloride in tacrolimus (3 mg/kg bd. wt.) induced nephrotoxicity in Wistar albino rats.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>Sodium (mg/dl)</th>
<th>Potassium (mg/dl)</th>
<th>Chloride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>0.407±0.11</td>
<td>0.11</td>
<td>0.66</td>
</tr>
<tr>
<td>2</td>
<td>Disease Control</td>
<td>1.607±0.09</td>
<td>0.09</td>
<td>2.548</td>
</tr>
<tr>
<td>3</td>
<td>Standard (Silymarin 100 mg/kg, BW, p.o.)</td>
<td>0.247±0.05</td>
<td>0.943±0.19</td>
<td>121</td>
</tr>
<tr>
<td>4</td>
<td>EECO (200 mg/kg, BW, p.o.)</td>
<td>1.302±0.17</td>
<td>1.023±0.17</td>
<td>134.7±9.13</td>
</tr>
<tr>
<td>5</td>
<td>EECO (400 mg/kg BW, p.o)</td>
<td>0.586±0.09</td>
<td>0.83±0.17</td>
<td>130.2±2.29</td>
</tr>
</tbody>
</table>

Table 3: Effect of EECO on Lipid peroxidase (µg/mg of protein) and GSH in kidney homogenate induced nephrotoxicity in Wistar albino rats.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Groups</th>
<th>LPO (µg/mg)</th>
<th>GSH (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>135.4±6.52</td>
<td>54.86±2.93</td>
</tr>
<tr>
<td>2</td>
<td>Disease Control</td>
<td>262.4±13.26</td>
<td>25.15±2.46</td>
</tr>
<tr>
<td>3</td>
<td>Standard (Silymarin 100 mg/kg, BW, p.o.)</td>
<td>196.7±6.74</td>
<td>44.32±1.57</td>
</tr>
<tr>
<td>4</td>
<td>EECO (200 mg/kg, BW, p.o.)</td>
<td>198.5±10.16</td>
<td>35.99±1.50</td>
</tr>
<tr>
<td>5</td>
<td>EECO (400 mg/kg BW, p.o)</td>
<td>187.3±18.59</td>
<td>43.08±1.627</td>
</tr>
</tbody>
</table>

divided into five group's six animals each, which was started prior to oral administration of tacrolimus and continued with the fourteen days tacrolimus treatment.

**Group-1**
Normal, received normal diet and water throughout the period of study.

**Group-2**
Disease control treated with (Tacrolimus, 3mg/kg, bd. wt., p.o.)

**Group-3**
Standard drug Silymarin (20mg/kg, bd. wt., p.o.), for consequence 14 days along with Tacrolimus (3mg/kg bd.wt.,p.o.)

**Group-4**
Rats were treated with EECO (100mg/kg, bd. wt., p.o.) for consequence 14 days along with Tacrolimus (3mg/kg bd.wt.,p.o.)

**Group-5**
Rats were treated with EECO (200mg/kg, bd. wt., p.o.) for consequence 14 days along with Tacrolimus (3mg/kg, bd. wt., p.o.).

After the duration of the study, the blood samples were collected from the retro-orbital sinus under anesthetic conditions, and the serum samples were prepared for biochemical estimations. At the same
time, the stomach region was incised, and the kidneys were isolated and the kidneys were subjected to histopathological studies.

**Biochemical estimation**

**Serum analysis**
The collected blood samples were centrifuged at 10,000 rpm for 10 minutes, and the serum samples were subjected to estimation of biochemical parameters like BUN, creatinine, uric acid, and electrolytes (Sodium, Potassium, Chloride).

**Kidney homogenate analysis**
The isolated kidneys were homogenized with homogenizer. The kidney homogenates were subjected to in-vivo antioxidant study using lipid peroxidation [LPO] and glutathione estimation, respectively.

**Histopathological studies**
The animals from all the respective groups were euthanized by using the CO₂ chamber at the end of the study, followed by the isolation of kidneys. The slides were prepared by staining with hematoxylin and eosin and observed under an electron micro-
scope (Swathi et al., 2011)

In-vivo antioxidant activity of cubeba Officinalis dried fruits

Lipid peroxidation [LPO]

The estimation of lipid peroxidation was done according to (Onoja et al., 2014). 2 ml of 10% of tissue homogenate was pipetted out. To this added 2 ml of 30% of Trichloroacetic acid followed by 2 ml of 0.8% thiobarbituric acid reagent (TBA). The test tubes were covered with cotton and were kept in the shaking water bath for 30 minutes at 80°C. Then the tubes were removed and retained in ice-cold water for another 30 minutes. They were then centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was calculated at 535 nm at 37°C against appropriate blank (excluding the homogenate), expressed as nmol formed per milligram of protein in the tissue was calculated using the formula-

Concentration = A X (V/E) X P

Where A is the volume of the solution, E is the extinction coefficient (1.56 x 105 m-1 cm-1), and P is the protein content of the tissue calculated as microgram of protein per milligram of the tissue.

Glutathione Estimation

Glutathione estimate has been performed as per (Kuchta et al., 2011). In the sodium chloride solution, 2 ml of 10% homogeneous was prepared, 2.5 ml of 0.02 m EDTA was added to homogeneous and shaken forcefully. 2 ml of this mixture was taken, and 4 ml of cold distilled water and 1 ml of 50% trichloroacetic acid was added and shaken for 10 minutes. After this, the contents were centrifuged at 3000 rpm for 15 minutes. 2 ml of the supernatant was mixed with 0.4 M TRIS buffer of pH 8.9. The entire solution was well blended, adding 0.1 ml of 0.01 M DTNB. The absorbance of the reagent blank without homogenate was read at 412 nm. Micro mol / mg wet tissue: [A/13600] X Dilution factor X 1000.

Statistical analysis

The data was determined using graph pad prism five software version 5.3. All values are expressed as mean ± S.E.M for six rats in each group. Comparisons made between ***p<0.001, **p<0.01, *p<0.05; Disease Control Vs. Normal, Treatment Vs., Disease control One-way ANOVA followed by Dunnett’s -t-test.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

The percentage yield of ethanolic extract was found to be 3.5%. The grades of qualitative phytochemical studies indicate the presence of phytoconstituents, i.e., phenolics, terpenoids, tannins, flavonoids, Steroids in ethanolic extract of Cubeba Officinalis dried fruits.

Acute toxicity studies

It was confirmed that the EECO is virtually nontoxic in normal rats and falls under the sort of GHS category 5, according to (Anupama & Hunda) 1/10th of the nonlethal dose is considered as an effective dose. From this (200 mg/kg. bd.wt.), & (400 mg/kg. bd.wt) were considered for further evaluation pharmacological studies.

Biochemical analysis

Estimation of BUN

The serum levels of the bun were increased significantly in the disease control group. The standard group (silymarin), low dose (200 mg/kg) and high dose (400 mg/kg) showed considerable fall in the BUN levels compared to the disease control group (table 1).

Estimation of serum creatinine

The serum creatinine levels were increased in the disease control group when compared to the normal group after the indication of the disease. After the treatment, the silymarin treated group showed a considerable fall in the levels, and a high dose (400 mg/kg) group showed a significant decrease in the creatinine levels in comparison with the disease control group (table 1).

Estimation of serum uric acid

Rise in the levels of serum uric acid due to the induction of the disease. The EECO at the high dose was found to be more effective in reducing the levels when compared to the low dose and the silymarin Group (Table 1).

Effect on serum sodium

As depicted in table 2, the standard contributed to the significant fall in the levels of sodium levels, whose levels were increased much higher due to the induction of the disease. While low dose and high dose also lowered the levels to a certain extent.

Effect on serum potassium

The high dose of EECO decreased the levels of serum potassium, which is slightly similar to the levels of the normal group (table 2). While the silymarin and the low dose also reduced the levels to a lower extent.

Effect on serum chloride
Significant rise in the serum chloride levels followed by its amelioration with the administration of different doses of EECO and the silymarin. The high dose of EECO was found to be effective compared to the low dose and the silymarin treated groups (Table 2).

**In-vivo antioxidant activity**

**Lipid peroxidation**

The EECO showed considerable antioxidant potential. The low dose of the EECO was found to be more effective when compared to the standard treated group, and the high dose treated group (Table 3).

**Glutathione estimation**

The low dose of EECO ie, 200 mg/kg, was found to have effective antioxidant activity. While the standard and the high dose of EECO also has better antioxidative potential (Table 3).

**Histopathological studies**

Morsy et al. (2013) In [Figure 1 (a-e)], the histopathological modifications in the kidney segments in the various groups are shown. The key segments in the control group and Silymarin disclosed ordinary glomeruli structure, proximal convoluted tubules, and distal convoluted tubules in the cortex and medulla. Figure 1 [a, c], on the other hand, kidney sections in groups treated with tacrolimus showed some histopathological changes in glomeruli and the urinary tubules such as leukocytic infiltrations, congestion in blood vessels and marked degeneration in proximal and distal convoluted tubules and [Figure 1 b] at the concentration 3 mg/kg bodyweight for 14 days. In compare; kidney sections in rats treated with TAC + EECO revealed a better improvement in glomerular damage with minimal degeneration in tubular cells and the corpuscles shown as normal structure [Figure 1 d-e].

Drug-induced nephrotoxicity is the main precursor for kidney diseases, including acute renal failure and chronic renal failure (Aiswarya et al., 2018). Immunosuppressive agents suppress the immune system in order to avoid graft rejections after organ transplantation. These agents, especially tacrolimus and cyclosporine, are associated with the high risk of toxicities, including renal toxicity, neurotoxicity, etc. (Refaie et al., 2016). After the induction of the nephrotoxicity using tacrolimus, there was a significant reduction in the blood urea nitrogen (BUN) serum creatinine and a raise in serum uric acid (Soumya et al., 2011). After the administration of the test drug, that is, ethanolic extract of (EECO) there was a considerable reduction in the levels of BUN, uric acid, and creatinine, and electrolytes levels were balanced. This may be due to the improved glomerular filtration rate which is caused by the administration of EECO (Olagunju et al., 2009).

**CONCLUSIONS**

The present study demonstrated the ameliorative effect of ethanolic extract of *Cubeba Officinalis* dried fruits against tacrolimus induced nephrotoxicity in Wistar albino rats. The promising results obtained in the present research have led to scientific proof for the ethnopharmacological data on *Cubeba Officinalis* dried fruits at the dose of 400mg/kg. Bd. Wt. Significantly decreases the nephro toxic response of tacrolimus. Hence further studies are required to find promising lead molecule and targeted proteins.

**REFERENCES**


