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

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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(400 mg/kg),uric acid levels from 1.822±0.249 to 1.092±0.306 (200 mg/kg) and 0.806±0.181 (400 mg/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.

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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 Officinalis*, also known as Piper cubeba or Tailed pepper (English), tokamiriyalu (Telugu), kabab chini (Hindi), etc. *Cubeba Officinalis* 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 untried animal room ought to be 22ºC (+ 3ºC). 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 dragendroff, 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 vanillin sulphuric 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 *EECO* (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.

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

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

S.no	Groups	Sodium (mg/dl)	Potassium (mg/dl)	Chloride (mg/dl)
S.no	Groups	Sodium (mg/dl)	Potassium (mg/dl)	Chloride (mg/dl)
1	Normal	0.407±0.11	0.66±0.104	117±5.74
2	Disease Control	1.607±0.09	2.548±0.293	259.8±6.42
3	Standard (Silymarin 100 mg/kg, BW, p.o)	0.247±0.05	0.943±0.19	121±5.137
4	EECO (200 mg/kg, BW, p.o)	1.302±0.17	1.023±0.174	134.7±9.13
5	EECO (400 mg/kg BW, p.o)	0.586±0.09	0.83±0.17	130.2±2.29

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

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

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

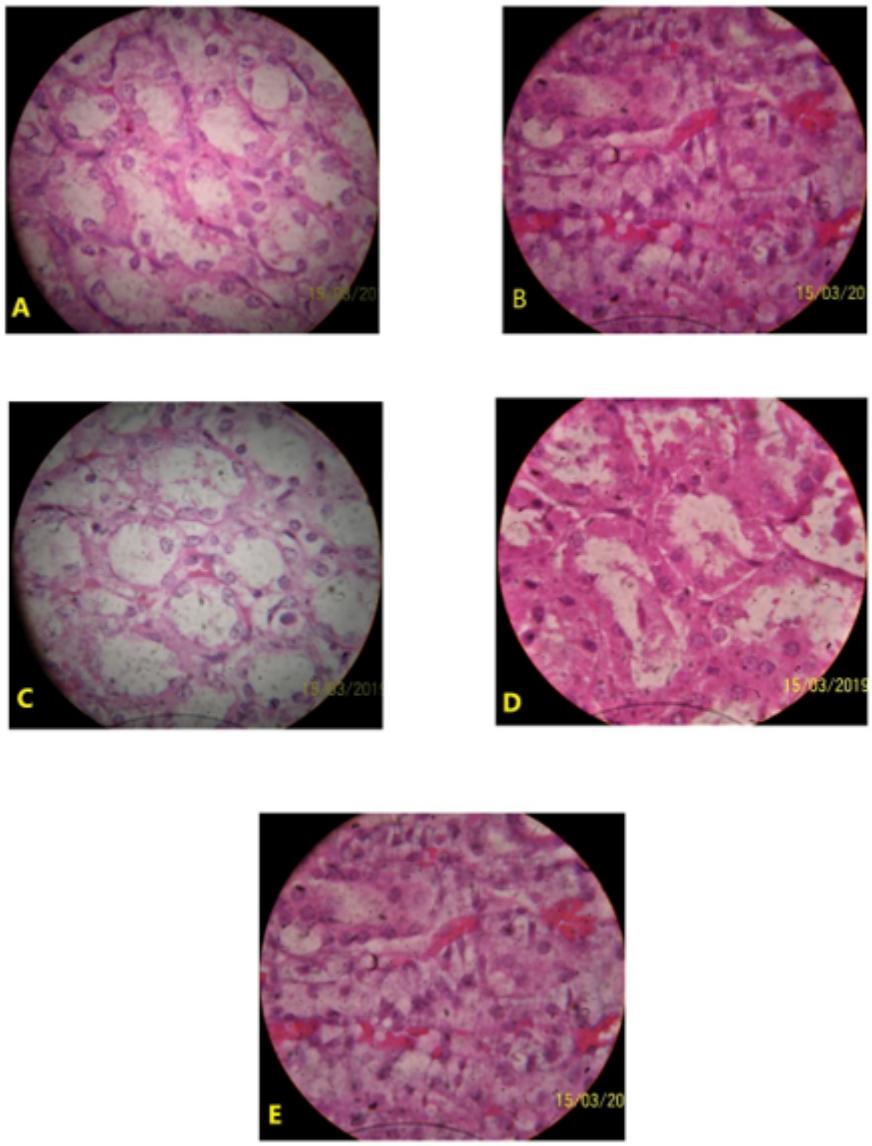

Figure 1: Photo micrographs of kidney sections in the different groups under study stained with H&E. [Figure 1A:Control
Figure 1B:Disease control
Figure 1C:Standard
Figure 1D:EECO (200 mg/kg)
Figure 1E:EECO (400 mg/kg)]

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 CO_2 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 2ml of 30% of Trichloroacetic acid followed by 2ml 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 80^0c. 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^oc against appropriate blank (excluding the homogenate), expressed as n moles 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 105m-1cm-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. 2ml 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 0.4M 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) $1/10^{th}$ 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 (400mg/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 (400mg/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 nephrotoxic response of tacrolimus. Hence further studies are required to find promising lead molecule and targeted proteins.

REFERENCES

Aiswarya, N., Rashmi, R., Preethi, R., ., J., Chandran, S., Teerthanath, V., Sunil, T., ., B., Rakesh, P., ., R. 2018. Nephroprotective Effect of Aqueous Extract of Pimpinella anisum in Gentamicin Induced Nephrotoxicity in Wistar Rats. Pharmacognosy Journal, 10(3):403–407.

Gijsen, V. M. G. J., Madadi, P., Dube, M. P., Hesselink, D. A., Koren, G., Wildt, S. N., De 2012. Tacrolimus-induced nephrotoxicity and genetic variability: A review. Annals of Transplantation, 17(2):111–121.

Kalra, P., Karwasra, R., Nag, T., Gupta, Y. K., Singh, S. 2017. Protective effect of Emblica Officinalis fruit extract on cisplatin-induced nephrotoxicity in female rats. Bulletin of Faculty of Pharmacy, 37(9):1492–1497.

Kuchta, A., Pacanis, A., Kortas-Stempak, B., Çwiklińska, A., Ziętkiewicz, M., Renke, M., Rutkowski, B. 2011. Estimation of Oxidative Stress Markers in Chronic Kidney Disease. Kidney and Blood Pressure Research, 34(1):12–19.

Marcinčáková, D., Falis, M., Schusterová, P., Váczi, P., Marcinčák, S., Legáth, J. 2018. Protective Role of Agrimonia eupatoria L. in Heavy Metal Induced Nephrotoxicity. Folia Veterinaria, 62(2):13–18.

Morsy, M. A., Ibrahim, S. A., Amin, E. F., Kamel, M. Y., Rifaai, R. A., Hassan, M. K. 2013. Curcumin Ameliorates Methotrexate-Induced Nephrotoxicity in Rats. Advances in Pharmacological Sciences, pages 1–7.

Naughton, C., *et al.* 2008. Drug-induced nephrotoxicity. American Family Physician, 78(6):743–750.

Olagunju, J. A., Adeneye, A. A., Fagbohunka, B. S., Bisuga, N. A., Ketiku, A. O., Benebo, A. S., Adeleke, G, A. 2009. Nephroprotective activities of the aqueous seed extract of Carica papaya Linn. in carbon tetrachloride-induced renal injured Wistar

rats: a dose-and time-dependent study. Biol Med, 1(1):11–20.

Onoja, S. O., Omeh, Y. N., Ezeja, M. I., Chukwu, M. N. 2014. Evaluation of the In Vitro and In Vivo Antioxidant Potentials of Aframomum melegueta Methanolic Seed Extract. Journal of Tropical Medicine, pages 1–6.

Refaie, M. M. M., Amin, E. F., El-Tahawy, N. F., Abdelrahman, A. M. 2016. Possible Protective Effect of Diacerein on Doxorubicin-Induced Nephrotoxicity in Rats. Journal of Toxicology, pages 1–9.

Soumya, P. S., Kannappan, P., Ravikumar, G., Kalaiselvi, M., Duraisamy, G., Uma, C. 2011. Nephroprotective effect of Aerva lanata against mercuric chloride-induced renal injury in rats. J Pharm Res, 4(8):2474–2476.

Swathi, N., Sreedevi, A., Bharathi, K. 2011. Evaluation of Nephroprotective Activity of Fruits of Ficus hispida on Cisplatin-Induced Nephrotoxicity. Pharmacognosy Journal, 3(22):62–68.