Studies on the Nephroprotective and Nephrotoxicity Effects of Aqueous Extract of *Cymbopogon Citratus* (Lemon Grass) on Wistar Albino Rats

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Abstract

This study was carried out to investigate the nephroprotective and nephrotoxicity effects of aqueous extract of *Cymbopogon citratus* at a graded dosages(200mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg, 4000mg/kg and 5000 mg/kg) using wistar strain of albino rats. In the determination of the effects of graded dosages of aqueous extract of *Cymbopogon citratus* on the creatinine, total protein and albumin levels, no significant ($p<0.05$) difference was observed in all treated groups when compared with control. Lipid peroxidation was determined by measuring the malondialdehyde level in serum, the result showed no significant ($p<0.05$) difference in all treated groups as compared with control. Similar result was obtained in haematological parameters : packed cell volume (PCV), platelets, total white blood cell count (TWBC) and red blood cell (RBC) results as the values obtained in the extract treated groups was still within normal biological values with respect to control. Histopathology analysis result showed that lemon grass administration caused no observable toxicity in the kidney of albino rats as no visible lesion was seen in the electron photomicrograph of the extract treated groups when compared with the control. In conclusion, aqueous extract of *Cymbopogon citratus* possessed nephroprotective properties.

Keywords: Reactive oxygen species; Nephrotoxicity; aqueous*; Cymbopogon citratus;* kidney; Haematological parameters; Histopathology

INTRODUCTION

Medicinal plants are source of a great economic value (Reische, 1998). The administration of herbal preparations without any standard dosage, coupled with a scarcity of adequate scientific studies on their safety, has raised concerns regarding their toxicity on some organs like the kidney (Tanko *et al.*, 2007). Here comes the opportunity to study the effect of *Cymbopogon citratus* for proper guidance on consumption and further therapeutic uses.

Cymbopogon is a tall, aromatic perennial grass that is native to tropical Asia. *C. citratus* is known as Guatemala, West Indian, or Madagascar lemongrass. *C. flexuosus* is known as cochin lemongrass, British Indian lemongrass, East Indian lemongrass, or French Indian verbena (Blumenthal, 1998). *C. citratus* is cultivated in the West Indies, Central and South America, and tropical regions. The linear leaves can grow up to 90 cm in height and 5 mm in width (Blanco *et al.*, 2009).

As folk medicine in certain parts of Nigeria use the essential oil as an insect repellant. In certain medications, it is used for mental illness (Ebomoyi, 1986). It is an antifungal, antitoxicant and deodorizing agent. In combination with other herbs, it has large use as cure for Malaria (Gbile, 1986). It is used as an antispasmodic, antiemetic and analgesic, as well as for the management of nervous and GI disorders and the treatment of fever (Blumenthal, 1998). In India, it is commonly used as an antitussive, anti rheumatic, and antiseptic. It is usually ingested as an infusion made by pouring boiling water on fresh or dried leaves. In Chinese medicine, it is used in the treatment of headaches, abdominal pain, and rheumatic pain (Girón *et al.*, 1991).

Fresh *C. citratus* grass contains approximately 0.4% volatile oil. The oil contains 65% to 85% citral, a mixture of 2 geometric isomers, geraniol and neral. Related compounds geraniol, geranic acid, and nerolic acid have also been identified (Torres, 1993). One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*) is citral (3,7-dimethyl-2,6-octadien-1-al) (Balbaa and Johnson, 1955; Banthorpe *et al.*, 1976).

Thus, the purpose of this study, therefore, is to investigate the toxicological effects of aqueous extract of lemon grass *(Cymbopogon citratus)* on kidney using albino rats as laboratory models.

Materials and methods

Cymbopogan citratus was harvested and collected freshly from a native farms and authenticated in Environmental Biology Laboratory, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo.

Preparation of plant extract

The fresh plant was washed, chopped into pieces and air-dried at room temperature. The dried plant part was milled into powder and weighed. The Plant powder was soaked in 90% absolute ethanol for 72 hours with intermittent shaking. Then, it was filtered through a muslin clothe and later Whatman No. 1 filter paper. The resulting filtrate was evaporated under reduced pressure using a rotary evaporator and there after freeze dried to get powder form aqueous extract. The yield was stored in a refrigerator (4°C) till when needed (Onoagbe *et al.*, 1999).

Chemicals and Reagents

All chemicals were of an analytical grade and are supplied from sigma chemical co. USA. Distilled water was used in all biochemical assays.

Experimental animal

Male albino rats (Wistar strain) weighing between 109-170g, purchased from the central animal house of University of Ibadan were used for the study.

Acclimatization: 15 days prior to dosing.

Identification of animals: By cage number.

Diet: Pelleted feed

Water: Potable drinking water

Housing & Environment: 4 animals each in a group

Determination of the weight of animals

The weights of the animals were weighed using an electronic weighing balance every 7 days to verify and quantitate the change in weight over the period of administration.

Animal ethics

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments.

Experimental design

Group I: Normal control (distilled water)

Group II: 200mg/kg *Cymbopogon citratus*

Group III: 500mg/kg *Cymbopogon citratus*

Group IV: 1000mg/kg *Cymbopogon citratus*

Group V: 2000mg/kg *Cymbopogon citratus*

Group VI: 4000mg/kg *Cymbopogon citratus*

Group VII: 5000mg/kg *Cymbopogon citratus*

Method of administration

Oral administration of the extracts through the use of oral gavage.

Duration of treatment: 30 days

Chemicals and reagents preparation

All chemicals were if an analytical grade and are supplied from sigma chemical co. USA. Distilled water was used in all biochemical assays.

Blood Biochemistry

Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis free clear serum for the analysis of creatinine (Bartels *et. al.* 1972), total protein (Gornal *et al.* (1949), albumin (Bacon, 1947) and Malondialdehyde (Rice-Evans *et al*., 1986)

Haematology

The method used as the impedance method for determining the packed cell volume, WBC, RBC, and platelets data. The analysis cycle, the sample is aspirated, diluted and mixed before the determination for each parameter is performed.

Histopathology

Small pieces of tissues were collected in 10% formaldehyde solution for histopathological study. The pieces of the liver was soaked in formalin for 6 hrs, embedded in paraffin wax and the sections were made about 4-6µm in thickness. They were stained with hematoxylin and eosin and photographed (Arthur and John, 1978).

Statistical analysis

The experimental results were expressed as the mean ± S.E.M. Statistical significance of difference in parameters amongst groups was determined by One way ANOVA followed by Duncan's multiple range test. $P<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Table 1: Effects of oral administration of aqueous extract of *Cymbopogon citratus* on serum kidney functions enzymes in normal wistar albino rats.

Group (mg/kg)	Creatinine (µmol/l)	Malondialdehyde (Unit/gprotein) (10^{-6})	TotalProtein (g/dl)	Albumin (g/dl)

Control	532.57 ± 39.81[b]	21.07 ± 0.49[a]	11.24 ± 2.14[b]	3.44 ± 0.12[a]
200	524.80 ± 33.52[b]	23.01 ± 0.31[a]	13.53 ± 0.36[b]	4.47 ± 0.71[a]
500	461.55 ± 55.93[a]	23.54 ± 0.92[a]	10.94 ± 1.56[b]	5.51 ± 0.92[b]
1000	583.15±77.71[b]	27.10 ± 0.41[ab]	7.93 ± 1.04[a]	5.19 ± 0.48[b]
2000	470.95 ± 6.43[a]	39.53 ± 0.47[c]	11.05 ± 0.23[b]	4.49 ± 0.60[a]
4000	240.2 ± 40.59[a]	32.10 ± 0.53[b]	7.71 ± 0.04[a]	7.45 ± 0.10[c]
5000	924.25 ± 32.03[c]	30.80 ± 0.44[b]	8.32 ± 2.25[a]	7.45 ± 0.13[c]

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different ($p < 0.05$)

The determination of the effects of graded dosages aqueous extract of *Cymbopogon citratus* on the creatinine, total protein and albumin levels showed an increase but not significant ($p<0.05$) difference in all treated groups when compared with control.

Also, lipid peroxidation was determined by measuring the malondialdehyde level in serum, the result showed no significant ($p<0.05$) difference in all treated groups as compared with control.

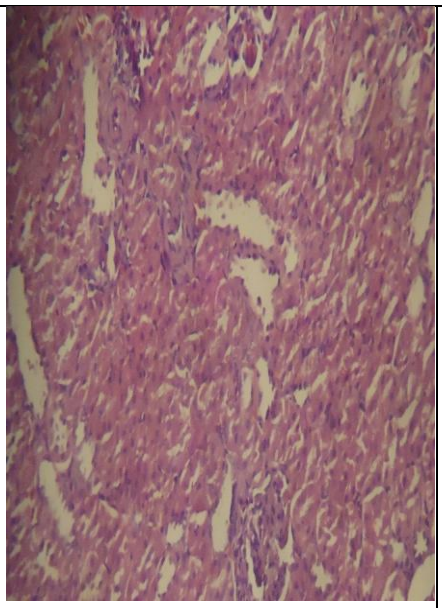
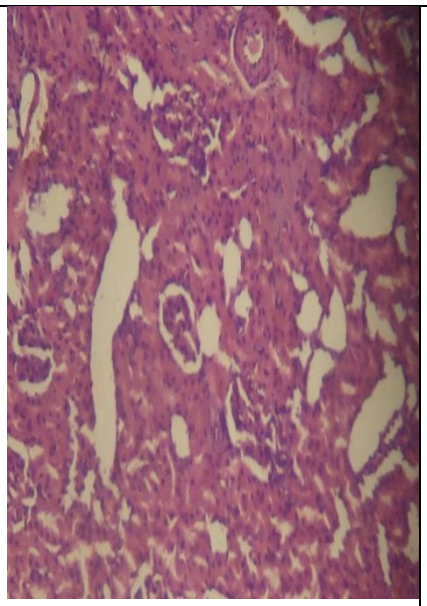
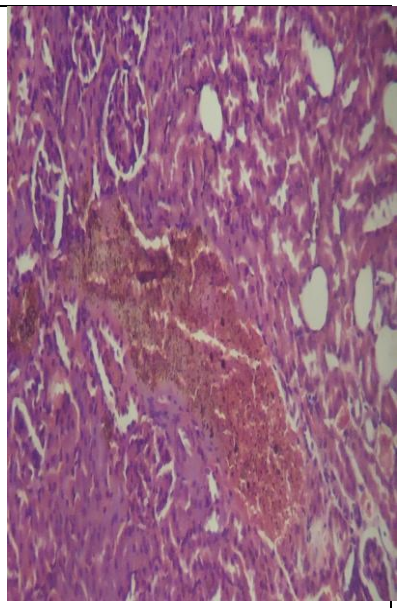
TABLE 2: Effects of oral administration of aqueous extract of *Cymbopogon citratus* on Haematological parameters in normal rats.

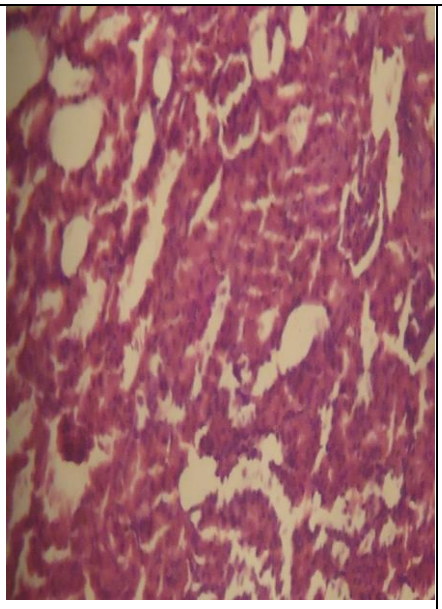
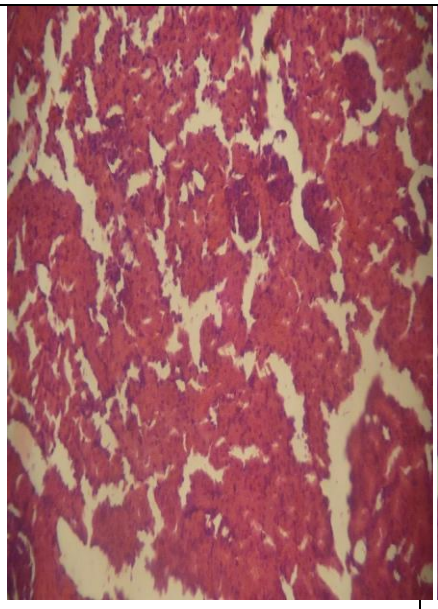
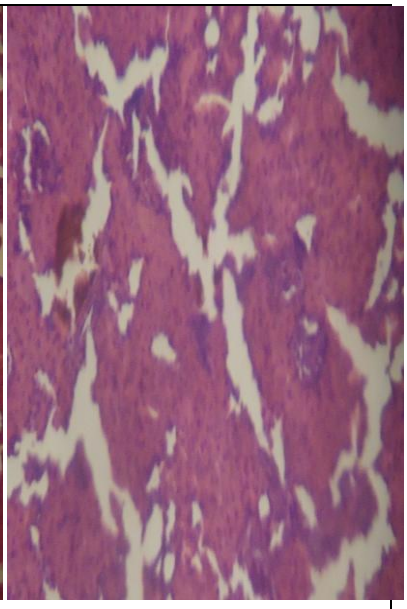
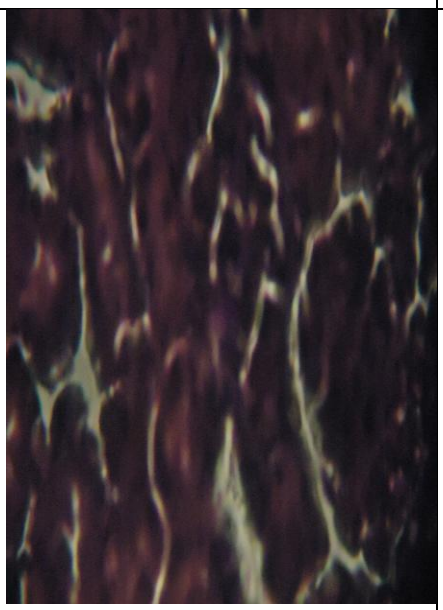
Group (mg/kg)	**PCV (%)**	**PLATELETS (cmm)/1 X 10^4**	**TWBC (10^{12}/mm^3) X 10^3**	**RBC (10^{12}/ml)**
Control	49.67 ± 3.86[b]	68.00 ± 1.17[ab]	4.50 ± 0.53[b]	8.79 ± 0.70[b]
200	51.00±3.61[b]	79.70 ± 1.86[b]	5.00 ± 1.27[b]	8.89 ± 0.75[b]
500	56.00 ± 5.66[c]	76.15 ± 1.52[b]	6.15 ± 2.19[a]	9.46 ± 0.90[c]
1000	47.50±2.12[a]	60.95 ± 0.60[a]	4.00 ± 0.85[b]	8.75 ± 0.31[b]
2000	48.00± 1.00[b]	84.47 ± 1.36[c]	3.93 ± 2.25[c]	7.55 ± 0.06[a]
4000	48.50± 3.54[b]	96.10 ± 1.63[c]	5.35 ± 1.06[a]	9.04 ± 0.24[c]
5000	46.30 ± 2.06[a]	67.40 ± 3.46[a]	4.89 ± 0.90[b]	8.92 ± 0.61[bc]

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different ($p < 0.05$)

In table II, Haematological investigations revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; However, the increase or decrease ($p<0.05$) in the values obtained were significant was still within normal biological and laboratory limits or the effect was not dose dependent. In this haematological evaluation, marked decrease in RBC and PCV were observed in the ethanolic extract treated group. The decrease in RBC was an indication of changes in the rate of the RBCs production. In this context, the possibility that the extract does have the potential to stimulate erythropoietin release in the kidney was likely. A decrease was also noted in the platelets levels in extract in the group treated with 1000mg/kg and 2000mg/kg respectively.

PLATE 1: Showing the Histopathology of the kidney of albino rats in various groups

		
Group I: Control	**Group II: 200mg/kg**.	**Group III: 500mg/kg**

		
Group IV: 1000mg/kg	**Group V: 2000mg/kg**	**Group VI: 4000mg/kg**
		
Group VII: 5000mg/kg		

From the plates above, group I represent the Control group of animal treated only with distilled water and the result showed no visible lesion. The 200mg/kg treated group showed no visible lesions as the collecting ducts appear dilated. Meanwhile, other treated groups (500mg/kg, 2000mg/kg, 4000mg/kg and 5000mg/kg) showed similar result of no visible lesion when compared with the control.

DISCUSSION

The administration of herbal preparations without any standard dosage, coupled with a scarcity of adequate scientific studies on their safety, has raised concerns regarding their toxicity (Saad *et al.*, 2006). To determine the safety of drugs and plant products for human use, toxicological evaluations are carried out on various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dosage in humans. The highest overall concordance of toxicity in animals with humans is with hematological, gastrointestinal and cardiovascular adverse effects (Olson *et al.*, 2000), whiles certain adverse effects in humans, especially hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals. Furthermore, it is quite difficult to ascertain certain adverse effects in animals, such as headache, abdominal pain, dizziness and visual disturbances. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals to humans. Hence the need to study the effect of *Cymbopogon citratus* for proper guidance on consumption.

Previous results on acute toxicity study of both aqueous extract of lemon grass (*Cymbopogon citrates*), no mortality was recorded in any of the experimental groups in 24 hours. According to toxicity classes of Hodge and Sterner (2005), any compound with oral LD_{50} (rat) of 5000mg/kg or more should be considered as practically harmless. Diets containing tannins at low dosages (0.15 – 0.2%), have been shown to improve well – being of the human body (Shiavone *et al.*, 2008). Significant toxicity is usually as a result of suicide attempt or inappropriate self-administration for therapeutic purposes (Raffi and Mark, 2009).

From table 1 above, aqueous extract *Cymbopogon citratus* causes no changes in the serum total protein titer when compared with the control. The fact that proteins present several features as potentially interesting biomarkers of toxicity they might serve as peripheral indicators of toxic events in relatively inaccessible target organs **(**Bernard *et al.*, 1995). Protein titers stability after *C. citratus* extract administration demonstrate the fact that this plant did not exhibited any protein degradation leading to propose a non toxic effect at the levels primary organ, in this case the liver.

To ascertain the oxidative status of the experimental animals treated with *extracts of C. citratus*, serum MDA levels were assessed (Table 1), the effects of aqueous extracts of *C. citratus* on the oxidative status of normal rats were monitored at pre-determined intervals in the serum for 4 weeks by measuring the concentration of malondialdehyde (MDA) (lipid peroxidation). For the

aqueous extract, there was a significant ($p>0.05$) decrease in the level of malondialdehyde (MDA) at all dosages when compare with control. And no any significant ($p<0.05$) difference was observed in the aqueous extracts treated group at 4000mg/kg dosages when compared with the control.

Oxidative stress is characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems (Adewole and Caxton-Martins, 2006).

Jyoti *et al.* (2004) reported that *Ocimum sanctum* extracts administered to normal rabbits for 30 days significantly reduced serum MDA levels. Several reports also indicate that hypoglycemic plants reduced MDA levels of streptozotocin/alloxan-induced diabetic rats (Adewole and Caxton-Martins, 2006; Mahdi *et al*., 2003; Paris and Umamaheswari, 2000), the reductions observed in our study agree with this. Taken together the results for serum MDA levels indicate that the administration of these plant extracts did not exert lipid peroxidation, in some instances they were even protective against it. Prakasam *et al.* (2005) reported that *Casearia esculenta* root extract restored the increased kidney MDA levels in streptozotocin-induced diabetic rats to non-diabetic. Thus, from our results, the cell membrane integrity is being maintained by the administration of the aqueous extract of *C. citratus*

In table 2, Haematological investigations revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; However, the increase or decrease ($p<0.05$) in the values obtained were significant was still within normal biological and laboratory limits or the effect was not dose dependent. In this haematological evaluation, marked decrease in RBC and PCV were observed in the aqueous extract treated group and another decrease in RBC and WBC in the aqueous extract treated group as compared to the control. The decrease in RBC was an indication of changes in the rate of the RBCs production. In this context, the possibility that the extract does have the potential to stimulate erythropoietin release in the kidney was likely. A decrease was also noted in the platelets levels in both extracts in the group treated with 1000mg/kg and 2000mg/kg respectively. This is similar to results obtained with some other plants (Polenakovic and Sikole, 1996; Sanchez-Elsner *et al*., 2004). From this result, the extract did not significantly alter the calculated RBC indices which were indicative of its minimal effect on the size of RBC and in Hb weight per RBC. This implies that ethanolic extract of *Cymbopogon citratus* does not possess the potential to induce anaemia. Inflammatory process is characterized by the involvement of multiple inflammatory cells of the WBC (Kytridis and Manetas, 2006). WBC and indices relating to it

such as lymphocytes usually show increase in activity in response to toxic environment (Robins, 1974). In this study, WBC was not significantly altered while lymphocytes, the main effectors cells of the immune system (McKnight *et al.*, 1999) showed marginal increase thus suggesting that the extract only exerted minimal challenge on the immune system of the animals.

The effects of anaemia are greatly influenced by its severity, duration and rate of development (Taiwo & Anosa 1995; Macfarlane *et al*, 2001). It may thus be safe to conclude that the ethanolic extract of the leaves of *Cymbopogon citratus* can prevent toxic effects on the red blood cells of rats.

In plate 1, histological studies showed that aqueous extract (200 mg/kg, 500 mg/kg, 1000 mg/kg, 2000mg/kg, 4000mg/kg and 5000 mg/kg body weights) of *C. citratus* was safer as no adverse effects were observed in the kidneys examined.

The histopathological results showed that no degenerative conditions and no necrotic changes in the tubular epithelia of the kidney with cellular infiltration was observed in the all the treated groups when compared with control. This effect agree with the theory of target organ toxicity (Heywood, 1981) since the kidney is the organ of excretion (Parke, 1982). The histological effects observed in this experiment is in contrast with the report of Manjrekar *et al. (*2008) who observed that P. amarus induced deleterious changes on the renal tubules and testes of male rats (Manjrekar *et al.*, 2008). It is also in contrast with the reported effects of damiana (*Turnera diffusa*) on matured Wistar rats where distortion of the renal cortical structures, reduced number and size of the renal corpuscles were observed (Enaibe *et al.*, 2007).

Apart from that, histological analysis was done to further confirm the alteration in cell structure of organs. The histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organs (OECD, 1995).

Apart from that, the acute toxicity study conducted on *C. fistula* pod extract and histological examination of the organs of rat treated with extract at a dose of 1000 mg/kg revealed that there was no potential toxicity or damage to the cell structure of liver, kidney and testes. Also there was no necrosis, inflammatory reaction, fibrosis or local fatty degeneration observed in kidney and the arrangement of cell structure almost similar to the organs of rats in control groups (Akanmu *et al.*, 2004). The morphology of kidney cell in both control and treated groups are normal and no structural damages were observed.

Also in contrast to our result, the study conducted by Alade *et al*. (2009) revealed the histology of kidney observed with focal proximal tubular epithelial necrosis, meanwhile there was

variation in the lung between the control and treated with rat *B. monandra* leaf extract at dose 4 g/kg. Apart from that, the study conducted by Akanmu *et al*. (2004) on *C. fistula* pods extract revealed that there were slight changes in the histology of kidney from the rat treated with extract at dose 1000 mg/kg where some of the glomeruli and the proximal tubules was observed to widen without any injury compared to the control.

Nephroprotective effects of other plants have also been reported such as naringenin (50 mg/kg/day) decreased the toxicity of cadmium and preserved the normal histological structure of the renal tissue (Renugadevi and Prabu, 2009) and *Allium ascalonicum* provided protective effects against cyclosporine induced renal damage (Wongmekiat *et al*., 2008).

CONCLUSION

In conclusion, ethanolic extract of *Cymbopogon citratus* (Lemon grass) whole plant materials possessed nephroprotective properties as no adverse toxicity was seen in the kidney of the treated rats and thus recommended to be taken because it has many beneficial effects in human health.

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