RESEARCH ARTICLE

PROTECTIVE AGENT, KIGELIA AFRICANA FRUIT EXTRACT, AGAINST CISPLATIN-INDUCED KIDNEY OXIDANT INJURY IN SPRAGUE-DAWLEY RATS

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Abstract: The protective effect of methanol extract of *Kigelia africana* fruit extract (KAFE) against cisplatin-induced renal toxicity in male rats was studied. Over 28 days, cisplatin-treated rats suffered significant loss in body weight ($p<0.05$), elevation in blood urea nitrogen (BUN) ($p<0.001$) and serum creatinine (Scr) ($p<0.001$) levels as well as tubular necrosis. However, KAFE alone and as a prophylaxis significantly ($p<0.05$) increased these parameters. Though post-treatment of animals with KAFE after cisplatin did not restore serum catalase activity it was lower than those receiving cisplatin alone. These results suggest that KAFE may protect against cisplatin-induced renal toxicity hence might serve as a novel combination agent with cisplatin to limit renal injury.

Keywords: *Kigelia africana*, cisplatin, catalase, BUN, creatinine, histology.

INTRODUCTION

Kigelia Africana (*Lam Benth*) is a tropical African plant widely grown and distributed in South, Central and West Africa. It belongs to the family of *Bignoniaceae* and commonly called the Sausage tree because of its huge fruits. In Nigeria, it is called 'pandoro' (Yoruba), 'uturubien' (Ibo) [1] and 'Hantsar giiwaa' [2] (Hausa). The tree is widely grown as an ornamental plant in tropical regions for its decorative flowers [3]. The sausage tree has a long history of use by rural African communities especially for its medicinal properties [4]. The fruits are believed to be a cure for a wide range of ailments including rheumatism, snakebites, evil spirits and veneral diseases like syphilis. The fruits are a popular source of traditional medicine throughout Africa [4] and beyond [5].

Kigelia africana fruit pulp and extracts have been exploited in a variety of ways; traditional/folklore, dietary/herbal supplement, cosmeceutical, nutraceutical and pharmaceutical purposes. It has strong anti-oxidative effects against hepatotoxicity induced by paracetamol toxicity [6]. It is speculated that the antioxidant activity is attributed to the caffeic acid derivative [7, 8] and compounds unique to *Kigelia* [9]. Other notable bioactivities include its antimicrobial action against sexually transmitted diseases [10], anti-protozoal activity against *Plasmodium falciparium*, *Tripanosoma cruzi*, *Tripanosoma brucei* and *Leishmania major* [11, 12], anti-amoebic activity against *E. histolytica* [13] anti-diarrhoeal activity [14], anti-inflammatory/analgesic activity [15] and anticancer activity [16, 17].

The *Bignoniaceae* family is noted for the occurrence of iridoids, naphthoquinones, flavonoids, terpenes, tannins, steroids, saponins and caffeic acid [4, 11-13, 15-17] in the fruits, stem, leaves and roots.

The anti-oxidant actions of *Kigelia africana* have been attributed to the abundance of flavonoids [18, 19] and saponins in the fruits [20]. *Kigelia africana* is reported to have low acute systemic toxicity [5].

Cisplatin (cis-diaminedichloroplatinum II) has become one of the most effective and widely used anticancer agent against various forms of solid tumours of the testes, bladder, ovary, lungs, head and neck. However, its usage has been plagued with many side effects, chiefly nephrotoxicity and testicular damage [21-23] which limits the dosage that can be administered. Several strategies have been explored to reduce the side effects of cisplatin therapy including aggressive hydration with saline, the use of less intensive treatment/or analogues and often with mannitol dilution. However, all have shown limited success. The understanding of the mechanism(s) for this side effect should allow clinicians to prevent/or treat this problem better as well as allow higher doses of cisplatin to be administered for better curative treatments. Based on these findings and others, this work investigates the effect of administration of *Kigelia africana* fruit extract on cisplatin-induced renal toxicity in Sprague-Dawley rats.

MATERIALS AND METHODS

Plant materials

Matured fruit of *Kigelia africana*, harvested in November 2007, was obtained from the local market in Badagry, Lagos and authenticated at the Forestry Research Institute of Nigeria, Ibadan where a voucher specimen number- FHI/08257 was recorded for ease of identification. The fruits were washed, cut into small pieces, sun dried and coarsely powdered. The powdered fruit was weighed and kept for further phyto-chemical analysis/extraction.

Extraction of plant material

The powdered extract weighed 1.15 kg before Soxhlet extraction. Alcohol extraction yielded 115.33 g which represented 10.03%. This was stored in universal bottles and refrigerated at 4 °C prior to use.

Drugs and chemicals

All chemicals were standard laboratory reagents and solvents of Analar grade and were obtained from registered distributors hence used without further purification.

(a) Cisplatin solution (Platinol-AQ) used was manufactured by Korea United Pharm. Inc., Chungnam, Korea.

(b) Ketamine was manufactured by Rotex Medica, Trittau, Germany.

Acute toxicity test (LD_{50})

Forty male Sprague-Dawley rats (200 ± 20g) were obtained from the Animal House of the College of Medicine of the University of Lagos, Nigeria. The animals were randomly divided into eight groups of 5 animals per group. The animals were fed with pelleted rat chow and had free access to drinking water but were starved for 12 hours prior to testing. The extracts were orally administered as 100, 400, 800, 1600, 3200, 6400, 12800 mg/kg alcoholic extract of *Kigelia africana* fruit extract (KAFE). Group one was given distilled water (10 ml/kg) as control. General symptoms of toxicity and mortality were observed for 24 hours for any sign of delayed toxicity (Lorke, 1983).

Animal treatment

All experimental procedures were approved by the Departmental Ethical Committee of the College of Medicine, University of Lagos. Thirty five 10-12 weeks old male Sprague-Dawley rats weighing 180-200g were used for the experiments. The animals were obtained from the Animal House of the College of Medicine, University of Lagos. They were kept in the Animal Room of the Department of Anatomy under standard conditions of temperature (27-30°C), with a 12-hour light: 12-hour dark cycle to acclimatize for two weeks prior to the commencement of the experiment. All animals were allowed unrestricted access to water and commercial rat pellets and properly housed in wire mesh cages. Animals were divided into 7 groups (Group A, B, C, D, E, F and G) of five rats per group. Group A control rats received 1 ml normal saline. Group B animals received 100 mg/kg KAFE while group C received 500 mg/kg

KAFE. Group D received a single dose of 10mg/kg of cisplatin which has been used previously to induce toxicity in various animal studies [23]. The LD_{50} of cisplatin in rats is 12 mg/kg body weight [24, 25]. Group E animals were administered KAFE and cisplatin (10 mg/kg) together. Group F animals were given KAFE for two weeks before cisplatin (10 mg/kg) administration. Group G animals were given cisplatin (10 mg/kg) and KAFE was administered after two weeks. All treatment was administered orally through a metal oro-pharyngeal cannula except for cisplatin and normal saline that were given intraperitoneally.

Male Sprague-Dawley rats of approximately equal weight were chosen for the study to minimise the effect of inter-individual differences. External influences on kidney function were minimised through standardised animal care and injection of equal quantities of fluid for administration.

Collection of sera and urine

The cages had special provisions for urine collection (metabolic cages). Urine was collected by means of special canisters that were attached to the cages for the last night before the end of the experiment and analysed. At the end of the experiment, animals were anaesthetized by intraperitoneal injection of ketamine following which a laparotomy was done and the kidneys were clearly dissected out for further analyses. Blood was collected by cardiac puncture using a hypodermic syringe and delivered into plain bottles and centrifuged for 15 minutes at 3000 rpm using a bench centrifuge in order to separate the plasma. The plasma was collected and stored at 4 °C for subsequent assay.

Assessment of kidney function

Serum creatinine and urea were determined at 37 °C colorimetrically using the modified Jaffe method and the modified Berthelot-Searcy enzymatic method respectively. They were assayed using reagents obtained from assay kits (Human Gesellschaft fur Biochemica und Diagnostica, Germany. Kits number 10051 and 10505 respectively). Urinary protein was quantified by the Biuret method using bovine serum albumin as the standard.

Histopathological analysis

The kidneys were removed and fixed in 10% neutral buffered formalin for at least 24 hours. Tissues were processed for microscopic examination using a standard protocol and paraffin sections were stained with hematoxylin and eosin.

Statistical analysis

Results were expressed as means ± standard deviation and subjected to statistical analysis using the Student's t-test and analysis of variance (ANOVA) and the Turkey's post-hoc test. The significance level considered was $p < 0.05$.

RESULTS

LD_{50} Studies

The extract was well tolerated by the animals as there were no observable signs of acute toxicity effects like restiveness, seizures or dizziness after the administration of 400 mg/kg. However, at 6400 mg/kg, the animals showed signs of toxicity like jerks and writhes with 60% death. At 12,800 mg/kg, there was 80% death of the animals. The LD_{50} was estimated from a log-dose curve to be 3,981.07 mg/kg (Figure 1).

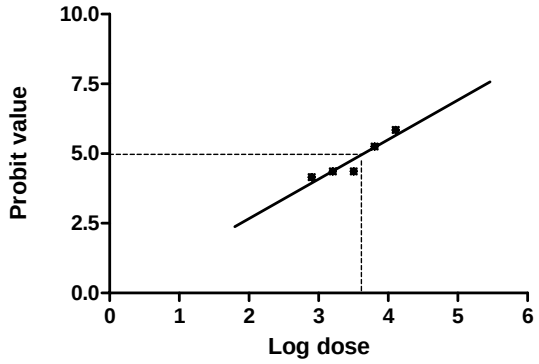


Figure 1. Log-dose response curve of the methanolic extract of *Kigelia africana* fruit extract

Body weight

Body weights of rats were significantly increased after 4 weeks in groups A ($p<0.05$), B ($p<0.01$), C ($p<0.001$) and F ($p<0.001$) while group D rats suffered a significant ($p<0.01$) loss in body weights. Though there was an increase in weight in group E, it was not statistically significant. Likewise the decreased body weight in group G was not statistically significant. Also, KAFE 100 mg/kg treatment alone and as prophylaxis prior to cisplatin recorded the highest percentage weight gain of 8.24 and 9.67 respectively (Table 1).

Functional nephrotoxicty indices such as blood urea nitrogen (BUN) and serum creatinine (Scr) were elevated in cisplatin-treated rats compared with the control. BUN was significantly higher in the cisplatin group ($p<0.001$) but lower in the KAFE (100 mg/kg) group ($p<0.001$). Pre-treatment with KAFE two weeks before cisplatin prevented the increases in BUN and as well as tubular damage (Figure 2). All the treatment groups recorded significant increases in urinary creatinine levels except group B receiving 100 mg/kg KAFE (Figure 3).

Table 1. Effects of KAFE and cisplatin on body weight of Sprgaue-Dawley rats after 4 weeks

Groups	Initial BW (g)	Final BW (g)	% Weight gain	% Weight loss
A	184.0±4.18	192.8±6.22$^{\alpha}$	4.56$^{\alpha}$	-
B	186.0±4.18	202.8±5.22$^{\beta}$	8.28$^{\beta}$	-
C	184.2±4.27	199.0±2.65$^{\gamma}$	7.44$^{\gamma}$	-
D	188.4±5.90	165.6±8.41$^{\beta}$	-	13.77$^{\beta}$
E	183.6±4.98	184.8±4.15	0.65	-
F	185.0±4.12	204.8±5.54$^{\gamma}$	9.67$^{\gamma}$	-
G	186.0±6.52	181.6±8.17	-	2.42

Where α: $p<0.05$; β: $p<0.01$; γ: $p< 0.001$; BW: body weight expressed as mean ± SD; n: number of rats

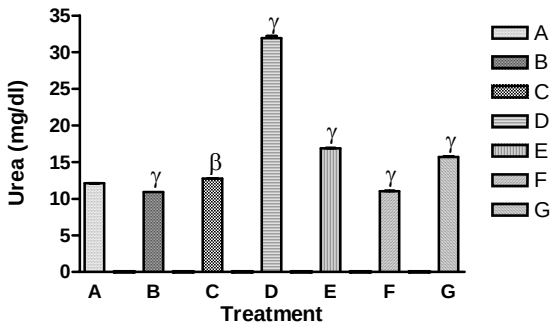


Figure 2. Effects of KAFE and cisplatin on blood urea nitrogen in Sprague-Dawley rats after 4 weeks, Where β: $p<0.01$; γ: $p< 0.001$

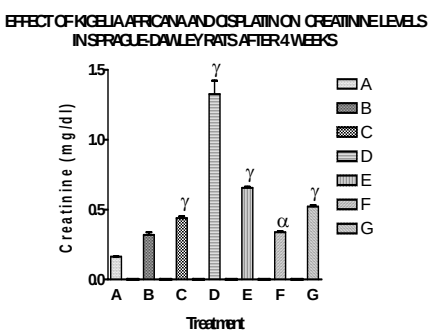


Figure 3. Effects of KAFE and cisplatin on urinary creatine levels in Sprague-Dawley rats after 4 weeks , Where α: $p< 0.05$; γ: $p< 0.001$

Urinary protein excretion was significantly elevated in the cisplatin group while KAFE (500 mg/kg) alone and KAFE pre-treatment lowered this parameter when compared with the control ($p<0.001$). Co-administration of KAFE and cisplatin did not significantly reduce urinary protein. Post-treatment with KAFE after cisplatin injection restored this parameter to almost normal (Figure 4).

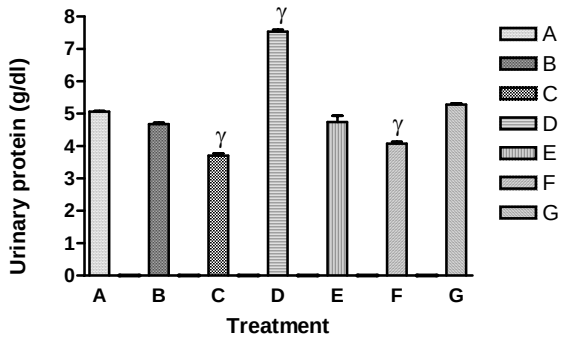


Figure 4. Effects of KAFE and cisplatin on urinary protein levels in Sprague-Dawley rats after 4 weeks, Where γ: $p< 0.001$

Catalase, which acts as a preventive antioxidant enzyme plays an important role in protection against the deleterious effects of lipid peroxidation was significantly ($p<0.001$) increased by KAFE (100 mg/kg) alone and as a prophylaxis. Cisplatin significantly ($p<0.001$) lowered this parameter. The serum catalase activity in groups E and G were equally lower than the control but higher than the cisplatin group (Figure 5).

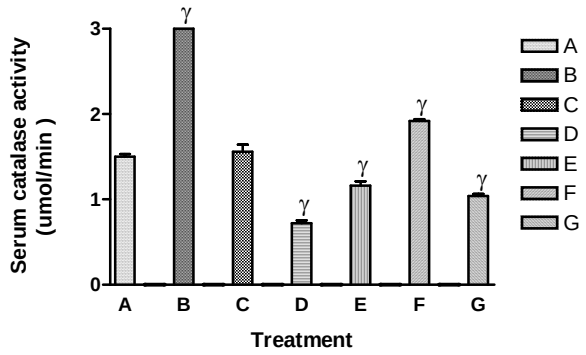
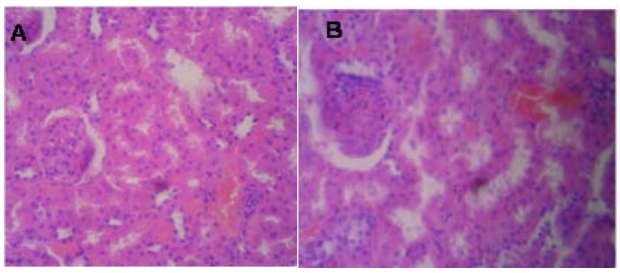


Figure 5. Effects of KAFE and cisplatin on serum catalase activity in Sprague-Dawley rats after 4 weeks, Where γ: $p< 0.001$

Histopathological changes

Histopathological sections of kidneys in control rats revealed adequate preservation of tubular structures with the presence of glomerular capsule. Animals receiving 100 mg/kg KAFE showed more cellularity than those receiving 500 mg/kg. There was distortion of renal architecture in cisplatin-treated rats as shown by degenerated tubular structures and less cellularity. Equally, sections of kidney tissue treated with KAFE prophylaxis produced similar histological patterns similar to those of the controls. However, administration of KAFE two weeks after cisplatin treatment failed to restore the histological patterns to normal. Sections showed degenerated/atrophic renal tubules and marked oedema (Figure 6).



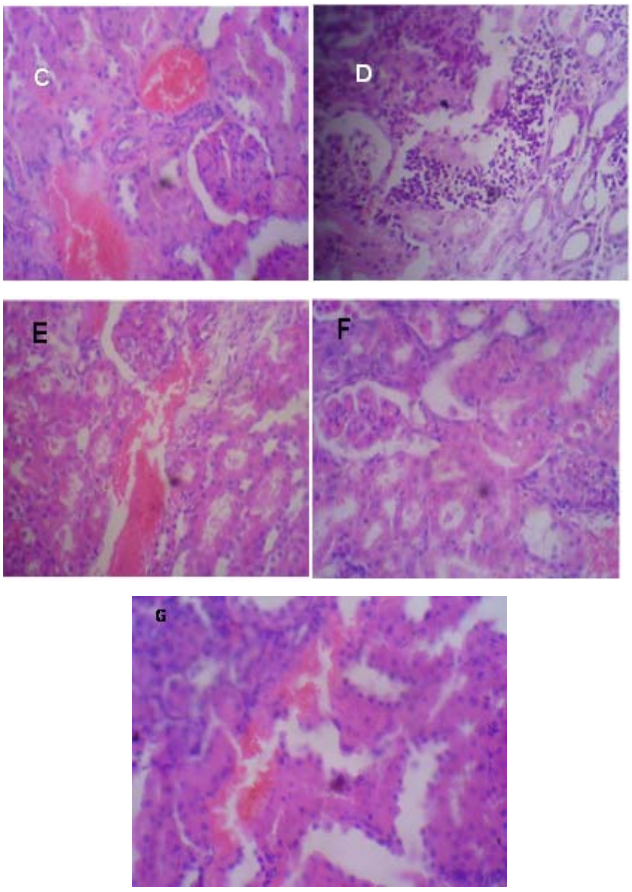


Figure 6. Histological sections of kidney (H&E x400) of group A (control); group B (100 mg/kg KAFE); group C (500 mg/kg KAFE); group D (Cisplatin 10 mg/kg); group E (CISPLATIN +KAFE); group F (100 mg/kg KAFE followed by Cisplatin); group G (Cisplatin 10mg/kg followed by KAFE)

Mortality

Five of the ten rats that were originally started in the cisplatin treated group died. Two of the seven rats in the cisplatin and *Kigelia africana* group died as well as two in the last group treated with KAFE after cisplatin. Other groups recorded nil mortality.

DISCUSSION

The kidney plays a major role in the maintenance of constant volume and composition of the extracellular fluid (ECF) hence does the basic functions of glomerular filtration, tubular reabsorption and tubular secretion [26]. Kidney function can be evaluated by a number of methods. Male rats are particularly a suitable animal model for evaluating tubular lesions because their intra-renal enzyme distribution is similar to that in man [27].

Cisplatin, a chemotherapeutic agent has been successfully used in clinical oncology against diverse types of animal and human malignancies [28]. It is an efficient platinum-derived alkylating agent which acts against proliferating and resting cells [29]. DNA-damaging agents usually have less toxicity in non-proliferating cells, yet the quiescent proximal tubule cells are selectively damaged by cisplatin via mechanisms that remain the focus of current investigations. However, studies suggest that inflammation, oxidative stress injury and apoptosis probably explain part of cisplatin injury [30].

The result of this study showed that the decline in body weight was attenuated by pre-treatment with KAFE at 100 mg/kg suggesting that KAFE could posses some protective constituents that prevented the loss of weight in the experimental animals following cisplatin administration. Polyuria uniformly accompanies cisplatin administration and occurs in two distinct phases. Phase one occurs within the first two days after administration but resolves spontaneously. It is characterised by decreased urine osmolality but stable glomerular filtration rate (GFR). Phase two starts between three to four days after administration and is

characterised by decreased GFR, urea cycling effect which causes derangement in ionic metabolism of sodium, potassium, magnesium and calcium [30]. We adduce that these concomitant losses in ions/fluids might be responsible (in part) for the weight loss. It could also be as a result of diminished appetite of the rats treated with cisplatin.

KAFE also produced impressive survival benefits in this study. It is not unlikely that the improved survival in the group receiving *Kigelia africana* prophylaxis followed by cisplatin relates to its nephroprotective effect.

That KAFE reduced the extent of cisplatin-induced nephrotoxicity is evidenced by the significant reduction in serum urea concentration as well as marked reduction in urinary protein which agrees with the work of Badary et al. [31].

Morphological and physiological studies have identified the renal tubule systems as the site of maximum cisplatin damage; therefore any direct protective effect of KAFE would be apparent on the tubule system. The histological results reveal widespread acute tubular necrosis, focal segmental glomerular degeneration in cisplatin treated rats. This concurs with previous reports that revealed degeneration, necrosis and desquamation of epithelial cell with consequent cyst formation and interstitial fibrosis [30] in cisplatin treated animals. However, KAFE at a low dose produced more cellularity in glomerular histology and pre-treatment with KAFE ameliorated the overt changes induced by cisplatin.

Oxidative stress has been recognised as a major pathway of cisplatin nephrotoxicity [32]. Antioxidant enzymes are inhibited by cisplatin and renal activities of catalase, superoxide dismutase and glutathione are reduced significantly. The reactive oxygen species (ROS) produced directly act on cell components including lipids, proteins and DNA and destroy their structure. The consequence of this is oxidative stress and cell death. KAFE is rich in flavonoids and saponins which have been ascribed strong antioxidants properties. It could possibly have a renoprotective effect in cisplatin-treated rats by exerting its beneficial effects via modulating the antioxidant system. Together, the results suggest the beneficial effects of using a free radical scavenger in modulating cisplatin-associated nephrotoxicty.

Oxidative stress-induced lipid peroxidation generates numerous electrophilic aldehydes that can attack many cellular targets. These products can also slow cell cycle progression of cancer cells and cause cell cycle checkpoint arrest, an effect that may interfere with the ability of the anticancer drugs to kill cancer cells. They may also inhibit drug-induced apoptosis by inactivating caspase activity which would diminish the efficacy of treatment. The use of antioxidants during chemotherapy may enhance therapy by reducing the generation of oxidative-stress induced aldehydes [33].

Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction proteins in the renal tubules [34, 35]. It is therefore important that the nephroprotective agent is present in renal tissue before damage occurs. This might explain why complete protection did not result when KAFE was given two weeks after administration of cisplatin. The acute renal failure indicated by increased Scr and BUN occurred before the development of tubular necrosis. These parameters are markers of glomerular filtration rate. However, it cannot be excluded that the enhancement of these parameters may be the result of tubular obstruction or tubular back-leak [36].

The mechanism(s) by which KAFE ameliorate cisplatin toxicity remains to be elucidated. We suppose they may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione [37]. Previous works by researchers have indicated that superoxide anions inactivate nitric oxide (NO) and that NO-dependent vascular relaxation is enhanced by superoxide dismutase [38, 39]. There is therefore the possibility that they could have maintained renal blood flow (RBF) as a result of preserved NO through scavenging of the superoxide anions. However, further research is needed to determine the exact mechanism of action. Also, whether nephrotoxicity of cisplatin is cumulative remains controversial [40] but creates another area for future research.

CONCLUSION

While research to providing critical clues to novel therapeutic interventions aimed at minimising cisplatin-induced nephrotoxicty with enhancement of its antineoplastic efficacy continues, our present finding suggests that *Kigelia africana* fruit extract offers such a strategy to counter/protect the ravages of reactive oxygen molecules in renal tissues and may be considered as potentially useful candidate in the combination chemotherapy with cisplatin.

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