Modulatory effect of Mangifera indica against carbon tetrachloride induced kidney damage in rats

Olufunsho AWODELE 1, Adejuwon Adewale ADENEYE 2, Sheriff Aboyade AIYEOLA 1, Adokiye Senibo BENEBO 3

1 Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Science, College of Medicine, University of Lagos, Ido-Araba, Surulere, Lagos State, Nigeria
2 Department of Pharmacology, Faculty of Basic Medical Science, Lagos State University College of Medicine, Ilaje G.R.A., Lagos State, Nigeria
3 Department of Pathology and Forensic Medicine, Faculty of Basic Medical Science, Lagos State University College of Medicine, Ilaje G.R.A., Lagos State, Nigeria

ABSTRACT
There is little scientific evidence on the local use of Mangifera indica in kidney diseases. This study investigated the reno-modulatory roles of the aqueous stem bark extract of Mangifera indica (MIASE) against CCl₄-induced renal damage. Rats were treated intragastrically with 125, 250 and 500 mg/kg/day MIASE for 7 days before and after the administration of CCl₄ (3 ml/kg of 30% CCl₄, i.p.). Serum levels of electrolytes (Na⁺, K⁺, Cl⁻; HCO₃⁻), urea and creatinine were determined. Renal tissue reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT), superoxide (SOD) activities were also assessed. The histopathological changes in kidneys were determined using standard methods. In CCl₄ treated rats the results showed significant (p<0.05) decreases in renal tissue SOD, CAT and GSH and significant (p<0.05) increases in MDA. The oral MIASE treatment (125–500 mg/kg) was found to significantly (p<0.05) attenuate the increase in serum electrolytes, urea and creatinine. Similarly, MIASE significantly (p<0.05) attenuated the decrease in SOD, CAT and GSH levels and correspondingly attenuated increases in MAD. Mangifera indica may present a great prospect for drug development in the management of kidney disease with lipid peroxidation as its etiology.

KEY WORDS: Mangifera indica; stem bark aqueous extract; carbon tetrachloride; reno-modulation; antioxidant

Introduction

Kidney diseases occur in all age groups with incidence between 1.5 per million and 3.0 per million in children (Fogo, 2007). Among the causes of kidney diseases are congenital abnormalities of the kidney and urinary tract, focal segmental glomerulosclerosis, hemolytic uremic syndrome, immune complex diseases (Foreman & Chan, 1988), exposure to drugs and chemicals. One of our previous studies (Awodele et al., 2010) and several other studies have however underscored the significant role of oxidative stress and lipid peroxidation in kidney diseases (Reeder et al., 2002; Reeder et al., 2008). In addition to its role in renal diseases, lipid peroxidation has also been documented to be one of the major mechanisms in the toxicity of drugs and environmental agents (Awodele & Akintonwa, 2012).

Traditional medicinal plants have been largely used in developing countries to supplement orthodox medicines and the use of these herbal preparations have been supported by the World Health Organization (WHO), provided their non-toxicity was established (WHO, 1985). Apart from using medicinal plants for curative purposes, several studies have shown the potentials of some medicinal plants in preventing and protecting against some systemic diseases and organ damage. Kaur et al. (2010) showed the modulatory role of alizarin from Rubia cordifolia L. against genotoxicity of mutagens; Kitagishi et al. (2012) demonstrated the protection offered by medicinal herbs against cancer mediated via the activation of tumor suppressor and Kumar et al., 2011 highlighted the immunomodulatory effects of some traditional medicinal plants. The commonest modulatory mechanism of these medicinal plants is via expression of antioxidants and scavenging of free radicals.
The tree *Mangifera indica* (family: Anarcardiaceae) is among the most economically and culturally important tropical rainforest medicinal plants in Asia and Africa, especially due to its edible fruits. It is widely known as Mango. Studies have reported *Mangifera indica* fruit (mango) to possess anti-diabetic, anti-oxidant, anti-viral, cardiotoxic, hypotensive, and anti-inflammatoryary properties (Barreto et al., 2008). The stem bark of *Mangifera indica* has been reported to exert several pharmacological activities with anti spasmodic, analgesic, antipyretic, anti-oxidant, anti-tumor, anti-viral, anti-diabetic, anti-bone resorption and immunomodulatory effects (Kumar et al., 2009). These findings are very encouraging and indicate that this herb should be studied more extensively to confirm the results and reveal other potential therapeutic effects. In African traditional medicine, in particular among Yoruba, Hausa and Igbo communities in Nigeria, various parts of *Mangifera indica* trees are used in the treatment of different human and veterinary diseases, including malaria (Ene et al., 2010), dysentery, cough, typhoid fever infection (Alo et al., 2012). It is also an anti-diuretic, anti-emetic and cardiac herb (Barreto et al., 2008). A preliminary ethnobotanical survey of its use conducted among traditional herbalists in Lagos metropolis (Southwest Nigeria) showed that hot and cold water infusion of *Mangifera indica* stem bark is highly valued in the local management of both liver and kidney diseases. Recently, the aqueous stem bark extract of *Mangifera indica* was reported to offer protection against CCL\(_4\)-induced hepatotoxicity in Wistar rats (Adeneye et al., 2015). Nevertheless, there is a dearth of scientific investigation into the possible protective role of the aqueous stem bark extract of *Mangifera indica* against nephrotoxicity. Thus the presented explorative study was aimed at confirming or refuting the value of the folkloric use of water infusion of *Mangifera indica* stem bark in the local treatment of renal diseases. Thus the reno-modulatory roles of 125–500 mg/kg/day of the *Mangifera indica* stem bark aqueous extract were investigated in CCL\(_4\)-induced nephrotoxicity in adult Wistar rats.

**Material and methods**

The plant collection and identification, preparation of the plant extract, qualitative phytochemical analyses of aqueous stem bark extract of *Mangifera indica* (MIASE), acute oral toxicity test of MIASE using preliminary dose test of up and down procedure were carried out as previously reported by Adeneye et al. (2015).

**Experimental animals**

The purchase of experimental animals, acclimatization, housing and feeding were done as documented in our previous study (Adeneye et al., 2015). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of Health (NIH Publication No. 85-23, revised 1996) for studies involving experimental animals.

**Experimental design**

Drug-induced renal toxicity models applied in conducting this study used 30% carbon tetrachloride dissolved in olive oil according to the modified method of Lu et al. (2002). The study was performed in two phases (chemopreventive and curative) with each phase involving 36 male Wistar rats. The rats in each model were grouped into six groups of six rats each – three control and three treatment groups (Adeneye et al., 2015).

**Induction of CCL\(_4\)-induced nephrotoxicity and oral drug treatment in the chemopreventive model**

In this model of chemically-induced nephrotoxicity, rats were randomly divided into 6 groups of 6 rats each so that the weight differences within and between groups did not exceed ±20% (Adeneye et al., 2015). The treatment protocols included: Group I (Control): 10 ml/kg of 0.9% normal saline; Group II: 10 ml/kg of 0.9% normal saline; Group III: 10 mg/kg of ascorbic acid; Group IV: 125 mg/kg of MIASE; Group V: 250 mg/kg of MIASE; Group VI: 500 mg/kg of MIASE.

The aforementioned oral treatments were applied for seven consecutive days and twenty-four hours after the last oral pretreatment with ascorbic acid and graded doses of MIASE, the rats in groups II–VI were treated with single intraperitoneal injection of 3 ml/kg of 30% CCL\(_4\) dissolved in olive oil. Ascorbic acid, being a known potent antioxidant and nephroprotectant, was used as standard reference drug. The treated rats were then sacrificed humanely forty-eight hours post-CCL\(_4\) treatment.

**Induction of CCL\(_4\)-induced nephrotoxicity and oral drug treatment in the curative model**

In this model of chemically-induced nephrotoxicity, the rats were also randomly divided into 6 groups of 6 rats each so that the weight differences within and between groups did not exceed ±20% (Adeneye et al., 2015). The treatment protocols included: Group I (Control) 1 ml/kg of 0.9% normal saline intraperitoneally; Group II: 3 ml/kg of 30% CCL\(_4\) i.p 1 hour before oral treatment with 1 ml/kg of 0.9% normal saline; Group III: 3 ml/kg of 30% CCL\(_4\) i.p 1 hour before oral treatment with 10 mg/kg ascorbic acid; Group IV: 3 ml/kg of 30% CCL\(_4\) i.p 1 hour before oral treatment with 125 mg/kg of MIASE; Group V: 3 ml/kg of 30% CCL\(_4\) i.p 1 hour before oral treatment with 250 mg/kg of MIASE; Group VI: 3 ml/kg of 30% CCL\(_4\) i.p 1 hour before oral treatment with 500 mg/kg of MIASE.

Each treatment lasted 7 days. Twenty-four hours after the last treatment on day 7, the rats were sacrificed humanely under diethyl ether anesthesia (Adeneye et al., 2015).

**Collection of blood samples and kidneys for renal tissue oxidative stress markers**

The blood samples and kidneys for renal tissue oxidative stress markers were collected using the methods as described by Adeneye et al. (2015).
Determination of kidney tissue antioxidant activities and lipid peroxidation
The methods described by Adeneye et al. (2015) were used to determine the superoxide dismutase, catalase, reduced glutathione and malondialdehyde activities.

Determination of serum renal function parameters
Serum creatinine determination
An aliquot of 0.5 ml of serum sample was added to 3.5 ml of picric acid. The mixture was centrifuged for 5 minutes. 3 ml of the supernatant was taken and to this 0.2 ml of 4N NaOH was added. The mixture was incubated for 1 minute and the absorbance was read at 520 nm. The concentration of creatinine was determined.

Serum urea determination
0.1 ml of serum sample was added into a universal bottle containing 19.9 ml of distilled water and the suspension was well shaken. 1 ml of the suspension was transferred into a test tube and 1 ml of color reagent was added followed by 1 ml of acid reagent. The mixture was heated in boiling water for 20 minutes. It was then cooled and the absorbance was read at 520 nm against blank.

Serum electrolyte determination
Serum levels of sodium, potassium, chloride, calcium, bicarbonate and phosphate were determined using the ISE 6000 BYY SFRI spectrophotometer. When powered on, the machine carries out self-calibration for all parameters. When calibration is complete, the sample is placed into the probe and the tun button on the machine is pressed on the screen of the machine. The machine aspirates the sample and beeps with a screen display “remove sample”. The machine then processes the sample and displays the result of the test. The results of the test are printed out, showing all the required electrolyte levels, namely: sodium, potassium, chloride, bicarbonate, calcium and phosphate.

Histopathology of the kidneys from treated rats
The remaining of the pair of kidneys harvested was gently but briskly rinsed in 0.9% normal saline and fixed in 10% formaldehyde. The kidneys were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). Photomicrographs were taken using an optical microscope (Nikon Eclipse 80i) with a digital camera (Nikon DS-Fi2). The slides were arranged in a traditional manner for comparison with control slides.

Statistical analysis
Statistical analysis was performed using Graph Pad Prism (Graph Pad Software – Version 5.0. Graph Pad Software Inc., La Jolla, California, U.S.A.). Data were expressed as mean ± S.D. for body weights and relative kidney weights and mean ± S.E.M. for biochemical and hematological assays. The data were analyzed using the one-way ANOVA for comparison between the control and treated groups and post hoc test conducted using Newman-Keuls’-test. The level of statistical significance was considered at p<0.05, p<0.001 and p<0.0001.

Results

Plant extraction and phytochemical analysis of Mangifera indica aqueous stem bark extract
A yield of 15% was obtained. Alkaloids, tannins, cardiac glycosides, flavonoids, phlobatannins, reducing sugars and saponins were contained in the extract as reported in our previous study (Adeneye et al., 2015).

Preliminary limit dose test of the up-and-down procedure of the acute oral toxicity test of MIASE in Wistar rats
Table 1 shows that doses of up to 5000 mg/kg of MIASE resulted in no mortality. However, behavioral toxicities such as body scratching, feed refusal, reduced locomotor activity, and watery stools were observed, as earlier reported in our study (Adeneye et al., 2015).

Effect of 125–500 mg/kg of MIASE on average body weight and relative organ weight in the chemopreventive model of CCl4-treated rats
Table 2 shows the effect of MIASE oral pretreatments on average body weight and relative organ weight of the kidney of CCl4-treated animals on days 1 and 7 of the experiment. Intraperitoneal treatment with CCl4 caused significant (p<0.0001) weight loss and non-significant weight reduction in the relative organ weight of the kidneys of CCl4-treated rats compared to control (Table 2). However, oral pretreatments with 125–500 mg/kg/day of MIASE and subsequent intraperitoneal treatment with CCl4 caused significant dose-dependent (p<0.05, p<0.001

| Groups     | Average body weight (g) on Day 1 | Average body weight (g) on Day 8 | Relative kidney weight |
|------------|----------------------------------|----------------------------------|------------------------|
| I          | 166.30±39.42                     | 173.20±36.03                     | 0.88±0.31              |
| II         | 201.50±22.59                     | 171.20±29.14                     | 0.69±0.23              |
| III        | 190.70±29.97                     | 150.50±29.04                     | 0.69±0.13              |
| IV         | 200.20±24.70                     | 184.00±29.31                     | 0.76±0.21              |
| V          | 196.00±25.88                     | 178.20±28.51                     | 0.74±0.20              |
| VI         | 204.30±14.39                     | 164.70±14.62                     | 0.76±0.25              |

Table 1. Sequence and results of the limit dose test of MIASE in young female Wistar rats.

| Test sequence | Dose (mg/kg) | Short-term result (48 h) | Long-term result (12 days) |
|---------------|-------------|--------------------------|---------------------------|
| 01            | 5000        | Survival                 | Survival                  |
| 02            | 5000        | Survival                 | Survival                  |
| 03            | 5000        | Survival                 | Survival                  |

Adeneye et al., 2015
Table 3. Effect of oral pretreatment with 125–500 mg/kg/day of MIASE on serum Na⁺, K⁺, Cl⁻, HCO₃⁻, urea and creatinine in CCl₄-treated rats.

| Groups          | Na⁺ (mmol/l) | K⁺ (mmol/l) | Cl⁻ (mmol/l) | HCO₃⁻ (mmol/l) | Urea (mmol/l) | Creatinine (µmol/l) |
|-----------------|--------------|------------|-------------|----------------|---------------|---------------------|
| I               | 146.5±0.76   | 11.38±0.97 | 113.90±1.65 | 18.63±1.31     | 11.23±0.53    | 58.92±3.86         |
| II              | 194.80±0.71  | 7.55±0.52  | 142.40±1.01 | 11.30±0.41     | 16.11±0.41    | 93.37±2.80         |
| III             | 142.90±1.30  | 11.58±0.42 | 105.70±1.48 | 17.43±0.68     | 8.33±0.39     | 9.42±4.47          |
| IV              | 162.50±1.10  | 13.97±1.79 | 126.80±4.49 | 13.22±0.94     | 15.48±1.39    | 65.10±3.65         |
| V               | 143.20±0.56  | 10.95±0.50 | 121.00±1.88 | 14.88±1.41     | 11.13±1.00    | 63.78±3.65         |
| VI              | 138.70±3.68  | 12.38±0.95 | 107.30±1.74 | 17.23±1.17     | 8.03±0.75     | 61.43±4.17         |

* represents a significant increase at p<0.0001 and # and † represent significant decreases at p<0.05 and p<0.0001, respectively, when compared to Group I values. a, b and c represent significant increases at p<0.05, p<0.01 and p<0.0001, respectively, when compared to Group II values. Group I: Control; Group II: 0.9% normal saline + CCl₄; Group III: ascorbic acid + CCl₄; Group IV: 125MIASE + CCl₄; Group V: 250MIASE + CCl₄; Group VI: 500MIASE + CCl₄

Table 4. Effect of oral pretreatment with 125–500 mg/kg/day of MIASE on renal function parameters in rats with CCl₄-chemoprevention

| Groups          | Na⁺ (mmol/l) | K⁺ (mmol/l) | Cl⁻ (mmol/l) | HCO₃⁻ (mmol/l) | Urea (mmol/l) | Creatinine (µmol/l) |
|-----------------|--------------|------------|-------------|----------------|---------------|---------------------|
| Group I: Control| 149.00±19.18 | 190.20±20.17 | 27.96±4.07 | 0.64±0.02      |               |                    |
| Group II: 0.9% normal saline + CCl₄ | 170.30±24.75 | 196.30±20.94 | 14.28±4.80 | 0.73±0.03      |               |                    |
| Group III: ascorbic acid + CCl₄ | 174.50±18.68 | 216.70±25.91 | 24.04±3.42 | 0.65±0.03      |               |                    |
| Group IV: 125MIASE + CCl₄ | 175.80±28.00 | 206.70±5.04 | 17.31±3.28 | 0.70±0.03      |               |                    |
| Group V: 250MIASE + CCl₄ | 144.20±4.67 | 179.20±5.04 | 24.46±6.77 | 0.74±0.02      |               |                    |
| Group VI: 500MIASE + CCl₄ | 149.30±12.31 | 194.60±19.05 | 27.64±2.99 | 0.67±0.02      |               |                    |

* and # represent significant decreases at p<0.05 and p<0.0001, respectively, when compared to Group I values while † and ‡ represent significant increases at p<0.05 and p<0.0001, respectively, when compared to Group II values.

Table 5. Effect of 125–500 mg/kg of MIASE on average body weight and relative kidney weight in the chemocurative model of CCl₄-treated rats.

and p<0.0001) further weight loss and non-significant alterations in the relative organ weights (kidneys) when compared with CCl₄-treated (Group II) rats (Table 2).

Effect of 125–500 mg/kg of MIASE on renal function parameters in rats with CCl₄-chemoprevention

CCl₄ treatment caused significant (p<0.05, p<0.0001) increases in serum Na⁺, K⁺, Cl⁻, urea and creatinine while causing significant (p<0.05, p<0.0001) decreases in serum HCO₃⁻ levels compared to control rats (Table 3). However, oral pretreatments with 125–500 mg/kg of MIASE significantly (p<0.05, p<0.0001) attenuated the increase in serum levels of Na⁺, K⁺, Cl⁻, urea and creatinine, while significantly (p<0.05, p<0.0001) increasing the serum HCO₃⁻ levels compared to CCl₄-treated rats (Table 3).

Effect of 125–500 mg/kg of MIASE on renal tissue antioxidative status in CCl₄ treated rats

Table 4 shows the effects of oral pre-treatments with 125–500 mg/kg/day of MIASE and subsequent intraperitoneal CCl₄ treatment on antioxidative markers (SOD, CAT, GSH and MAD) in the treated rats. CCl₄ treatment caused significant (p<0.05 and p<0.0001) decreases in renal tissue SOD, CAT and GSH while causing significant (p<0.001 and p<0.0001) increases in renal tissue MAD (Table 4). Oral pretreatment with 250 and 500 mg/kg of MIASE significantly (p<0.05 and p<0.0001) attenuated decreases in renal tissue levels of SOD, CAT and GSH while it significantly attenuating increases in renal tissue MAD levels (Table 4). These results were comparable to the effect recorded for the 10 mg/kg of the standard antioxidant (ascorbic acid) used. However, 125 mg/kg/day of MIASE did cause significant alterations in the renal tissue levels of SOD, CAT, GSH and MAD when compared to the effect in the CCl₄-treated rats of Group II (Table 4).

Histopathological results of oral pretreatment with 125–500 mg/kg MIASE on kidneys of CCl₄-treated rats

Figures 1–6 show the histopathological findings of oral pretreatments with 125–500 mg/kg/day of MIASE on the
renal tissue of CCl₄-treated rats. Single intraperitoneal treatment with CCl₄ caused glomerular atrophy with tubular swelling and necrosis (Figure 2) compared to normal renal architecture (Figure 1). With repeated daily oral pretreatments with 10 mg/kg of ascorbic acid and 125–500 mg/kg/day of MIASE, these histological changes were ameliorated and improved in a dose-related manner (Figure 3–6).

**Table 5** shows the effect of MIASE oral pretreatments on the average body weight and relative organ weight of the kidney of CCl₄-treated, days 1 and 7 of the experiment. Intraperitoneal treatment with CCl₄ caused significant (∗p<0.0001) weight loss changes and non-significant increase in the kidney relative weight of CCl₄-treated rats.

**Figure 1.** Sectional representation of normal rat kidney showing normal glomeruli and tubules with a localized single area of peritubular hemorrhage (hematoxylin & eosin stain, ×100 magnification).

**Figure 2.** Sectional representation of CCl₄-treated rat kidney showing focal glomerular atrophy and tubular congestion and necrosis (hematoxylin & eosin, ×400 magnification).

**Figure 3.** Sectional representation of CCl₄-treated rat kidney pretreated with 10 mg/kg/day of vitamin C showing normal glomerulus and mild tubular congestion (hematoxylin & eosin, ×400 magnification).

**Figure 4.** Sectional representation of CCl₄-treated rat kidney pretreated with 125 mg/kg/day of MIASE showing intact glomeruli and moderate tubular necrosis (hematoxylin & eosin, ×100 magnification).

**Figure 5.** Sectional representation of CCl₄-treated rat kidney pretreated with 250 mg/kg/day of MIASE showing intact glomeruli and moderate tubular congestion (hematoxylin & eosin, ×100 magnification).

**Figure 6.** Sectional representation of CCl₄-treated rat kidney pretreated with 500 mg/kg/day of MIASE showing intact glomeruli and mild tubular congestion (hematoxylin & eosin, ×100 magnification).
Kidney damage and roles of Mangifera indica

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Table 6. Effect of 125–500 mg/kg of MIASE on renal function parameters in CCl4-chemocurative rats.

| Groups          | Naa (mmol/l) | K+ (mmol/l) | Cl– (mmol/l) | HCO3– (mmol/l) | urea (mmol/l) | creatinine (µmol/l) |
|-----------------|--------------|-------------|--------------|----------------|---------------|---------------------|
| I               | 143.0±1.09   | 11.15±0.55  | 100.10±0.45  | 17.85±1.00     | 8.00±0.36     | 58.92±3.86          |
| II              | 172.20±1.59f | 17.45±1.7f  | 127.00±1.06f | 11.50±0.39f    | 18.22±0.07f   | 93.37±2.80          |
| III             | 142.90±0.60f | 9.80±0.15   | 106.10±0.46f | 16.70±0.76f    | 11.34±0.40f   | 59.42±4.47f         |
| IV              | 157.20±2.67f | 11.10±1.7f  | 116.90±0.59f | 12.12±0.35     | 17.34±0.30f   | 65.10±3.65f         |
| V               | 136.40±3.52f | 11.07±0.44f | 103.80±0.66f | 12.72±0.45     | 10.43±0.79f   | 63.78±3.65f         |
| VI              | 130.90±0.46f | 9.59±0.15f  | 100.40±1.36f | 16.72±0.44f    | 9.33±0.22f    | 61.43±4.17f         |

a represents a significant increase at p<0.0001 and b represents significant decreases at p<0.0001 when compared to Group I values. c and d represent significant decreases at p<0.05, p<0.001 and p<0.0001, respectively, while e and f represents a significant increase at p<0.0001 when compared to Group II values.

Group I: Control; Group II: 0.9% normal saline + CCl4; Group III: ascorbic acid + CCl4; Group IV: 125MIASE + CCl4; Group V: 250MIASE + CCl4; Group VI: 500MIASE + CCl4

Effect of 125–500 mg/kg MIASE on renal tissue antioxidant markers in CCl4-chemocurative rats

Table 7 shows the effects of 125–500 mg/kg of MIASE on renal tissue antioxidant markers (SOD, CAT, GSH and MAD) in the post-CT treated rats. CCl4 treatment caused significant (p<0.05 and p<0.0001) decreases of renal SOD, CAT and GSH while causing significant (p<0.0001) increases in renal MAD values (Table 8). However, post-CCl4 oral treatments with 125–500 mg/kg/day of MIASE significantly (p<0.05, p<0.001 and p<0.0001) reversed and improved the values of these markers when compared to values for the CCl4-treated rats, returning them near normal values (Table 7).

Histopathological results of post-CCl4 oral treatment with 125–500 mg/kg of MIASE on the renal tissue of CCl4-treated rats

Intraperitoneal CCl4 treatment was associated with severe tubular swellings, tubular lumen obliterations and tubular necrosis (Figure 8) when compared to normal renal architecture (Figure 7). With repeated post-CCl4 oral treatment with 10 mg/kg/day of vitamin C and 125–500 mg/kg/day of MIASE, there was a dose related amelioration in the CCl4-induced renal lesions (Figures 9–12).

Discussion

Exposure to carbon tetrachloride is on the increase due to environmental pollution. The exposure can come from the air, drinking water, foodstuffs and soil (ATSDR, 2005; IPCS, 1999). It could also be from certain industrial sites where carbon tetrachloride is still used or where previously industrial contamination had occurred (ATSDR, 2005). The liver and kidney are the major target organs for toxicity following acute inhalation or ingestion exposure to carbon tetrachloride (IPCS, 1998;1999). Liver damage can occur after 24 hours and in serious cases this can result in painful swollen liver, ascites, hemorrhages, hepatic coma and death (ATSDR, 2005; IPCS, 1999). Kidney damage with impairment in function normally occurs 2–3 weeks after exposure (IPCS, 1999), but in severe cases this can
develop within 1–6 days in association with liver failure (ATSDR, 2005). Due to the fatality of kidney damage in affecting optimal human functions, attention should concentrate on strategies preventing the occurrence of kidney disease more than on palliative management.

In the recent past, several research studies on preventive strategies of renal damage have been conducted. Mesery et al. (2009) demonstrated the chemopreventive and renal protective effects of ocosahexaenoic acid (DHA); Pracheta et al., (2012) showed the chemopreventive effect of hydroethanolic extract of Euphorbia neriifolia leaves against DENA-induced renal carcinogenesis in mice and Sharma & Janmeda (2012) documented the chemopreventive role of Euphorbia neriifolia (Linn) and its isolated flavonoid against N-nitrosodiethylamine-induced renal histopathological damage in male mice.

A preliminary ethno-botanical use survey conducted among traditional herbalists in Lagos metropolis (Southwest Nigeria) showed that hot and cold water infusion of Mangifera indica stem bark is highly valued in the local management of both liver and kidney diseases. However, this assertion has not been scientifically
investigated. Thus the present study investigated the reno-modulatory roles of the aqueous stem bark extract of *Mangifera indica* against CCl₄ induced renal damage using rodent models.

The result of acute oral toxicity (LD₅₀) study of aqueous stem bark extract of *Mangifera indica* showed no mortality at the maximum dose of 5000 mg/kg/body weight (Adeneye *et al.*, 2015). In an acute oral toxicity study by Ogbe *et al.* (2012), *Mangifera indica* was documented to be non-lethal in animals at doses of 5000 mg/kg body weight. These results may indicate that the aqueous leaf extract of *Mangifera indica* is safe (non-lethal) during acute oral administration. The dose of 2 g/kg was reported as the ceiling point for medicinal plant toxicity when administered orally in acute toxicity studies (Lu *et al.*, 1965; Adeneye *et al.*, 2012). But this safety affirmation is not applicable to long-term intake of medicinal plants. Behavioral toxicities manifested by the treated rats included body scratching, feed refusal, reduced locomotor activity, and watery stools.

Exposure of rodents to CCl₄ in the present study showed significant (*p<0.05, p<0.0001*) increases in serum Na⁺, K⁺, Cl⁻, urea and creatinine, while causing significant (*p<0.05, p<0.001*) decreases in serum HCO₃⁻ levels. These findings corroborate the previous data on CCl₄ induction of renal damage (ATSDR, 2005; IPCS, 1999). The consistent damage of CCl₄ on renal tissue may call for critical strategies to preserve/protect the kidneys of people who are occupationally or otherwise exposed to CCl₄. MIASE (125–500 mg/kg), as shown in this study, significantly (*p<0.05, p<0.001*) attenuated the increase in the serum levels of Na⁺, K⁺, Cl⁻, urea and creatinine, while significantly (*p<0.05, p<0.001*) increasing serum HCO₃⁻ levels. The results of histology of rat kidney tissues also revealed glomerular atrophy with tubular swelling and necrosis caused by CCl₄. MIASE at oral doses of 250 and 500 mg/kg protected the rat kidney tissues from gross architectural alterations and damage. The chemopreventive potentials of MIASE (125–500 mg/kg) as shown in this study may scientifically affirm the use of *Mangifera indica* in kidney diseases in folk medicine. The speculated mechanism of kidney damage by CCl₄ may occur via oxidative stress and lipid peroxidation, as shown by significant (*p<0.05 and p<0.001*) decreases in renal tissue SOD, CAT and GSH and significant (*p<0.001 and p<0.0001*) increases in renal tissue MAD. It may also be considered that MIASE (125–500 mg/kg) offered chemoprevention against kidney damage by enhancing the endogenous antioxidant activity in the treated rats, as implied in the significantly reduced (*p<0.05 and p<0.0001*) decreases in renal tissue levels of SOD, CAT and GSH and the attenuated increases in renal tissue MAD levels. It is also interesting to know that MIASE (125–500 mg/kg) demonstrated dose dependent chemocurative effects against CCl₄-induced renal damage, with the highest reversal of kidney damage activity at 500 mg/kg.

On balance then, *Mangifera indica* possesses some antioxidant agents (Pardo-Andreu *et al.*, 2006). The antioxidant properties of *Mangifera indica* could be attributed to its constituent flavonoids and other polyphenolics as these phytocomponents have been widely reported to possess antioxidant activities (Roy *et al.*, 2005; Yang *et al.*, 2010). It is thus possible to speculate that the antioxidant properties of this plant are responsible for its reno-modulatory activities, as documented in this study. Antioxidants preventing or reversing kidney damage had been reported in our earlier study where vitamins C and E were found to protect against renal and testicular damage caused by *Alstonia boonei* (Awodele *et al.*, 2010).

Based on the findings obtained in this study, it can be stated that *Mangifera indica* has the potential to prevent or reverse kidney damage. This readily available plant may offer great prospect for drug development in the management of acute renal disease, especially kidney disease with the etiology of oxidative stress and lipid peroxidation.

**Conflict of Interest:** There is no conflict of interest in this study

**REFERENCES**

Acute Oral Toxicity (2007) (OECD Test Guideline 425) (AOT): Statistical Programme (AOT425StatFgm), Version 1.0.

Adeneye AA, Awodele O, Aiyela SA, Benebo AS. (2015). Modulatory potentials of the aqueous stem bark extract of *Mangifera indica* on carbon tetra-chloride-induced hepatotoxicity in rats. *Journal of Traditional and Complementary Medicine* 5: 106–115.

Aebi H. (1984). Catalase in vitro. *Methods Enzymology* 105: 121–126.

Agency for Toxic Substances and Disease Registry (ATSDR) (2005). Toxicological Profile for Carbon Tetrachloride. US Department of Health and Human Services. Atlanta.

Alo M, Eze UA, Anyim C. (2012). *In vitro* antimicrobial activities of extracts of *Mangifera indica*, *Canica papaya*, and *Psidium guajava* leaves on *Salmonella typhi* isolates. *World Journal of Public Health Sciences* 1: 1–6.

Awodele O, Oreagba IA, Omoda S, Teixeira da Silva JA, Osunkalu VO. (2012). *Moringa oleifera* Lam. (Moringaceae). *Journal of Ethnopharmacology* 139: 330–336

Awodele O, Osunkalu VO, Akinde RO Teixeir da Silva JA, Okunowo WO, Odogwu EC, Akintonwa A. (2010). Modulatory Roles of Antioxidants against the Aqueous Stem Bark Extract of *A.boonei* (Apocynaceae) induced Nephrotoxicity and Testicular Damage. *International Journal of Biomedical and Pharmaceutical Sciences* 4: 78–80.

Awodele O and Akintonwa A. (2012). Investigation of lipid peroxidation as probable mechanism of rifampcin toxicity in vivo. *Annals of Neurosciences* 19: 68–70.

Barreto JC, Trevisan MTS, Hell WE, Erben G, De Britto ES, Pfundstein B, Wurtele G, Spiegelhalder B, Owen RW. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves and peel of Mango (*Mangifera indica* L.). *Journal of Agricultural and Food Chemistry* 56: 5999–5610.

Buege JA, Aust SD. (1978). *Microsomal lipid peroxidation. Method Enzymology* 52: 302–310.

El-Mesery ME, Al-Gayyar MM, Salem HA, Darweisha MM, El-Mowafy AM. (2009). *Chemopreventive and renal protective effects for docosahexaenoic acid (DHA): implications of CRP and lipid peroxides. Cell Division* 4: 6

Ene AC, Atawodi SE, Ameh DA, Kwanashie HO, Agomo PU (2010). Locally used plants for malaria therapy among the Hausa, Yoruba and Ibo communities in Maiduguri, Northeastern Nigeria. *Indian Journal of Traditional Knowledge* 9: 486–490.

Fogo AB. (2007). Mechanisms of progression of chronic kidney disease. *Pediatric Nephrology* 22: 2011–2022.

Foreman JW and Chan JC. (1988). Chronic renal failure in infants and children. *Journal Pediatric* 113: 793–800

International Programme on Chemical Safety (IPCS) (1998). Carbon Tetra-chloride health and safety guide; WHO. Geneva.
International Programme on Chemical Safety (IPCS) (1999). Carbon Tetrachloride. Environmental Health Criteria 208, WHO. Geneva.

Kakkar P, Das B, Viswanathan PN. (1984). A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry Biophysics 21: 130–132.

Kaur P, Chandell M, Kumar S, Kumar N, Singh B, Kaur S. (2010). Modulatory role of alizarin from Rubia cordifolia L. against genotoxicity of mutagens. Food Chemical Toxicology 48: 320–325.

Kitagishi Y, Kobayashi M, Matsuda S. (2012). Protection against cancer with medicinal herbs via activation of tumor suppressor. Journal of Oncology. doi: 10.1155/2012/236530, 7 pages.

Kumar BD, Mitra A, Manjunatha M. (2009). In vitro and in vivo studies of anti-diabetic Indian medicinal plants: A review. Journal of Herbal Medicine and Toxicology 3: 9–14.

Kumar SV, Kumar SP, Kumar DP. (2011). Immunomodulatory effects of some traditional medicinal plants. Journal of Chemical and Pharmaceutical Research 3: 675–684.

Lu KL, Tsai CC, Ho LX, Lin CC, Chang YS. (2002). Preventive effect of the Taiwan folk medicine Ixeris laevigata var. oldhami on α-naphthyl-isothiocyanate and carbon tetrachloride-induced acute liver injury in rats. Phytotherapy Research 16: S45–S50.

Lu FC, Jessup DC, Lavallée A. (1965). Toxicity of pesticides in young versus adult rats. Food Cosmetic Toxicology 3: 591–596.

Ogbe RJ, Adenkola AY, Anefu E. (2012). Aqueous-ethanolic extract of Mangifera indica stem bark effect on the biochemical and hematological parameters of Albino rats. Archives of Applied Science Research 4: 1618–1622.

Pardo-Andreu GL, Sanchez-Baldoquin C, Avila-Gonzalez R, Yamamoto ET, Revilla A, Uyemura SA. (2006). Interaction of Vimang (Mangifera indica L. extract) with Fe (III) improves its antioxidant and cytoprotecting activity. Pharmacological Research 54: 389–395.

Pracheta P, Sharma V, Singh L, Paliwal R, Sharma S, Yadav S, Sharma S. (2012). Chemopreventive effect of hydroethanolic extract of Euphorbia neriifolia leaves against DENA-induced renal carcinogenesis in mice. Asian Pacific Journal of Cancer Prevention 12: 677–683.

Reeder BJ, Hider RC, Wilson MT. (2008). Iron chelators can protect against oxidative stress through ferryl heme reduction. Free Radical Biology and Medicine 44: 264–273.

Reeder BJ, Svistunenko DA, Sharpe MA, Wilson MT. (2002). Characteristics and mechanism of formation of peroxide-induced heme to protein cross-linking in myoglobin. Biochemistry 41: 367–375.

Roy S, Sehgal R, Padhy BM, Kumar VL. (2005). Antioxidant and protective effects of latex of Calotropis procera against alloxan-induced diabetes in rats. Journal of Ethnopharmacology 102: 470–473.

Sedlak L, Lindsay RH. (1968). Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellman’s reagent. Anal of Biochemistry 25: 1192–1205.

Sharma V, Janmeda P. (2012). Chemopreventive role of Euphorbia neriifolia (Linn) and its isolated flavonoid against N-nitrosodiethylamine-induced renal histopathological damage in male mice. Toxicology International 20: 101–107.

Sofowora A. (1993). Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Ibadan: Spectrum Books Ltd., 150.

Sun M, Zigman S. (1978). An improved spectrophotometric assay of superoxide dismutase based on epinephrine anti-oxidation. Anal of Biochemistry 90: 81–89.

WHO (1985). The WHO traditional medicine programme: policy and implementation. International Traditional Medicine Newsletter 1: 1–5.

Yang J, Li Y, Wang F, Wu C. (2010). Hepatoprotective effects of apple polyphenols on CCl4-induced acute liver damage in mice. Journal of Agriculture and Food Chemistry 58: 6525–6531.