

Phytochemical Screening, *in vitro* Antioxidant Capacity and Nephro-protective effects of Combined Extract of *Psidium guajava* and *Carica papaya* Leaves in rats intoxicated with Cadmium

ABSTRACT

Aim: Toxic effects arising from heavy metals and other contaminants in the environment are presently been tackled by the use of medicinal plants. Thus, this study evaluated the protective effects of combined extract of *Psidium guajava* and *Carica papaya* leaves on kidney injury occasioned by Cd toxicity. **Materials and Methods:** Phytochemical investigation of the combined extract revealed the presence of tannins, saponins, flavonoids, triterpene, steroids, alkaloids, cardiac glycoside, anthocyanins, and anthraquinone and the the extract displayed excellent antioxidant capacity *in vitro*. Eighteen adult female rats were divided into three groups: Group 1 (Control, received feed and water only); Group 2 (Cadmium, received single dose of cadmium, 30mg/kg body weight at the start of the experiment) and Group 3 (Cadmium + combined leaf extract, received cadmium as in group 2 above and 200mg/kg body weight of extract, daily for two weeks). **Results:** Intoxication with Cd causes significantly ($p < 0.05$) increased in the levels of lipid peroxidation and in the activity of glutathione peroxidase. Cd intoxication also depleted glutathione (GSH), super oxide dismutase (SOD) and catalase (CAT) reserves and brought about a significant increase in serum urea, creatinine, sodium and potassium. However a significant ($p < 0.05$) restoration in the levels of GSH, SOD, CAT, urea, creatinine, sodium and potassium was observed in Cd-intoxicated rats administered the extracts as compared to the untreated rats. **Conclusion:** The results reveal that Cd causes nephro-toxicities manifested via lipid peroxidation, increase in serum levels of urea, creatinine, sodium and potassium ions and that the combined extract of *Psidium guajava* and *Carica papaya* leaves possess adequate antioxidant power against Cd-induced nephro-toxic effects.

KEYWORDS: *Psidium guajava*, *Carica papaya*, Phytochemicals, antioxidant capacity

INTRODUCTION

Cadmium (Cd), a toxic metal, finds use in different industrial applications (such as pigments, fertilizers, plastics, electroplating and battery), which led to increased human exposure and serious environmental contamination.^[1,2] Cellular damage to bone, testis, liver and kidney following acute and chronic Cd exposures have been reported, with the kidney and liver being most susceptible.^[3] As a vital organ in the body, the kidney's role in storage, secretion, metabolism and detoxification of xenobiotics makes it a prime target of heavy metal toxicity.^[4,5] Cadmium-induced nephro-toxicity is mediated via elevation in oxidative stress as a result of

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enhanced production of free radicals and reduction in the activity of tissue endogenous antioxidant defense systems.^[6]

Given the fact that medicinal plants possess inert chemo-therapeutic capabilities, are readily available, cost effective and natural, researchers (over the past decade) have been keen on the application of plant products and extracts in the amelioration of toxicity generated by drugs, pesticides, heavy metals and other environmental pollutants.^[7-13]

Psidium guajava L (Guava) and *Carica papaya* (pawpaw) leaves are used traditionally to cure various diseases (wounds, toothache, coughs, sore throat, malaria, inflamed gums, etc).

^[14,15,16,17] They have been reported to possess adequate antioxidant, anticancer, anti-inflammatory and anti-bacterial properties. ^[7, 18-20] However, no study has reported the *in vitro* or *in vivo* antioxidant prowess of combined extract of both leafs.

Multi-plant extracts utilization has found expression in ethnomedicine over time and has been scientifically proven to be beneficial, over single-plant extracts, due to synergistic effects of the compounds in the extracts.^[21,22] Therefore, this research investigated the *in vitro* antioxidant capacity as well as the nephro-protective prowess of combined extract of *Psidium guajava*) and *Carica papaya* leaves.

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MATERIALS AND METHODS

Reagents/Chemicals

Chemicals and reagents used were of analytical grade supplied by reputable vendors. Assay kits for serum urea, creatinine, potassium and sodium assays were products of Randox Laboratories Ltd.

Plant Material

Fresh leaves of Guava and pawpaw were collected from Kiagbodo, Burutu Local Government Area, Delta State. The sample of the plants were identified by a botanists (ECU/03/2023), the leaves were sorted to eliminate any dead matter and other unwanted particles and then air dried for (2) weeks and ground into powder using an electric dry mill.

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Extraction Method

The extraction was done using a simple maceration as described by Clark and Omo-Udoyo^[23]. Exactly 200 grams of the powdered leaves was engrossed inside a stoppered flask with 1L of ethanol. The mixture was left at room temperature accompanied with frequent stirring for 72 hours. This was followed by filtration with the aid of Whatman filter paper No.1. This process was repeated for five times to ensure exhaustive extraction of the plant material. The cumulative filtrate was then concentrated with the aid of a rotary evaporator at 40⁰ C to yield a viscous mass which was air-dried at room temperature.

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Phytochemical screening

Qualitative phytochemical screening for saponins, steroids, tannins, alkaloids, anthraquinone, flavonoids, anthocyanins, cardiac glycoside, and triterpene in the extract were done following standard procedures as described by Harborne^[24].

Determination of Antioxidant Prowess of the extract

In vitro antioxidant properties of the extract were done using standard protocols as shown in Table 1.

Table 1: Methods used in the Determination of Antioxidant Capacity of the extract

Assay	Authority	Principle/Remark
Ferric Reducing Antioxidant Power (FRAP)	Oyaizu ^[25]	The concentration of extract having a ferric reducing ability equivalent to that of 1 μmol FeSO_4 was used as FRAP parameter and the reducing power of sample was expressed as equivalent concentration of FeSO_4 .
2,2 diphenyl-1 - picrylhydrazyl (DPPH) Free Radical scavenging	Brand <i>et al.</i> ^[26]	EC_{50} value is the effective concentration of sample that could scavenge half (that is 50%) of the DPPH radicals.
Total Antioxidant Activity (TAA)	Orak ^[27]	Linoleic acid solution (5.0 ml) 6 mg/mL in 99% methanol was prepared. To this, 1 ml of the extract was added and the resulting mixture was incubated for 10 min at 37 °C. Thereafter, 0.1 ml of this solution was added to 4.7 ml of 75% ethanol, 0.1 ml of 0.1 M ammonium thiocyanate and 0.1 ml of 20 mM ferrous chloride in 3.5% HCl solution. The reaction was then allowed to stand in the dark for 5 min at 30 °C. Thereafter, the absorbance was read at 500 nm.
Metal Chelating Activity (MCA)	Ponmozhi <i>et al.</i> ^[28]	For the assay, 100 μl of standard and sample were added to a solution of 100 μL Ferric chloride (1 mM). Then the reaction was initiated by adding of 250 μL of 1 mM ferrozine.
Hydrogen Peroxide Scavenging Activity (HPSA)	Akinpelu <i>et al.</i> ^[29]	H_2O_2 solution (4 mM) was first prepared in 0.1 M (pH 7.4) phosphate buffer. To 0.6 ml of this solution was added 0.1 ml of sample. The resulting solution was then incubated for 10 min and thereafter its absorbance was read at 230 nm against blank solution (H_2O_2 without sample).

Experimental Animals and Experimental Design

A total of 18 adult female rats weighing between 150 ± 20 g were used for the study. They were acclimatized in the experimental house for one week before the commencement of the experiment.

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The rats were further divided into 3 groups containing 6 rats each, as follows;

Group 1: Control group (received feed and distilled water only)

Group 2: Cd group (received single dose of cadmium, 30mg/kg body weight at the start of the experiment)

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Group 3: Cd + Extract group (received cadmium as in group 2 above and 200mg/kg body weight of combined extract of *Psidium guajava* and *Carica papaya* leaf extract, daily for two weeks).

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The toxicant (Cd), the combined extract of guava leaf (*Psidium guajava*) and pawpaw leaf (*Carica papaya*) were administered to the animals orally by syringe through gastric intubation. All the animal experiments were carried out in accordance with the guidelines of the institution's animal ethical committee (ECU/EC/05/23).

Sample Collection

After two weeks of administration, the animals in each group were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture into plain bottles, allowed to clot and centrifuged at 300g 10min. Serum obtained was used for analysis. The kidney was obtained and weighed and 1g portion of each was homogenized in phosphate buffer and centrifuged at 600g for 10mins. Supernatants collected were stored frozen until used for analysis.

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A portion of the kidney was also fixed for histological examination.

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Serum Kidney Function assays

The levels of urea, creatinine, sodium and potassium ions in the serum of the experimental animals were analyzed following the instruments in the various Randox assay kits. The urease method of Weatherburn^[30] was employed in the determination of serum urea, while the method of Bartels and Bohmer^[31] was used in determination the level of serum creatinine. Serum electrolytes, sodium and potassium were determined according to the methods of – and – respectively.

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Assessment of Oxidative Stress markers in the Kidney

Using kidney tissue homogenate, the activities of catalase (CAT), superoxide dismutase (SOD) and Glutathione peroxidase (GPx) as well as the levels of lipid peroxidation and glutathione (GSH) were measured using standard protocols as shown in Table 2 below:

Table 2: Methods used in the Assessment of Oxidative Stress markers in the Kidney

Assay	Authority	Principle/Remark
Catalase Activity	Sinha ^[32]	dichromate mixed with acetic acid is converted to chromic acetate upon heating along with hydrogen peroxide. The chromic acetate so formed is thereafter measured spectrophotometrically at 570-610nm.
Superoxide Dismutase (SOD)	Misra and Fridovich ^[33]	the autoxidation of adrenaline is inhibited by SOD at pH 10.2. One unit of SOD activity is therefore defined as the quantity of the enzyme that can bring about 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.
Lipid Peroxidation	Varshney and Kale ^[34]	determined by assessing the formation of Malonaldehyde (MDA) produced when membrane fatty acid and react with a chromogenic reagent to yield a pink coloured complex measured spectrophotometrically.
Glutathione (GSH)	Beutler <i>et al.</i> ^[35]	When 5'5'-dithiobis-(2-nutriobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds, a relatively stable (yellow) colour is obtained called 2-nitro-5-thiobenzoic acid, which

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		possesses molar absorption at 412nm.
Glutathione peroxidase (GPx)	Paglia and Valentine ^[36]	The assay is based on the reaction catalyzed by Glutathione Peroxidase (GPx) in which Glutathione (GSH) is oxidized by Cumene Hydroperoxide. Oxidized Glutathione (GSSG) is converted to the reduced in the presence of Glutathione Reductase (GR) and NADPH with a concomitant oxidation of NADPH and NADP ⁺ . The decrease in absorbance at 340nm is measured.

Tissue Histopathological Examination

Tissue samples were promptly fixed in formalin and processed for light microscopic inspection. The tissue blocks were then sliced into serial slices, which were then deparaffinized and stained with hematoxylin. The kidney microscopic architecture of experimental rats was histologically studied on hematoxylin stained slides. Images of stained tissues were obtained using a digital microscopic eyepiece, Brunel light microscope, 20 mega pixels (Brunel SP35 Digital Trinocular) linked to a computer's USB connection.

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Data Analysis

The results obtained from this study were analysed by one way analysis of variance (ANOVA), followed by Least Significance Difference (LSD) test to ascertain the difference between the mean value of the measured parameters in the respective test and control groups. A significant change was considered acceptable at $P < 0.05$. Results of the biochemical estimations are reported as means \pm SD.

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RESULTS

Phytochemical screening of combined extract of *Psidium guajava* and *Carica papaya* Leaves

The result of the qualitative phytochemical screening of the combined extract of *Psidium guajava* and *Carica papaya* leaves is shown in Table 3. Cardiac glycoside, anthraquinone saponins, and reducing sugars were detected as well as alkaloids, Flavonoids, anthocyanins, triterpene and tannins.

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Table 3: Phytochemical screening of combined extract of *Psidium guajava* and *Carica papaya* Leaves

Tests	Combined extract of <i>Psidium guajava</i> and <i>Carica papaya</i> Leaves
Cardiac glycoside	++
Anthraquinone	++
Saponins	++
Reducing Sugara	++
Alkaloids	++
Flavonoids	++
Anthocyanins	++
Triterpene	+
Tannins	+

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In vitro Antioxidant Properties of combined extract of *Psidium guajava* and *Carica papaya* Leaves

Fig. 1 shows the *in vitro* antioxidant properties of combined extract of *Psidium guajava* and *Carica papaya* leaves. The ability of the combined extract of *Psidium guajava* and *Carica papaya* leaves to trap free radicals produced by stable DPPH' free radical (12.28 ± 1.2) was higher than that of the standard, ascorbic acid (14.22 ± 2.1). The combined leaf extract also recorded a higher total antioxidant activity ($73.6 \pm 1.9\%$) than the standard (ascorbic acid) ($50.33 \pm 1.3\%$). Conversely, the combined extract had a Ferric Reducing Antioxidant Power (FRAP) ($28.48 \pm 2.6\%$) lower than that of ascorbic acid ($38.28 \pm 3.0\%$). No significant difference was

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observed in the hydrogen peroxide scavenging activity of the combined leaf extract and that of standard ascorbic acid. On metal chelating ability, the combined leaf extract recorded a significantly higher ability ($52.43 \pm 2.8\%$) than ascorbic acid ($24.60 \pm 2.8\%$).

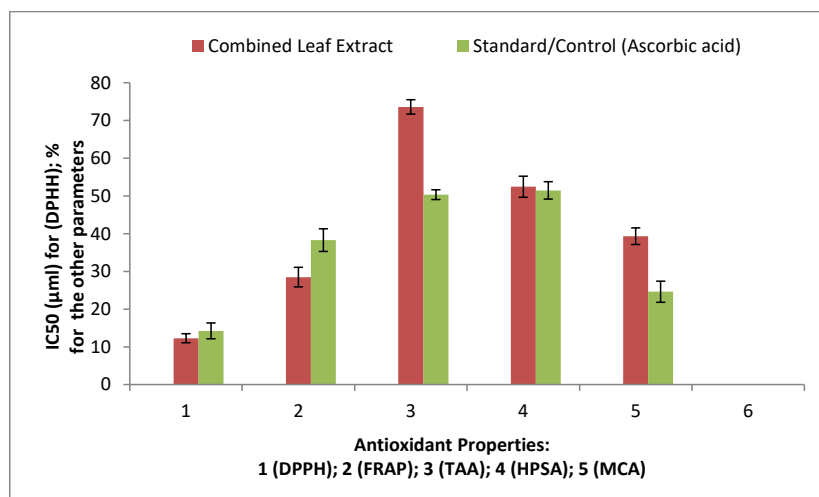


Fig.1: In vitro Antioxidant Properties of combined extract of *Psidium guajava* and *Carica papaya* Leaves

Effects of Combined Extract of *Psidium guajava* and *Carica papaya* Leaves on Oxidative Stress markers in the Kidney of rats intoxicated with Cadmium

The effects of combined extract of *Psidium guajava* and *Carica papaya* leaves on Oxidative Stress markers in the Kidney of rats intoxicated with cadmium is presented in Table 4.

Intoxication with Cd alone (group 2) significantly ($p < 0.05$) stimulated increased peroxidation ($79.69 \mu\text{mole/mg protein}$) in comparison with un-intoxicated rats (Group 1) ($28.60 \mu\text{mole/mg protein}$) and intoxicated rats that were given the combined leaf extract (Group 3) ($55.38 \mu\text{mole/mg protein}$). The result indicates that significant ($p < 0.05$) protection against lipid

peroxidation was offered to Cd-intoxicated rats by the ingestion of the combined extract of *Psidium guajava* and *Carica papaya* leaves. The activity of glutathione peroxidase in the kidney of the experimental rats in the groups followed a trend similar to the result obtained for lipid peroxidation. The activity of the enzyme was significantly elevated in Cd-intoxicated rats (group 2) compared with the control and rats given the extract.

The level of glutathione (GSH) in control (42.12 μ mole/mg protein) was significant higher than in the rats intoxicated with Cd (20.30 μ mole/mg protein), indicating that exposure to Cd depleted the GSH reserves of the animals. However a significant restoration in the levels of GSH was observed in Cd-intoxicated rats administered the extracts (Group 3) (39.75 μ mole/mg protein) as compared to the untreated rats (Group 2) (20.30 μ mole/mg protein). A similar pattern was observed in SOD and CAT activities.

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Table 4: Effects of Combined Extract of *Psidium guajava* and *Carica papaya* Leaves on Oxidative Stress parameters in the Kidney of rats intoxicated with Cadmium

GROUPS	GSH (μ mole/mg protein)	MDA (μ mole/mg protein)	SOD (μ mole/mg protein)	CAT H ₂ O ₂ consumed/ min/mg protein	GPx (μ mole/mg protein)
Group 1 (Control)	42.12 \pm 1.91 ^a	28.60 \pm 2.82 ^a	35.33 \pm 1.28 ^a	22.06 \pm 1.72 ^a	30.46 \pm 2.33 ^a
Group 2 (Cadmium only)	20.30 \pm 2.34 ^b	79.69 \pm 4.01 ^b	16.83 \pm 1.67 ^b	11.02 \pm 2.35 ^b	41.49 \pm 2.54 ^b
Group 3 (Cd+ Guava and Pawpaw Leaf Extract)	39.75 \pm 2.14 ^a	55.38 \pm 4.03 ^c	36.68 \pm 2.22 ^a	17.58 \pm 1.36 ^c	31.89 \pm 3.02 ^a

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Values are given as mean \pm standard deviation (SD) of three determinations. Values on the same column with different superscripts differ significantly ($p < 0.05$).

Effects of Combined Extract of *Psidium guajava* and *Carica papaya* Leaves on Kidney

Function Parameters of rats intoxicated with Cadmium

The effects of combined extract of *Psidium guajava* and *Carica papaya* leaves on kidney function parameters of rats intoxicated with Cadmium is shown in Table 5.

Table 5: Effects of Combined Extract of *Psidium guajava* and *Carica papaya* Leaves on Kidney Function Parameters of rats intoxicated with Cadmium

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)	Na ⁺	K
Group 1 (Control)	85.3±6.5 ^a	0.76±0.12 ^a	24.33±3.5 ^a	1.3±0.10 ^a
Group 2 (Cadmium only)	125±5.0 ^b	1.91±0.04 ^b	72.00±5.3 ^b	4.1±0.26 ^b
Group 3 (Cd+ Guava and Pawpaw Leaf Extract)	96±5.3 ^c	1.2±0.10 ^c	54±3.6 ^c	2.2±0.25 ^c

Values are presented as mean ±standard deviation (SD) of three determinations. Values on the same column with different superscripts differ significantly ($p<0.05$).

Serum urea level in the control rats was 85.3±6.5. This was significantly elevated ($p<0.05$) to 125±5.0 in rats intoxicated with Cd (Group 2) and thus Cd administration brought about a significant increase in serum urea. When Cd-intoxicated rats were treated with guava and pawpaw leaf extracts (Group 3), there was a significant decrease ($p<0.05$) in the level of serum urea compared to rats exposed to Cd alone (Group 2), indicating that the combined leaf extract significantly lowered serum urea levels. Similarly, serum creatinine level in the control (0.76±0.12), was significantly elevated ($p<0.05$) to 1.91±0.04 in rats exposed to Cd (Group 2) again, showing that Cd intoxication brought about a significant increase in serum creatinine. When Cd-intoxicated rats were treated with combined extract of guava and pawpaw leaves (Group 3), there was a significant reduction ($p<0.05$) in the level of serum creatinine (1.2±0.10) compared to rats intoxicated with Cd alone (Group 2), indicating that the extract significantly

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lowered serum creatinine level. A similar pattern was observed in values recorded for the serum electrolytes (sodium and potassium).

Effects of Combined Extract of *Psidium guajava* and *Carica papaya* Leaves on the histology of the kidney of rats intoxicated with Cadmium

Figures 2-4 show effects of combined extract of *Psidium guajava* and *Carica papaya* leaves on the histology of the kidney of rats intoxicated with cadmium.

Normal renal corpuscle (long arrow) and visible interstitial and tubules (short arrow) were seen in the kidney of rats that were not intoxicated with Cd (Fig. 2). However, the histology of rats intoxicated with Cd (Fig 3) revealed that the atrophied renal corpuscles were slightly distorted (long arrow) accompanied with necrosis of the tubules (short arrow). As shown in Fig. 4, treatment of Cd-intoxicated rats with the combined extract of *Psidium guajava* and *Carica papaya* leaves normalized the histology of the kidney as normal renal corpuscle (long arrow) with interstitial and tubules (short arrow) were visible (Figure 4).

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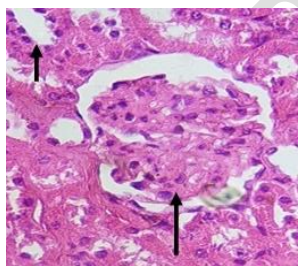


Fig. 2 (Group 1 – Control)

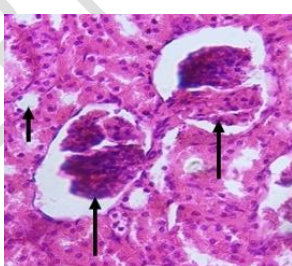


Fig. 3 (Group 2: Cd)

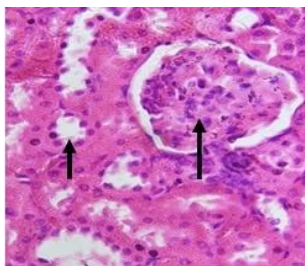


Fig. 4 (Group 3: Cd and Extract)

DISCUSSION

The maintenance of body homeostasis and excretion of harmful metabolic waste products are important functions of the kidneys [37]. Toxic effects arising from heavy metals and other contaminants in the environment are presently being tackled by the use of medicinal plants. Thus, this study examined the protective effects of combined extract of *Psidium guajava* and *Carica papaya* leaves on kidney injury occasioned by Cd toxicity.

The phytochemical investigation of the combined extract revealed the presence of tannins, saponins, flavonoids, triterpene, steroids, alkaloids, cardiac glycoside, anthocyanins, and anthraquinone, which can be attributed to the combinatorial power of the extract, as no study has reported all these metabolites in either guava or pawpaw leaf extracts screened separately. Ayoola et al. [38] reported the absence of alkaloids and anthraquinones in the ethanolic leaf extract of *P. guajava* and triterpene was not reported in a screening test on *C. papaya* carried out by Alexander et al. [39]. Alkaloids were not detected in the screening of methanolic extract of *P. guajava* leaf reported by Jani et al. [40] and a report by Khadam et al. [41] showed that saponins were not detected in ethanolic extract of *C. papaya* leaves. The advantage of combining the two leaves can also be seen considering the report by Alexander et al. [39] that flavonoids are highly concentrated in *P. guajava* while *C. papaya* has more tannin. The combined extract is therefore very rich in both metabolites. These phytochemicals have been shown to elicit diverse positive pharmacological actions in living systems including, nephro-protective and antibacterial capabilities, as well as the ability to scavenge free radicals. [19,41-43] Thus, it was not surprising that the combined of *Psidium guajava* and *Carica papaya* leaves demonstrated strong *in vitro* antioxidant capacity that was even higher than standard ascorbic acid in some of the assays (Fig. 1). The result also agrees with the work of Jani et al. [40] and Ayoola et al. [38] who reported strong

antioxidant potentials against DPPH by *Psidium guajava* and *Carica papaya* leaves extract respectively.

Toxic injury to the kidneys is commonly assessed by the estimation of certain biomarkers in the serum such as urea and creatinine.^[5] When proteins are broken down, urea is produced as waste product, which undergoes glomerular filtration with subsequent passive excretion in the urine. As such, urea is a reliable index of renal function. On the other hand, creatinine originates from the breakdown of muscles, whose level in the serum can be used as sign of normal kidney function^[5,44]. In this study, a significant increase in the serum levels of creatinine, sodium, urea and potassium was observed, all indicating Cd-induced oxidative damage to the kidney and it is in agreement with the earlier reports.^[45-48]

It has been shown that although Cd, unlike other heavy metals, cannot generate free radicals directly, it can generate them indirectly by displacing essential elements such as iron and copper from membranes. Displaced iron and copper then generate free radicals through the Fenton reaction.^[4,49,50] Cd-induced inhibition of complex III in the respiratory chain has also been shown as one mechanism through which Cd-injury is propagated.^[51] Excess accumulation of free radicals beyond the buffering abilities of tissue antioxidant system results in lipid peroxidation.^[52,53] This was witnessed in this study, as rats administered Cd alone (Group 2) had significantly heightened lipid peroxidation accompanied by depletion of tissue endogenous antioxidant enzymes and molecules such as SOD, CAT, GSH and GPx. This finding is in line with reported oxidative injury of Cd in the kidney.^[3,48,54-57] SOD and CAT are vital enzymes used in the elimination of singlet oxygen, hydrogen peroxide and other reactive oxygen species. SOD, often called upon as first line of defense against invading free radicals facilitates the neutralization of superoxide. Hydrogen peroxide produced from this reaction is subsequently

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neutralized by CAT.^[58,59] Significant lowering in GSH levels, linked with enhanced peroxidation of cellular membranes as observed in this study, can result in negative changes in the normal histology of kidney tissues^[57] and that probably explains the observation, that Cd intoxication caused slight distortion of renal corpuscles atrophied and necrosis of the renal tubules.

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The positive amelioration of Cd-induced nephrotoxicity by the combined extract of guava and pawpaw leaves, revealed by the restoration of serum levels of urea, creatinine, sodium and potassium, restoration of enzymatic and non enzymatic antioxidant molecules (SOD, CAT, GPx, GSH) and normalization of kidney histology point to the antioxidant powers of the extract. The nephro-protective prowess of plants has been attributed to antioxidant compounds they contain^[60-63] According to Adeneye and Benebo^[64], plants with medicinal properties are able to

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mitigate the harmful effects of free radicals by deploying the antioxidant powers of flavonoids, alkaloids, saponins and other polyphenolic compounds and phytochemicals. Adithya *et al.*^[65] showed that lipid peroxidation is reduced when phenolic compounds donate hydrogen atoms to free radicals, thereby halting free radical chain reaction. This study, being the first to do so, has successfully shown the nephro-protective powers of combined extract of *Psidium guajava* and *Carica papaya* leaves in rats intoxicated with Cd.

CONCLUSION

This study has succinctly demonstrated that cadmium causes nephro-toxicities manifested via lipid peroxidation, increase in serum levels of urea, creatinine, sodium and potassium ions and histological changes in the kidney and that the combined extract of *Psidium guajava* and *Carica papaya* leaves possess adequate antioxidant power against Cd-induced nephro-toxic

effects. The ability of the extract to offer nephro-protection is attributed to the abundant phytochemicals in it, which are able to mitigate free radicals occasioned by Cd exposure.

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Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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