Original Article

Comparative Phytochemical Screening, *In Vivo* Antioxidant and Nephroprotective Effects of Extracts of Cassava Leaves on Paracetamol-intoxicated Rats

Israel O. Okoro, Helen E. Kadiri, Eferhire Aganbi

Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria

Abstract

The phytochemical screening, antioxidant, and nephroprotective effects of methanol and acetone extracts of cassava (*Manihot esculenta* Crantz) leaves were comparatively investigated using standard procedures. Fifty-four male Wistar rats (albino) were divided into nine groups of six rats each. Group 1 = negative control (normal untreated rats + normal saline); group 2 = positive control (rats + 2 g/kg bw acetaminophen + normal saline), groups 3, 4, and 5 = 200 mg/kg bw, 100 mg/kg bw, and 50 mg/kg bw of methanol extract, respectively, + 2 g/kg bw acetaminophen; groups 6, 7, and 8 = 200 mg/kg, 100 mg/kg bw, and 50 mg/kg bw of acetone extract, respectively, + 2 g/kg bw acetaminophen; and group 9 = 100 mg/kg silymarin + 2 g/kg bw acetaminophen. The phytochemical screening of the methanol and acetone leaves extracts showed the presence of flavonoids, alkaloids, saponins, anthocyanins, tannins, and triterpene, whereas, cardiac glycoside, steroids, and anthraquinone were absent in both extracts. Acetaminophen administration significantly elevated the levels of serum urea, creatinine, sodium, and potassium with a corresponding decrease in the levels of total protein, albumin, and calcium in the group 2 rats compared with that in the group 1 rats. Similarly, the levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione, and glutathione S-transferase were significantly less in the acetaminophen-intoxicated group than that in the negative control group. However, pretreatment with either extracts, dose dependently prevented the acetaminophen-induced derangement of the aforementioned parameters. The extracts showed antioxidant activity similar to the reference drug (silymarin). Comparatively, the methanol extract gave higher *in vivo* antioxidant and nephroprotective effects than the acetone extract. The results suggest the extracts of cassava leaves have high nephroprotective potential and may be based on their phytoconstituents and antioxidant activity.

Keywords: *Acetaminophen,* in vivo *antioxidant,* Manihot *esculenta, nephroprotective*

Introduction

Acetaminophen is frequently used as antipyretic agent, which has been said to cause uremia and renal tubular damage in high doses. An overdose of acute acetaminophen is reported to lead to renal necrosis and a fatal hepatic injury in humans and experimental rats.[1] Though high doses of acetaminophen are said to form a glucuronic acid conjugated bond, a substantial percentage is metabolized by cytochrome P_{450} system, which results in the generation of reactive toxic metabolites such as *N*-acetyl-*p*-benzoquinone imine (NAPQI) that interacts with the sulfhydryl groups in glutathione (GSH) molecule.[2] Thus, acetaminophen brings a depletion of GSH stores in the cell. The binding of cellular proteins and portions of remaining NAPQI initiates lipid peroxidation and finally induces kidney injury.[3] Hence, acetaminophen toxicity is evaluated by the amount of NAPQI formed and the insufficient GSH for acetaminophen detoxification. Even though nephrotoxicity is not too common compared to hepatotoxicity in cases of paracetamol overdose, renal damage and severe kidney failure can arise even when there is no liver injury,[1] and can cause death in both humans and animals.[4]

The kidney is an organ responsible for the maintenance of homeostasis and for the excretion of end products of metabolism, drugs, and chemicals. It regulates acid–base balance, mineral metabolism, osmotic pressure, and nitrogenous slags excretion. The kidney plays a special role in detoxification and elimination of xenobiotics, thereby making it susceptible to developing injuries, which have been associated with reactive oxygen species (ROS) resulting to renal oxidative stress and

How to cite this article: Okoro IO, Kadiri HE, Aganbi E. Comparative phytochemical screening, *in vivo* antioxidant and nephroprotective effects of extracts of cassava leaves on paracetamol-intoxicated rats. J Rep Pharm Sci 2019;8:188-94.

***Address for correspondence**:*
Dr. Israel O. Okoro,
Department of Biochemistry,
Faculty of Science, Delta State University, Abraka,
Delta State, Nigeria.
E-mail: israelik@yahoo.com

Access this article online

Website: www.jrpsjournal.com

DOI:10.4103/jrptps.JRPTPS_10_19

Quick Response Code:

failure.[5] Kidney disorders are said to result in 1 of 10 deaths, thereby raising the public health concerns in recent years about chronic kidney disease. Creatinine and urea are nitrogenous metabolites (nonprotein) usually cleared by the kidney from the body through glomerular filtration. The determination of either serum or plasma levels of these metabolites and electrolytes is commonly used as kidney function markers.[6]

Several herbs have shown nephroprotective abilities and this has awakened global interest in this regard. The target is majorly to protect and prevent injuries to kidney and improve the restoration of tubular cells. Some nephroprotective phytoconstituents from diverse plant extracts have also been reported.[7] Similarly, certain nutraceuticals such as alpha lipoic acid and probiotic have shown tremendous nephroprotective effect against paracetamol-induced kidney failure in male rats.[8]

Manihot esculenta Crantz, a plant of the Euphorbiaceae family, is widely distributed in tropics. It is commonly called cassava, manioc, and tapioca.[9] The plant is known to be drought tolerance with unusual ability to adapt to several weather and soil conditions. The leaves of *M. esculenta* have been said to contain various phytochemicals such as flavonoids (e.g., kaempferol, rutin, and quercetin), alkaloids, tannins, phlobatannins, anthraquinones, saponins, anthrocyanosides, and reducing sugars. The fresh leaves also contain lotaustralin, cyanogenic glycosides, and linamarin.[10] The medicinal values of the plant have been explored by different researchers; its antioxidant, antidiarrheal, anthelmintic, antimicrobial, antihemorrhagic, antipyretic, analgesic, and anti-inflammatory properties have also been reported.[11-13] Cassava leaves are valuable as food and feeds for both human and animals.[14] The leaves of cassava are usually consumed regularly as vegetable and as spinach in parts of Africa.[15,16] Although the leaves contain cyanogenic glycosides, which can be toxic when consumed in the crude form, but appropriate preparation before consumption could reduce the cyanide contents and make them safe. Previous reports showed that pounding of cassava leaves or allowing them to stand for 5 h in the shade or washing them three times with water could reduce the cyanide content to 72%, 88%, and 99%, respectively.[17,18] The leaves are also used in folk medicines for the treatment of rheumatism, headaches, fever, loss of appetite, conjunctivitis, ringworm disease, tumor, abscesses, and sore.[19] However, in the literature, no report about the effects of cassava leaves extracts against paracetamol-induced nephrotoxicity is available. Therefore, we comparatively investigated the *in vivo* antioxidant and nephroprotective effects of methanol and acetone extracts of cassava (*M. esculenta* Crantz) leaves extracts against paracetamol-induced nephrotoxicity in rats. Also, the phytochemical screening of the extracts was carried out.

Materials and Methods

The cassava (*M. esculenta*, Crantz) leaves were collected from site III of the Delta State University, Abraka, Nigeria. They were authenticated and assigned a voucher number (UBH_M372) by Dr. H. A. Akinnibonsu of the Department of Plant Science, University of Benin, Benin City, Edo State, Nigeria.

Extract preparation

The leaves were washed with water and air dried for 10 days and grounded to powder using electric blender. The powder (50 g) was cold macerated with 200 mL of methanol in airtight container with intermittent shaking for 24 h. It was then filtered through muslin cloth and thereafter through Whatman No. 1 filter paper and the filtrate was concentrated at 45°C under reduced pressure in a rotary evaporator to obtain crude methanol extract. The above process was repeated using acetone to obtain crude acetone extract. The extracts were initially stored in airtight bottles at 4°C until usage and were reconstituted in distilled water to obtain the concentrations (50 mg/kg bw, 100 mg/kg bw, and 200 mg/kg bw) used for the study.

Animals and treatment

The male Wistar rats (54 rats) used were obtained from the department of anatomy of the Delta State University and were acclimatized for 14 days before the experiment. The rats were fed on standard diet (Top Feeds, Sapele, Nigeria) and water *ad libitum* while the study lasted. The animals were cared for in accordance with the guiding principles for care and use of laboratory animals.[20] They were randomly grouped into nine groups of six animals each and treated as follows:

Group 1 received distilled water only (negative control), group 2 received distilled water and acetaminophen, group 3 was treated with 200 mg/kg bw of methanol extract and acetaminophen, group 4 was treated with 100 mg/kg bw of methanol extract and acetaminophen, group 5 was treated with 50 mg/kg bw of methanol extract and acetaminophen, group 6 was treated with 200 mg/kg bw of acetone extract and acetaminophen, group 7 was treated with 100 mg/kg bw of acetone extract and acetaminophen, group 8 was treated with 50 mg/kg bw of acetone extract and acetaminophen, and group 9 was treated with silymarin and acetaminophen. Treatment for all groups was carried out for 7 days and on the 7th day, a single dose of 2 g/kg bw of acetaminophen was orally administered 30 min after the last treatment with extract or standard drug to rats in groups 2–9. All rats were anesthetized with chloroform 24 h after their final respective administrations. Blood was collected by cardiac puncture and allowed to cloth before centrifuged to collect the serum. Kidneys were removed and washed with saline solution (ice cold). The kidney tissues were processed for the various enzymatic and other biochemical assays.

Serum renal function analysis

Serum samples were used for renal function tests by assaying for urea, creatinine, protein, and albumin, using standard diagnostic kits (from Randox Laboratories, Ardmore, UK), whereas the sodium, potassium, and calcium levels were tested according to procedures of kits supplied by Teco Diagnostics.

Nephrochemical studies

Kidney tissue homogenates were used for the various biochemical studies. Protein concentration of the kidney tissue was determined by the method of Lowry *et al.*[21] The kidney tissue MDA levels were measured according to the method by Placer *et al.*[22] and expressed as nmol/g tissue. Kidney catalase (CAT) was determined according to the method of Aebi,[23] superoxide dismutase (SOD) by the method of Kakkar *et al.*;[24] glutathione peroxidase (GPx) by the method of Lawrence and Burk[25] method; the method of Habig *et al.*[26] was used for the determination of glutathione S-transferase (GST); and GSH levels were measured by the method of Sedlak and Lindsay.[27]

Acute toxicity (LD50): The oral acute toxicity studies of both extracts of the plant were carried out according to the method by Lorke[28] using 13 rats.

Phytochemical screening

The qualitative phytochemical tests were performed for the extracts following standard procedures as aforementioned.[29,30] The screening was carried out for the presence of flavonoids, alkaloids, saponins, anthocyanins, tannins, cardiac glycoside, steroids, anthraquinone, and triterpene.

Results

Serum renal function parameters

Table 1 shows the effects of cassava leaf extract and silymarin on serum renal function parameters. Intoxication with acetaminophen caused significant elevation in the serum levels of urea, creatinine, sodium, and potassium when compared with the negative control rats, whereas significant lower levels of total protein, albumin, and calcium were observed in the positive control group relative to the negative control group. However, pretreatment with either extracts or the standard drug for 7 days significantly ($P < 0.05$) prevented the acetaminophen-induced elevation in the urea, creatinine, sodium, and potassium levels in a dose-dependent manner. Similarly, the pretreatment also prevented and attenuated ($P < 0.05$) the effect of acetaminophen in the kidney levels of total protein, albumin, and calcium. Thus, the extracts were effective as the reference drugs in attenuating the acetaminophen-induced nephrotoxicity in the animals. The methanol extract was comparatively ($P < 0.05$) more effective than the acetone extract at 100 and 50 mg/kg bw for urea level, 200 and 100 mg/kg bw for creatinine, 100 mg/kg bw for protein, 200 mg/kg bw for albumin level, and 100 mg/kg bw for calcium level.

Oxidative stress markers

The effects of cassava leaf extracts on oxidative stress markers determined from kidney tissue homogenates are shown in Table 2. Higher MDA level was observed in the acetaminophen-intoxicated, untreated rats (positive control) when compared with that in the negative control. However, pretreatment with either the extracts or the standard drug caused significant ($P < 0.05$) reduction in the MDA levels relative to the positive control group in a dose-dependent manner. On the contrary, significantly ($P < 0.05$) lower levels of SOD and CAT were seen in the positive control rats compared to that in the negative control, but pretreatment with the extracts also brought about significant ($P < 0.05$) elevation in levels of enzymes when compared with the untreated positive control group. Similarly, significantly ($P < 0.05$) lower levels of GPx, GSH, and GST were observed in the positive control group when compared with that in the negative control rats, whereas pretreatment with either methanol or acetone extract prevented the acetaminophen-induced reduction in the levels of these antioxidants in the kidney. Also, pretreatment with the standard drug, silymarin, caused increase in these antioxidants levels. Comparatively, the methanol extract was more effective ($P < 0.05$) than the acetone extract at 200 and 100 mg/kg for CAT level, 200 and 50 mg/kg for GSH, and 100 and 50 mg/kg for GST level.

Acute toxicity (LD50): The estimated minimum lethal dose (LD50) of the acetone and methanol extracts was determined and was found to be greater than 2000 mg/kg (>2000 mg/kg).

Table 1: Effects of cassava leaf extracts on serum renal function parameters

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Total protein (mg/dL)	Albumin (mg/dL)	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mg/dL)
GRP-1	12.92 ± 0.69b	0.52 ± 0.08b	6.75 ± 0.20b	2.89 ± 0.13b	107.33 ± 9.05b	4.04 ± 0.16b	10.26 ± 0.70b
GRP-2	26.94 ± 0.83a	1.89 ± 0.12a	3.60 ± 0.23a	1.75 ± 0.12a	156.15 ± 8.90a	8.02 ± 0.20a	4.83 ± 0.35a
GRP-3	14.31 ± 0.62bf	0.60 ± 0.07bf	6.16 ± 0.08abf	2.56 ± 0.13abf	112.08 ± 9.02bf	4.81 ± 0.19abf	9.10 ± 0.54abf
GRP-4	17.21 ± 0.87abg	1.03 ± 0.08abg	5.33 ± 0.39abg	2.41 ± 0.07abf	125.11 ± 11.99bf	5.67 ± 0.43abg	8.00 ± 0.30abg
GRP-5	21.67 ± 0.83abh	1.62 ± 0.06abh	4.02 ± 0.28ah	2.03 ± 0.11ag	147.25 ± 8.54af	7.01 ± 0.38ah	6.21 ± 0.58abh
GRP-6	15.21 ± 0.76abk	0.87 ± 0.07abk	6.00 ± 0.74bk	2.25 ± 0.13abk	119.15 ± 5.80abk	4.92 ± 0.34abk	9.34 ± 0.61bk
GRP-7	22.05 ± 0.78abl	1.50 ± 0.19al	4.60 ± 0.49al	2.12 ± 0.12ak	140.04 ± 9.31ak	5.61 ± 0.27abl	7.00 ± 0.25abl
GRP-8	24.20 ± 0.96abl	1.70 ± 0.11al	3.87 ± 0.74am	2.00 ± 0.11ak	151.03 ± 8.16ak	7.41 ± 0.25am	6.01 ± 0.27abm
GRP-9	12.11 ± 0.62b	0.48 ± 0.07ab	6.51 ± 0.46b	2.81 ± 0.19b	104.21 ± 6.21b	4.22 ± 0.24b	10.01 ± 0.64b

*Down the columns: a ($P < 0.05$) vs. negative control; b ($P < 0.05$) vs. positive control

**Comparison between groups 3, 4, 5 (methanol extract) and groups 6, 7, 8 (acetone extract); values with dissimilar superscripts down a column are statistically different ($P < 0.05$)

Table 2: Effects of cassava leaf extracts on oxidative stress markers

Parameter	MDA (nmol/g tissue)	SOD (U/g protein)	CAT (U/g protein)	GPx (U/g protein)	GSH (nmol/g tissue)	GST (nM/min/mg protein)
GRP-1	40.20 ± 1.34b	21.98 ± 1.73b	17.61 ± 1.13b	133.57 ± 11.40b	34.26 ± 1.96b	69.07 ± 2.18b
GRP-2	91.29 ± 4.46a	5.75 ± 0.57a	6.88 ± 0.65a	47.35 ± 3.34a	10.09 ± 0.48a	39.69 ± 1.64a
GRP-3	43.11 ± 2.29bf	19.07 ± 1.37bf	16.03 ± 0.86bf	128.11 ± 9.30bf	32.16 ± 0.50bf	66.35 ± 2.31bf
GRP-4	56.39 ± 6.14abg	12.42 ± 1.26abg	15.22 ± 0.76bf	119.21 ± 4.64bf	25.35 ± 1.72abg	61.16 ± 1.29abg
GRP-5	70.05 ± 2.37abg	8.12 ± 0.63abh	12.08 ± 1.03abg	84.15 ± 7.09abg	18.15 ± 1.05abh	53.61 ± 2.17abh
GRP-6	49.15 ± 1.97abk	16.75 ± 1.40abk	14.12 ± 0.53abk	125.55 ± 7.52bk	27.19 ± 1.49abk	63.62 ± 2.46bk
GRP-7	64.81 ± 2.00abl	10.04 ± 0.43abl	13.11 ± 0.89abk	106.02 ± 8.68bl	23.08 ± 0.76abl	54.69 ± 2.40abl
GRP-8	74.04 ± 2.08abm	7.09 ± 0.57abm	11.20 ± 0.82abk	75.06 ± 6.36abm	20.72 ± 0.63abm	45.15 ± 2.57abm
GRP-9	41.04 ± 2.40b	20.43 ± 1.13b	16.22 ± 0.69b	125.10 ± 6.71b	31.26 ± 0.79ab	65.22 ± 2.33b

*Down the columns: a ($P < 0.05$) vs. negative control; b ($P < 0.05$) vs. positive control
**Comparing between groups 3, 4, 5 (methanol extract) and groups 6, 7, 8 (acetone extract); values with dissimilar superscripts down a column are statistically different ($P < 0.05$)

Phytochemical components of cassava leaf extracts

Chemical constituents of cassava leaf extracts are shown in Table 3. The results revealed the presence of flavonoids, alkaloids, saponins, anthocyanins, tannins, and triterpene. However, cardiac glycoside, steroids, and anthraquinone were absent in both extracts.

Table 3: Chemical constituents of cassava leaf extracts

Sr. no.	Chemical constituents	Acetone extract	Methanol extract
1	Flavonoids	+	++
2	Alkaloids	+	++
3	Saponins	+	++
4	Anthocyanins	+	++
5	Tannins	++	+
6	Cardiac glycoside	-	-
7	Steroids	-	-
8	Anthraquinone	-	-
9	Triterpene	+	++

Discussion

The kidney is a vital organ with a dominant role in maintaining homeostasis by excretion of waste products of metabolism. Medicinal plants are regularly used in preventing or treating certain ailments and are known to play a valuable role in human health. Nephrotoxicity is a toxic effect of certain substances such as chemicals and some drugs, which results in kidney damage.[31] Although methanol and acetone are two solvents with wide margin of polarity, there have been conflicting reports about their potential for extracting phytoconstituents. Although Truong *et al.*[32] and Felhi *et al.*[33] reported maximum phenolic and flavonoid contents for methanol from their findings, Sharaibi and Afolayan[34] and Ngo *et al.*[35] gave a contrary report from their work, that is, the phytochemical constituents, such as flavonoids and saponins, were higher in acetone extract than that in methanolic extract. Hence, methanol and acetone extracts were compared in this study.

In this study, the phytochemical investigation of the extracts revealed the presence of flavonoids, alkaloids, saponins, anthocyanins, tannins, and triterpene. However, cardiac glycoside, steroids, and anthraquinone were absent in both extracts. The results partly agree with previous reports by Anbuselvi and Balamurugan,[36] on the phytoconstituents in methanol and acetone extracts of cassava leaves. Although saponins were reportedly absent in the early report, they were found to be present in this study, whereas steroids and cardiac glycosides reportedly found in the acetone extract in earlier report were not found in either extract in this study. The observed differences in phytochemical composition of the previous report and our results may be due to differences in environmental conditions, which are dependent on the soil nature and level of plant nutrient and the presence and nature of environmental pollutants.[37] Furthermore, we observed from the results that the concentration of phytoconstituents contained in methanol extract of *M. esculenta* leaves was slightly different from that in the acetone extract of the same sample. Thus, the concentration of tannins was more in acetone extract than that in the methanol extract. On the contrary, the presence of alkaloids, saponins, and flavonoids detected was more in the methanol extract than that in the acetone extract. The medicinal values of tannins, which include anti-inflammatory and wounds healing, have been ascribed to its stringent properties. Equally, the wound-healing activity of saponin has been reported.[38] Flavonoids have been reported to prevent drug-induced nephrotoxicity as a result of its strong antioxidant activity. Similarly, certain flavonoids and triterpenoids have been said to show nephroprotective effect due to their antioxidant properties.[39]

Nephrotoxic effect is frequently recognized by estimating specific and reliable biomarkers such as serum urea and serum creatinine. Urea is a major product of protein metabolism. It is totally filtered by the glomerulus, and then excreted passively in the urine at high concentrations. Thus, the level of urea in serum is often used as a useful indicator of renal function, whereas creatinine, an end product of muscle breakdown, on the contrary, is constantly removed by the kidneys. Also, the concentration of creatinine in serum is an indicator of renal function, and an increase in serum level of creatinine is a

signal of abnormal functioning of the kidneys.[40] In this study, we noticed that the administration of acetaminophen (2 g/kg) resulted in significant nephrotoxicity as shown by elevation in serum urea, creatinine, sodium, and potassium levels, which is in agreement with earlier reports.[40] However, a decrease in the levels of total protein, albumin, and calcium was observed with the administration of high-dose acetaminophen, which was consistent with earlier reports.[41,42] However, pretreatment with the extracts or silymarin caused a decrease in the acetaminophen-induced enhancement of the aforementioned serum parameters (urea, creatinine, sodium, and potassium levels). Likewise, we observed that the administration of cassava leaves extracts, particularly, at the highest dose for 7 days led significantly to an increase in the levels of total protein, albumin, and calcium. The results reveal that oral administration of the extract offered a dose-dependent protection against the acetaminophen-induced nephrotoxicity in rats. Thus, pre-administration of cassava leaves extract significantly and dose dependently inhibited the acetaminophen-induced elevated serum biomarkers (urea, creatinine, sodium, and potassium). The observed inhibition was of comparable effects with the standard drug (silymarin). Adrian *et al.*[43] reported that the administration of extract of *M. utilissima* leaves to cadmium-induced mice caused a reduction in the levels of serum creatinine and urea.

There exists a correlation between oxidative stress and nephrotoxicity in various experimental models.[44] In this experiment, MDA level was noted to be high significantly on treatment with paracetamol when the positive control animals were compared with the negative control group. This observation about the MDA level is in agreement with the reports of Mostafa *et al.*[45] An acute paracetamol overdose has been reported to elevate lipid peroxidation level and overwhelms renal tissue antioxidant defense mechanisms.[46]

SOD and CAT are said to be very important enzymes reported to be involved in enhancing the effects of metabolism of oxygen. SOD is regarded as the first line of defense the organism uses in fighting against the harmful effects from cellular oxygen radicals through the scavenging of ROS by catalyzing dismutation of superoxide to yield oxygen and hydrogen peroxide.[47] In this study, a decreased level of CAT was noticed in the paracetamol-intoxicated rats, which has been linked with increased lipid peroxidation and weakening of the body antioxidant defense system because of paracetamol overdose.[48] CAT is important for detoxification of cellular oxygen and hydrogen peroxide (H_2O_2)-derived free radicals. Also, in the paracetamol-intoxicated group, levels of SOD, CAT, GPx, GSH, and GST were significantly less than the negative control group, which was an indication of oxidative stress to the kidney. Previous reports indicated that intracellular GSH plays a crucial role in the detoxification of APAP and also prevents APAP-induced toxicity in both liver and kidney cells. Also, it has been reported that generation of ROS often precedes the reduction of intracellular GSH and cellular damage in APAP-induced hepatotoxicity.[49]

Earlier, it has been reported that acetaminophen intoxication leads to significant decrease in hepatic and renal activities of antioxidant enzymes; GSH, GR, GPx, CAT, and GST.[45,50] Similarly, Roy *et al.*[51] observed an increase in the levels of urea, creatinine, and MDA and a decrease in the levels of antioxidant enzymes, such as SOD, CAT, and GSH, in rats treated with 550 mg/kg acetaminophen for 14 days. Also, Sabiu *et al.*[42] observed significant increases in serum concentrations of urea, creatinine, uric acid, potassium, and sodium in acetaminophen-intoxicated rats.

However, pretreatment with the extract dose dependently prevented the acetaminophen-induced oxidative stress observed in the positive control group. Thus, the extracts of cassava leaves dose dependently showed antioxidant property similar to the standard drug (silymarin) and inhibited oxidative stress. Comparatively, the methanol extract gave higher *in vivo* antioxidant effects than the acetone extract of the leaves and also showed better nephroprotective effects than the acetone extract in a dose-dependent manner, which is in agreement with the report of Adrian *et al.*[43] on the effect of the extract of *M. utilissima* leaves against cadmium-induced mice.

Paracetamol kidney damage is mediated by its deacetylation to *p*-amino phenol excreted in urine. Thus, *p*-amino phenol plays a key role in pathogenesis of paracetamol induced kidney damage. Conjugates of GSH formed in the liver are implicated in paracetamol-induced kidney toxicity.[52]

The obtained results in this study may be described on the basis of the observed *in vivo* antioxidant properties of the leaves extracts as pretreatment with the extracts, or silymarin significantly prevented the acetaminophen-induced oxidative stress, which was evident by decreased MDA levels in renal tissue and increments to near normal in the other oxidative stress marker parameters examined. Various studies revealed that the nephroprotective activities of plants are due to the presence of antioxidant compounds in them.[53] Though the possible mechanism(s) of cassava leaves extracts against acetaminophen-induced nephrotoxicity was not investigated in this study, the possible mechanism of protection of the extracts may be mediated through free radical scavenging and/or antioxidant activities. Reports have shown that medicinal plants having nephroprotective properties often mediate their protection through free radical scavenging and/or antioxidant activities as a result of their high concentration of alkaloids and flavonoids.[54] Likewise, the hepatoprotective and nephroprotective effects of saponins against carbon tetrachloride–induced toxicity have been reported.[55]

Conclusion

On the basis of results obtained from this study, it could be concluded that the extracts of cassava leaves have high nephroprotective potential based on their antioxidant activity and phytoconstituents. Thus, the leaves of *M. esculenta* could be a good source of natural pharmaceutical nephroprotective products.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Jones AF, Vale JA. Paracetamol poisoning and the kidney. J Clin Pharm Ther 1993;18:5-8.
2. Abdel-Zaher AO, Abdel-Hady RH, Mahmoud MM, Farrag MM. The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. Toxicology 2008;243:261-70.
3. El-Shafey MM, Abd-Allah GM, Mohamadin AM, Harisa GI, Mariee AD. Quercetin protects against acetaminophen-induced hepatorenal toxicity by reducing reactive oxygen and nitrogen species. Pathophysiology 2015;22:49-55.
4. Sarumathy K. A protective effect of *Caesalpinia sappan* on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. J Pharmacol Toxicol 2011;1:2.
5. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol 2012;2012:465897.
6. Tietz N. Kidney function and disease. In: Tietz Fundamentals of Clinical Chemistry. 6th ed. London, UK: UB Sauders; 2008. p. 360-659.
7. Roy S, Das K, Mandal S, Pradhan S, Patra A, Nandi DK. Crude root extract of *Asparagus racemosus* ameliorates acetaminophen induced uremic rats. Int J Pharm Sci Res 2013;4:3004-12.
8. Pradhan S, Mandal S, Roy S, Mandal A, Das K, Nandi DK. Attenuation of uremia by orally feeding alpha-lipoic acid on acetaminophen induced uremic rats. Saudi Pharm J 2013;21:187-92.
9. Jansz ER, Uluwaduge DI. Biochemical aspects of cassava (*Manihot esculenta* Crantz) with special emphasis on cyanogenic glucosides—A review. J Natn Sci Council Sri Lanka 1997;25:1-24.
10. Ebuehi OAT, Babalola O, Ahmed Z. Phytochemical, nutritive and antinutritive composition of cassava (*Manihot esculenta*) tubers and leaves. Nigerian Food J 2005;23:40-6.
11. Jayasri P, Narandra ND, Elumalai A. Evaluation of antihelmintic activity of *Manihot esculenta* leaves. Int J Curr Pharm Res 2011;4:115-6.
12. Okeke CU, Iweala F. Antioxidant profile of *Dioscora rotundata Manihot esculenta* Aloe vera. J Med Res Technol 2007;4:4-10.
13. Popoola TOS, Yangomodou OD, Akintokun AK. Antimicrobial activity of cassava seed oil on skin pathogenic microorganism. Res J Med Plant 2007;1:60-4. 1167-73.
14. Latif S, Müller J. Potential of cassava leaves in human nutrition: A review. Trends Food Sci Technol 2015;44:147-58.
15. Achidi UA, Ajayi OA, Bokanga M, Maziya-Dixon B. The use of cassava leaves as food in Africa. Ecol Food Nutr 2005;44: 423-35.
16. Nkunzimana J, Zee JA, Turgeon-O'Brien H, Marin J. Potential iron bioavailability in usual diets of the Imbo Region of Burundi. J Agricult Food Chem 1996;44:3591-4.
17. Montagnac JA, Davis CR, Tanumihardjo SA. Processing techniques to reduce toxicity and antinutrients of Cassava for use as a staple food. Compr Rev Food Sci Food Saf 2009;8:17-27.
18. Bradbury JH, Denton IC. Mild method for removal of cyanogens from cassava leaves with retention of vitamins and protein. Food Chem 2014;158:417-20.
19. Bahekar SE, Kale RS. Antidiarrheal activity of ethanolic extract of *Manihot esculenta* Crantz leaves in Wistar rats. J Ayurveda Integr Med 2015;6:35-40.
20. Olfert ED, Cross BM, McWilliams AA. Guide to the Care and Use of Experimental Animals. 2nd ed. Ottawa, Canada: Canadian Council on Animal Care; 1993. p. 82-93.
21. Lowry OH, Rosenbrough NF, Farr AL, Randall JL. Protein measurement with the Folin Phenol reagent. J. Biol. Chem 1951;193:265-75.
22. Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem 1966;16:359-64.
23. Aebi H. Catalase. In: Bergmeyer HU, editor. Methods in Enzymatic Analysis. New York: Academic Press; 1983. p. 276-86.
24. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130-2.
25. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 1976;71:952-8.
26. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.
27. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192-205.
28. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983;54:275-87.
29. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, UK: Chapman & Hall; 1973.
30. Trease GE, Evans WC. Pharmacognosy. London, UK: Baillaiere-Tindals; 1989. p. 687-9.
31. Saxena R, Masood M, Khan F, Qureshi Z, Rathore M. Effect of *Dalbergia sissoo* leaves on aminoglycosides induced nephrotoxicity in experimental rats. Int J Pharmacol Pharm Sci 2016;3:5-14.
32. Truong D, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. J Food Qual 2019;8178294:1-9.
33. Felhi S, Daoud A, Hajlaoui H, Mnafgui K, Gharsallah N, Kadri A. Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. Food Sci Technol Campinas 2017;37:483-92.
34. Sharaibi OJ, Afolayan JA. Phytochemical analysis and toxicity evaluation of acetone, aqueous and methanolic leaf extracts of *Agapanthus praecox* Willd. Int J Pharm Sci Res 2017;8: 5342-8.
35. Ngo TV, Scarlett CJ, Bowyer MC, Ngo PD, Vuong QV. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis*. L J Food Qual 2017;9305047:1-8.
36. Anbuselvi S, Balamurugan T. Phytochemical and antinutrient constituents of cassava and sweet potato. W J Pharm Pharm Sci 2014;3:1440-9.
37. Ubani CS, Oje OA, Ihekogwo FNP, Eze EA, Okafor CL. Effect of varying soil minerals and phytochemical parameters on antibacterial susceptibility of *Mitracarpus villosus* ethanol extracts; using samples from south east and south-southern regions of Nigeria. Glob Adv Res J Microbiol 2012;1:120-5.
38. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr J Biotechnol 2006;5:357-61.
39. Takeoka GR, Dao LT. Antioxidant constituents of almond (*Prunus dulcis*) (Mill). Hulls J Agric Food Chem 2003;51:496-501.

40. Lesely AS, Levey AS. Measurements of kidney function. Med Clin North Am 2005;89:457-73.
41. Egbung GE, Odey OD, Atangwho IJ. Effect of *Vernonia calvoana* extract on selected serum kidney function biomarkers of acetaminophen treated Wistar rats. Asian J Biochem 2017;12:99-104.
42. Sabiu S, O'Neill FH, Ashafa AOT. Membrane stabilization and detoxification of acetaminophen-mediated oxidative onslaughts in the kidneys of Wistar rats by standardized fraction of *Zea mays* L. (Poaceae), Stigma maydis. Evid-Based Complementary Altern Med 2016;2046298:1-14.
43. Adrian, Fachrial E, Almahdy A, Syaifullah S, Zein R. The effect of ion Cd(II) in the kidney of experimental rats and utilization of cassava leaves (*Manihot utilisima*) as antidote. Res J Pharm Biol Chem Sci 2016;7:1228-32.
44. Neeraj KG, Sharad M, Tejram S, Abhinav M, Suresh PV, Rajeev KT. Evaluation of anti-apoptotic activity of different dietary antioxidants in renal cell carcinoma against hydrogen peroxide. Asian Pac J Trop Biomed 2011;2:57-63.
45. Mostafa FAA, Salem AA, Elaby SM, Nail NS. Protective activity of commercial citrus peel extracts against paracetamol induced hepato-nephro toxicity in rats. J Chem Bio Phy Sci Sec 2016;6:070-83.
46. Abdel-Zaher AO, Abdel-Rahman MM, Hafez MM, Omran FM. Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. Toxicology 2007;234:124-34.
47. Hussein SA, Ragab OA, El-Eshmawy MA. Protective effect of green tea extract on cyclosporine A-induced nephrotoxicity in rats. J Biol Sc 2014;14:248-57.
48. Yousef MI, Omar SA, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food Chem Toxicol 2010;48:3246-61.
49. Manov I, Hirsh M, Iancu TC. Acetaminophen hepatotoxicity and mechanisms of its protection by N-acetylcysteine: A study of Hep3B cells. Exp Toxicol Pathol 2002;53:489-500.
50. Okokon JE, Simeon JO, Umoh EE. Nephroprotective activity of *Homalium letestui* stem extract against paracetamol induced kidney injury. J Exp Integr Med 2016;6:38-43.
51. Roy S, Pradhan S, Das K, Mandal A, Mandal S, Patra A, *et al*. Acetaminophen induced kidney failure in rats: A dose response study. J Biol Sci 2015;15:187-93.
52. Li C, Liu J, Saavedra JE, Keefer LK, Waalkes MP. The nitric oxide donor, V-PYRRO/NO, protects against acetaminophen-induced nephrotoxicity in mice. Toxicology 2003;189:173-80.
53. Nandave M, Ojha SK, Joshi S, Kumari S, Arya DS. *Moringa oleifera* leaf extract prevents isoproterenol-induced myocardial damage in rats: Evidence for an antioxidant, antiperoxidative, and cardioprotective intervention. J Med Food 2009;12: 47-55.
54. Adeneye AA, Benebo AS. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. J Ethnopharmacol 2008;118:318-23.
55. Jeong TC, Kim HJ, Park J, Ha CS, Park JD, Kim S, *et al*. Protective effects of red ginseng saponins against carbon tetrachloride induced hepatotoxicity in Sprague-Dawley rats. Planta Medica 1996;63:136-40.