



Cnidoscopus aconitifolius leaf extract and ascorbate confer amelioration and protection against dimethyl nitrosamine-induced renal toxicity and testicular abnormalities in rats

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ABSTRACT

Extracts of *Cnidoscopus aconitifolius* (CA) have been reported to possess medicinal properties ranging from potential hepatoprotective, anti-diabetic, and anti-cardiovascular. In our previous study, gas chromatography mass spectroscopy check of CA extract showed the inclusion of 2,4-bis(1,1-dimethylethyl)-phenol, a phenolic phyto-compound that constitutes about 45 %, carotene and linoleic acid sources, and silicon-rich components. Hence we compare the preventive and ameliorative potentials of CA with ascorbate in dimethyl nitrosamine (DMN)-induced renal toxicity and sperm abnormalities in rats. Renal toxicity was investigated by quantifying the levels and activities of endogenous antioxidant parameters. Renal damage marked by significant reduction in GSH level, as well as significant elevation in MDA concentration, and activities of GPx, GST, CAT, and SOD were restored after the intervention of CA and ascorbate. Also, there was decrease in live sperm, sperm concentration, sperm gross and individual motility, and normal sperm morphology, following DMN administration. Based on the gathered results, it is concluded that ascorbate and CA demonstrate comparable ameliorative and protective effects against DMN-induced renal and testicular toxicities in rats.

1. Introduction

Dimethyl nitrosamine (DMN) is formed as a by-product of industrial activities that utilize either nitrites or nitrates or both, and amines under specific pH conditions [1]. This is as a result of inadvertent production when alkylamines, specifically di- and trimethylamine combine and react with nitrous acid, nitrite salts or nitrogen oxides [2]. Therefore, DMN may be seen in wastes of industries such as pesticide and rubber manufacturing, food production, leather tanning, foundries, sewage treatment plant effluent, and dye production, all of which are released into surrounding water. DMN has also been discovered in the emissions and exhaust of diesel vehicle [3].

Nitrosamines, a strong carcinogen in our environment, has been reported to stimulate the production of lethal and reactive oxygen species (ROS) leading to oxidative stress, disturb the antioxidant protecting system and cellular injury, which can be one among the factors within the etiology of cancer [4,5]. In oxidative stress, ROS are generated in biological system, and these ROS can destroy DNA causing damage to chromosomes. It also oxidizes cellular thiols and removes electrons from

unsaturated fatty acids to begin the destruction of membrane lipids [6]. Moreover, overproduction of ROS propels the oxidative damage which add to the over one hundred diseases in humans including atherosclerosis, liver disease, diabetics, coronary heart disease, cancer, neurodegenerative disorder, and also play vital role in the process of aging [7,8]. In normal physiological conditions, cellular ROS production needs to be checked by the action of antioxidant enzymes and proteins. As a result of their potential deleterious effects, too much of the ROS produced must be quickly removed by a variety of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) etc. These are needed substances which have the capacity to shield cells from destructions caused by ROS-induced oxidative stress [9]. These they do by scavenging free radicals and inhibiting lipid peroxidation [10].

We have numerous underutilized native and indigenous plants that are edible and can serve as food [11]. Nigeria is blessed with herbal resources, and one of these plants that are utilized locally is *Cnidoscopus aconitifolius* (CA), known as Chaya [12], belonging to the family of the arbore woody plant, a perennial evergreen shrub containing milky sap

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from a pinnate lobed leaves. The plant was first cultivated as a household leafy green vegetable in the Maya area of Guatemala and Southeast Mexico [13]. CA is usually consumed in Yoruba-land of Nigeria as vegetable, where we refer to it as *Iyana Ipaja*, while within a part of Southern Nigeria; it is called “Hospital Too Far” due to its health benefits. CA leaves have been shown to have a high content of ascorbate, β -carotene, protein, calcium, phosphorus, iron, thiamin, riboflavin, and niacin [14]. Some reported uses of the plant are as an antibacterial [15], treatment of anemia and protein energy malnutrition [16], anti-diabetic [17], and treatment of mega dose of paracetamol intoxication [17,18].

We therefore in this study, compared the effect of administrations of CA with ascorbate in renal toxicity and testicular abnormalities induced by DMN administration in male rats.

2. Materials and methods

DMN (purity $\geq 98\%$), also called N-nitrosodimethylamine ($C_2H_6N_2O$; CAS number 62-75-9) was bought from Sigma Chemical Co., Saint Louis, MO, USA, and is of analytical grade.

2.1. Plant material

Leaves of CA plant were harvested from the premises of University of Ibadan, Ibadan, Oyo State, Nigeria. The leaves were then identified by Professor Adebisi, a Forester at the University of Ibadan, Nigeria, where we have CA specimen already deposited.

2.2. Preparation of CA extract

The method of Oyagbemi and Odetola [18] was followed. Components of about six hundred grams of the fresh CA leaves were extracted using ethanol. The extracted components were concentrated under reduced pressure, followed by evaporation to dryness, which yielded 12.78 g. The dried extract was reconstituted in corn oil and administered to rats.

2.3. Qualitative phytochemical screening of CA

Bioactive phyto-compounds in CA extract were screened for alkaloids, phenols, glycoside, saponin, steroids, phlobatannin, flavonoids, and tannins by using standard procedures [19–22].

2.4. Quantitative phytochemical analyses

Total alkaloid and tannin present were quantified by following the procedure of Harborne [23] and Van-Burden and Robinson [24] respectively. Total saponin content was determined by following the method of Obadoni and Ochuko [25], while Folin-Ciocalteu photometric method was used for the quantification of total phenolic content [26].

2.5. Half maximal inhibitory concentration (IC_{50}) determinations of CA and ascorbate

IC_{50} was determined based on the ability of CA and ascorbate to inhibit nitric oxide (NO), ferric reducing antioxidant power (FRAP) [27], 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) [28], and hydrogen peroxide (H_2O_2) [29].

2.6. Fourier-transform infrared (FTIR) spectroscopic analysis of CA extract

FTIR analysis reveals the chemical bonds of compounds present in a particular plant. In this analysis, ten (10) milligram of dried CA powder was encapsulated in hundred (100) milligram of KBr pellet, for the preparation of translucent sample discs. CA sample was loaded in the

FTIR machine (Shimadzu, Japan), using a scan range from 400 to 4000 cm^{-1} , and a resolution of 4 cm^{-1} .

2.7. Elemental analysis compositions of CA extract

0.57 g of the extract of CA was weighed into a digestion tube. 10 ml of nitric acid (HNO_3) was added and heated at 350 °C until a clear mixture is formed. It was left to cool, and 20 ml of distilled water was added and then filtered. The resulting filtrate was used for the elemental analysis. Analysis of magnesium, sodium, manganese, zinc, nickel, iron, potassium, calcium, cadmium and lead was done using Atomic Absorption Spectrometer (Thermo Scientific S Series 712354 v1.27). The sample is aspirated and mixed with acetylene and air and burned in a flame to produce the individual atoms. Following absorption of ultraviolet light at the required wavelengths, metal atoms in the sample that are in the ground state are switched to a higher state, causing reduction in its intensity. Change in intensity is read, which is converted into an absorbance proportionate to the sample concentration by the computer software installed.

2.8. Proximate analysis of CA

Proximate compositions including moisture, ash, fat, carbohydrate, crude protein, and crude fiber of sample of CA were determined following the methods described by AOAC [30]. For Moisture content, 2 g of the sample was weighed into dried and weighed crucible, and put into a moisture extraction oven at 105 °C, and heated for 3 h. The dried sample was then put into desiccator, allowed to cool and was reweighed. This process was repeated until a constant weight was obtained. The difference in weight was calculated as a percentage of the original sample. For the ash content, 2 g of the sample was weighed into a crucible, heated in a moisture extraction oven for 3 h at 100 °C before it was transferred into a muffle furnace at 550 °C where it was allowed to turn white and free of carbon. Sample was removed from the furnace, cooled in a desiccator to a room temperature and reweighed immediately. The weight of the residual ash was calculated and expressed as percentage. For the crude protein determination, the micro Kjeldahl method was used. In this method, 2 g of the sample was mixed with 10 ml of concentrated sulphuric acid in a heating tube. A catalyst was added and the mixture was heated inside a fume cupboard. The resulting digest was transferred into distilled water, and 10 ml portion of the digest was mixed with equal volume of 45 % sodium hydroxide solution and was poured into a Kjeldahl distillation apparatus. The mixture was distilled, distillate collected into 4% boric acid solution that contained 3 drops of indicator. Following titration, nitrogen content was calculated and multiplied by 6.25 to obtain the crude protein content. For the fat content, 2 g of the sample was wrapped with a filter paper and put into the thimble fitted to a cleaned, dried, and weighed round bottom flask, containing 120 ml of petroleum ether. Sample was then heated with a heating mantle and allowed to reflux for 5 h and stopped. The thimble with the spent sample was weighed and the difference in weight was received as mass of fat and was expressed in percentage of the sample. For the determination of crude fibre, 2 g of the sample and 1 g of asbestos were put into 200 ml of 1.25 % sulphuric acid, and was boiled for 30 min. The content was then poured into Buchner funnel equipped with muslin cloth. Following filtration, the residue was put into 200 ml of boiled sodium hydroxide for 30 min, and transferred to the funnel and filtered. The filtrate was washed in alcohol three times, and the obtained material was further washed in petroleum ether three times. Obtained residue was poured in dried crucible and put in moisture extraction oven to obtain a constant weight. This was then weighed and the difference of weight was taken and expressed in percentage. For the carbohydrate content determination, this was calculated as the difference between 100 and the summation of other proximate parameters.

2.9. Animal studies

Total of fifty five (55) male Wistar rats that are 8 weeks old and weighing 200 g were purchased from College of Veterinary medicine of our university and used for this study following acclimatization for a period of two weeks. The permission and approval to use the animals were granted by the Departmental Committee on Animal Ethics.

2.10. Experimental design

Rats were separated into eleven (11) groups, each having five (5) rats. Group I (2 mL/kg corn oil for 7 days), Group II (200 mg/kg CA for 7 days), group III (400 mg/kg CA for 7 days), group IV (400 mg/kg ascorbate for 7 days), Group V (30 mg/kg DMN administered once for 48 h), Group VI (30 mg/kg single DMN dose for 48 h +200 mg/kg CA for 7 days), Group VII (200 mg/kg CA for 7 days +30 mg/kg single DMN dose for 48 h), Group VIII (30 mg/kg single DMN dose for 48 h +400 mg/kg CA for 7 days), group IX (400 mg/kg CA for 7 days +30 mg/kg single DMN dose for 48 h), group X (30 mg/kg single DMN dose for 48 h +400 mg/kg ascorbate for 7 days), and group XI (400 mg/kg ascorbate for 7 days +30 mg/kg single DMN dose for 48 h). All administrations were done orally except DMN that was administered intraperitoneally.

2.11. Samples collection

Rats were treated in accordance to the established guidelines for the care and handling of laboratory animals [31]. Following administrations, about 6 mL of blood was collected via the abdominal artery into clean EDTA and heparin bottles, the latter were spinned in a centrifuge for 10 min at 3000 rpm to obtain plasma, and separated into 1 mL tubes for the estimation of biochemical indices, while the latter was used for hematological studies. Spermatozoa were also collected, and various characteristics and morphologies were checked. Kidney and testis were also harvested from the rats; a portion of the kidney was excised and homogenized. The homogenate was centrifuged at 5000 rpm for 10 min, and the resulting supernatant was also separated and used for the estimation of oxidative stress parameters. Another portion of the kidney and testis were fixed in 10 % formal-saline for histopathology.

2.12. Spermatozoa collection

The spermatic cord was incised, and sperm samples were collected from the cauda epididymis minced in pre-warmed (37 °C) saline as described by Oyeyemi and Ubogoro [32], and samples were analyzed immediately for characteristics and morphologies.

2.13. Sperm motility

Sperm individual and gross motility was determined microscopically following their placement on glass slides, and results were expressed as percentage motility [33].

2.14. Sperm viability

Sperm viability study was performed using eosin/nigrosin stain as described by Acharya et al. [34]. Sperm located in the epididymis was eluted on a microscopic slide and stained. Live spermatozoa were not stained while the dead spermatozoa retain the stain. The live and dead spermatozoa were counted under Olympus light microscope using x 40 magnification and percentage viability ratio (live:dead ratio) was calculated.

2.15. Sperm count

Sperm count was done under a microscope immediately after sacrifice. Epididymis was homogenized in normal saline, and then mixed

with sodium bicarbonate-formalin in ratio 1–20. The hemocytometer chamber was filled with well diluted spermatozoa and was counted in 2 mm² of the chamber. The sperm cells were counted in 1 ml of fluid and multiplied by 100,000 [34].

2.16. Sperm morphology

This was assessed by smearing the spermatozoa on slides and allowed to air-dry, followed by staining with Walls and Ewas stain. The slides were then viewed at x100 magnification under Olympus light microscope. The normal and various forms of abnormal spermatozoa morphologies were counted and their percentage was calculated [35].

2.17. Measurement of renal function markers

Plasma concentrations of uric acid, creatinine, urea, and protein were estimated based on the procedures in their respective Cypress Diagnostic Kits, Langdorp, Belgium. Briefly, for the uric acid assay, the reaction mixture contained 25 µl of plasma and 1 ml of the working reagent. A standard tube was also prepared which contained 25 µl of the uric acid standard and 1 ml of the working reagent. They were mixed and incubated for 10 min at room temperature, and absorbance of sample and standard were read against blank, and from the absorbance, the concentration of uric acid was calculated. For urea, the reaction mixture contained 10 µl and 1 ml of the plasma and working reagent respectively, while the standard tube contained 10 µl and 1 ml of the urea standard and working reagent. They were mixed and incubated at room temperature for 10 min. After incubation, 1 ml of sodium hypochlorite was added to both tubes, mixed and incubated again for 10 min at room temperature. The absorbance of sample and standard were read against blank, and from the absorbance, the concentration of urea was calculated. For creatinine concentration determination, the reaction mixture was made of 100 µl of plasma and 1 ml of alkaline picric acid reagent, while the standard tube contained 100 µl of the creatinine standard and 1 ml of the alkaline picric acid reagent. The content of the tubes were mixed and absorbance were read after 30 and 90 s of sample and standard addition. Creatinine concentration was calculated from the change in absorbance obtained. For total protein, the reaction mixture contained 25 µl and 1 ml of the plasma and biuret reagent respectively, while the standard tube contained 25 µl and 1 ml of the protein standard and biuret reagent. They were mixed and incubated at room temperature for 10 min. After incubation, the absorbance of sample and standard were read against blank, and from the absorbance, the concentration of total protein was calculated.

2.18. Hematological indices

White blood cell (WBC) and red blood cell (RBC) counts, as well as packed cell volume (PCV), were estimated using standard methods described by Dacie and Lewis [36]. Counting of total WBC was done using Turks fluid (1:20) and counted with a Neubauer counting chamber under a microscope using x10 magnification in an area of 4 mm². For RBC, diluted blood (1:200) with Hayem's fluid was counted with a Neubauer counting chamber under a microscope using x40 magnification in an area of 15 mm². PCV was estimated by adding blood sample to a bottle containing anticoagulant. Hematocrit tube was filled to 100 mm and was centrifuged for 30 min at 3000 rpm. The height of the RBC was read and expressed as a percentage of PCV.

2.19. Determination of renal MDA and GSH levels

Buege and Aust [37] method was followed for the determination of MDA. In this method, kidney sample (0.1 ml) was added to trichloroacetic acid-thiobarbituric acid hydrochloric acid (TCA/TBA/HCl) (1:1:1 ratio) reagent (2 ml), boiled for 15 min, and cooled. After centrifugation at 3000 rpm for 10 min, the absorbance of the supernatant was read at

532 nm against a blank. MDA concentration was estimated using the molar extinction coefficient of $1.55 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$. GSH concentration was estimated following the Moron et al. [38] method. Briefly, 1:1 of the kidney sample and sulphosalicylic acid were mixed together, centrifuged at 3000 rpm for 5 min. 0.5 ml of the resulting supernatant was added to a mixture containing 4 ml of 0.1 M phosphate buffer (pH 7.4) and 0.5 mL of Ellman's reagent, and the absorbance was read at 412 nm.

2.20. Determination of renal GST, GPx, SOD, and CAT activities

Catalase activity was determined using the protocol of Sinha [39]. The reaction mixture (2.5 mL) contained 0.01 M phosphate buffer (pH 7.0), kidney sample (0.25 mL) and 2 M H_2O_2 (1 mL). The reaction was stopped by the addition of 0.5 mL dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios) at 0, 1, 2, and 3 min, followed by heating in boiling water, and then cooled. The absorbance was read at 570 nm. Method of Misra and Fridovich [40] was followed for the estimation of SOD. Briefly, the reaction mixture involves 0.2 mL of the kidney sample, 2.5 mL of 0.05 M sodium bicarbonate buffer (pH 10.2), and 0.3 mL of adrenaline. Mixture was shaken thoroughly and absorbance was read at 480 nm. The unit of enzyme activity is amount of enzyme required for 50 % inhibition of the auto oxidation of adrenaline. Method of Rotruck et al. [41] was used for the determination of GPx activity. Added together were 600 μL of distilled water, 500 μL of kidney sample, 100 μL of sodium azide, 200 μL of reduced glutathione, 500 μL of phosphate buffer, and 100 μL of hydrogen peroxide. Incubation of mixture was done for 3 min, and 500 μL of TCA was added. After centrifugation, 1000 μL of supernatant was added to di-potassium hydrogen phosphate and Ellman's reagent. Absorbance was then read at 412 nm. Method of Habig et al. [42] was followed for the determination of GST activity. Briefly in this method, 30 μL of reduced glutathione, 2.79 mL of 0.1 M phosphate buffer (pH 6.5), 30 μL of kidney sample, and 150 μL of 2,4-dinitrochlorobenzene (CDNB) were added together. They were mixed and absorbance was read at 340 nm each minute for 3 min.

2.21. Kidney and testis histopathology

The formal-saline fixed kidney and testis tissues were paraffin embedded, sectioned and adhered onto the surface of glass slides, stained with haematoxylin–eosin dye and then viewed and read under a microscope at x 100 magnification by a Pathologist, at the Department of Veterinary Pathology, of our university.

2.22. Statistical analysis of data

Mean \pm Standard Error of Mean (SEM) was used to express the results. One-way analysis of variance (ANOVA) was used to determine the differences among the groups, using Statistical Package for Social Sciences (SPSS) software, version 17.0. *P*-values < 0.05 were chosen to be statistically significant.

3. Results

3.1. Qualitative phytochemical screenings of CA

Results in Table 1 show the presence of alkaloids, phenols, glycoside, saponin, steroids, tannin, and so on, the most abundant of all being alkaloids and phenols.

3.2. Quantitative phytochemical screenings of CA

Following quantification of phytochemicals in CA (Table 2), quantity of total phenol and tannin recorded were 405.56 ± 16.67 GAEmg/g and 38.70 ± 0.50 GAEmg/g respectively. Also, 0.234 ± 0.00 g/g and 0.316 ± 0.08 g/g were recorded for saponin and alkaloid respectively.

Table 1

Qualitative phytochemical screenings of ethanolic leaf extract of *Cnidoscopus aconitifolius*.

Phytochemicals	Quality
Alkaloids	+++
Phenols	+++
Glycoside	++
Saponin	++
Steroids	++
Phlobatannin	++
Flavonoids	+
Tannin	++

+++ appreciable amount; ++ moderate amount; + minute amount.

Table 2

Quantitative phytochemical screenings of ethanolic leaf extract of *Cnidoscopus aconitifolius*.

Phytochemicals	Quantity
Saponin (g/g)	0.234 ± 0.00
Alkaloid (g/g)	0.316 ± 0.08
Tannin (GAEmg/g)	38.70 ± 0.50
Total phenol (GAEmg/g)	405.56 ± 16.67

Values are mean of 2 replicates; GAE = Gallic acid equivalent.

3.3. IC_{50} determination of CA and ascorbate

Table 3 shows the results of IC_{50} of *in vitro* antioxidant models obtained for CA and ascorbate. For NO and FRAP, CA had close and comparable effects with ascorbate, while for DPPH, ABTS, and H_2O_2 , ascorbate demonstrated a better effect than CA.

3.4. FTIR results

Following the subsection of CA extract to FTIR analysis, twenty (20) peak values were obtained, ranging from 418.57 cm^{-1} to 3400.62 cm^{-1} . Some of the bonds detected (Table 4) were O–H stretch of phenols and alcohol (3400.62 cm^{-1}), C–H stretch of alkanes (2926.11 and 2854.74 cm^{-1}), Si–H bond of silane (2362.88 cm^{-1}), C=O stretch of α,β -unsaturated aldehydes and ketones (1708.99 cm^{-1}), C–N stretch of aromatic amines (1276.92 cm^{-1}), C–O stretch of alcohols, carboxylic acids, esters, and ethers (1178.55 cm^{-1}), C–N stretch of aliphatic amines (1060.88 cm^{-1}), C–H bond of aromatics (873.78 cm^{-1}), and so on (Table 4).

3.5. Mineral compositions of CA extract

Analysis of the extract of CA for mineral or elemental compositions and their quantities (Table 5) revealed that the leaf of the plant possesses calcium, sodium, magnesium, and potassium (which are all macronutrients) in large amounts (Table 5). The amounts recorded were 5556.23, 2078.58, 1252.35, and 1230.74 mg/dL respectively. Other minerals seen were iron (242.61 mg/dL), nickel (19.89 mg/dL), zinc (18.35 mg/dL), manganese (4.51 mg/dL), cadmium (3.53 mg/dL) and

Table 3

IC_{50} of *Cnidoscopus aconitifolius* and ascorbate on some *in vitro* models.

<i>In vitro</i> models	IC_{50} (mg/dL)	
	<i>Cnidoscopus aconitifolius</i>	Ascorbate
NO	9.14	6.83
DPPH	20.47	7.06
ABTS	15.95	6.71
H_2O_2	18.19	5.67
FRAP	17.87	14.48

Values are mean of 3 replicates.

Table 4
FTIR spectral peak values and functional groups of CA extract.

S/NO	Peak Values (cm ⁻¹)	Bonds	Functional Groups
1	418.57	Unknown	Unknown
2	526.58	C-Br stretch	Alkyl halides
3	601.81	C-Br stretch	Alkyl halides
4	669.32	-C = C-H: C-H bend	Alkynes
5	837.13	C-Cl stretch	Alkyl halides
6	873.78	C-H	Aromatics
7	1060.88	C-N stretch	Aliphatic amines
8	1178.55	C-O stretch	Alcohols, carboxylic acids, esters, ethers
9	1209.41	C-H wag (-CH ₂ X)	Alkyl halides
10	1276.92	C-N stretch	Aromatic amines
11	1305.85	C-O stretch	Alcohols, carboxylic acids, esters, ethers
12	1402.30	C-C stretch (in-ring)	Aromatics
13	1460.16	C-H bend	Alkanes
14	1629.90	N-H bend	1° amines
15	1708.99	C = O stretch	α,β-unsaturated aldehydes, ketones
16	2127.55	-C = C- stretch	Alkynes
17	2362.88	Si-H	Silane
18	2854.74	C-H stretch	Alkane
19	2926.11	C-H stretch	Alkane
20	3400.62	O-H stretch, H-bonded	Alcohols, phenols

Table 5
Elemental compositions of CA extract.

Elements	Concentration (mg/dL)
Magnesium (Mg)	1252.35
Sodium (Na)	2078.58
Manganese (Mn)	4.51
Zinc (Zn)	18.35
Nickel (Ni)	19.89
Iron (Fe)	242.61
Potassium (K)	1230.74
Calcium (Ca)	5556.23
Cadmium (Cd)	3.53
Lead (Pb)	25.72

lead (25.72 mg/dL).

3.6. Results of proximate compositions of CA

Proximate analysis (Table 6) revealed the presence of carbohydrate (42.75 %), Ash (33.24 %), Fat (12.21 %), Moisture content (8.77 %), crude fibre (2.89 %) and crude protein (0.16 %).

3.7. Effects of treatments with CA and ascorbate on sperm profiles and morphologies

DMN administration affected sperm cells of the animals by decreasing and increasing the number of live and dead sperm cells

Table 6
Proximate analysis of CA extract.

Parameters	Amount (%)
Moisture content	8.77 ± 0.44
Ash	33.24 ± 0.09
Fat	12.21 ± 0.02
Crude fibre	2.89 ± 0.08
Crude protein	0.16 ± 0.01
Carbohydrate	42.75 ± 0.29

Values are expressed as mean ± SEM of 2 replicates.

respectively (Table 7). Sperm gross motility, individual motility and sperm count were also altered compared with the controls (Table 7). Also, following exposure to DMN, various morphological abnormalities such as sperm cells with coiled tail around head, bent tail, free head, free tail, dwarf tail, and defect on the head were recorded compared with the controls (Table 8). Comparing the effects of CA extract with ascorbate, the latter had a better effect on the sperm parameters by increasing the number of live cells, sperm gross and individual motility, and sperm count (Table 7), as well as on the morphological parameters when compared with DMN only (Table 8).

3.8. Effects of treatments with CA and ascorbate on hematological parameters

Following DMN administration, PCV, RBC and WBC counts were significantly decreased compared with the controls (Table 9). Treatments with extract and ascorbate significantly increased the PCV (Table 9), but the 200 mg/kg of CA had a better effect than 400 mg/kg. Pre-treatment with 200 mg/kg significantly increased the number of RBC count, and again, 200 mg/kg of CA had the best effect compared with 400 mg/kg CA and treatments with ascorbate. Post-treatments with 200 mg/kg CA and 400 mg/kg ascorbate were able to significantly increase the WBC counts (Table 9) compared with DMN only, and the best effect was exerted by 200 mg/kg CA.

3.9. Effects of treatments with CA and ascorbate on some markers of renal function

Total protein level was reduced significantly, while the concentrations of urea, uric acid, and creatinine were significantly increased following administration of DMN to rats, compared with controls (Table 10). The concentrations of total protein were significantly increased by all treatments apart from post-treatment with 400 mg/kg CA (Table 10) compared with DMN only, and the effect of 200 CA was better than 400 CA. Also, apart from pre-treatments with the two doses of CA (200 and 400 mg/kg) that were not significant on the levels of urea, all other forms of treatments with CA and ascorbate significantly decreased the uric acid and creatinine concentrations (Table 10) compared with DMN only, and also, the effect of 400 CA was better than 200 CA.

Table 7
Effect of treatments with *Cnidioscolus aconitifolius* and ascorbate on some sperm profiles in DMN-induced toxicity.

Groups	Live/dead ratio (%)	Sperm gross motility	Individual motility (%)	Sperm count (millions/mL)
Control	70:30	+	60	14.50 ± 0.50 ^a
200 CA	80:20	+++	75	189.00 ± 70.00 ^c
400 CA	83:18	+++	80	91.00 ± 7.00 ^d
400 Vit C	85:15	+++	85	211.00 ± 13.00 ^e
DMN	65:35	++	60	37.00 ± 30.00 ^f
DMN + 200 CA	70:30	++	65	17.00 ± 13.00 ^{af}
200 CA + DMN	75:25	++	74	42.00 ± 16.00 ^f
DMN + 400 CA	65:35	++	65	69.00 ± 29.00 ^b
400 CA + DMN	70:30	++	69	48.50 ± 7.50 ^f
DMN + 400ASC	83:18	+++	82	154.00 ± 42.00 ^c
400ASC + DMN	80:20	+++	82	89.00 ± 7.00 ^d

Values are expressed as mean ± SEM; n = 5. Values with different superscript along the same column are statistically significant (p values < 0.05). DMN = dimethyl nitrosamine; CA = *Cnidioscolus aconitifolius*; ASC = Ascorbate.

Table 8Effect of treatments with *Cnidoscopus aconitifolius* and ascorbate on sperm morphologies in DMN-induced toxicity.

Groups	Normal (%)	CTAH (%)	BT (%)	FH (%)	FT (%)	Acrosome (%)	DT (%)
Conttroll	91.0	1.5	1.5	3.5	2.5	0.0	0.0
200 CA	92.0	0.0	2.5	1.5	3.0	0.0	1.0
400 CA	90.5	2.0	0.5	3.5	3.0	0.5	0.0
400 VitC	95.0	0.5	1.5	1.0	1.5	0.0	0.5
DMN	81.0	2.5	7.0	3.5	2.5	1.0	2.5
DMN + 200 CA	78.0	7.0	7.0	3.5	2.5	0.0	2.0
DMN + 400 CA	81.0	4.5	7.0	5.0	2.0	0.0	1.5
DMN + 400 VitC	86.5	2.0	2.5	4.5	3.0	0.5	1.0
DMN + 400ASC	79.0	2.0	5.5	6.0	6.0	0.0	0.5
DMN + 400ASC + DMN	96.0	0.5	1.0	0.0	0.0	0.0	2.5
DMN + 400ASC + DMN	89.5	3.0	2.0	2.5	0.5	1.0	1.5

Values are expressed as mean of 5 samples. CTAH = coiled tail around head; BT = bent tail; FH = free head; FT = free tail; Acrosome = defect on the head; DT = dwarf tail. DMN = dimethyl nitrosamine; CA = *Cnidoscopus aconitifolius*; ASC = Ascorbate.

Table 9Some haematological results of *Cnidoscopus aconitifolius* and ascorbate treatments in DMN-induced toxicity.

Groups	PCV (%)	RBC x 10 ⁶ μL ⁻¹	WBC x 10 ³ μL ⁻¹
Control	53.40 ± 2.25 ^a	95.95 ± 25.75 ^c	3.17 ± 0.15 ^{bc}
200 CA	55.80 ± 0.20 ^{ab}	64.63 ± 9.83 ^{ab}	2.25 ± 0.16 ^a
400 CA	57.00 ± 0.71 ^{ab}	73.45 ± 8.45 ^{bcd}	1.53 ± 0.09 ^a
400 VitC	52.80 ± 3.65 ^a	67.57 ± 5.81 ^{abcd}	1.67 ± 0.12 ^a
DMN	25.00 ± 3.61 ^c	52.40 ± 2.50 ^a	2.15 ± 0.35 ^a
DMN + 200 CA	60.50 ± 2.22 ^b	63.97 ± 2.00 ^{ab}	4.33 ± 0.41 ^d
DMN + 400 CA	59.33 ± 2.33 ^{ab}	85.40 ± 1.70 ^{de}	2.73 ± 0.15 ^{abc}
DMN + 400 VitC	55.67 ± 1.86 ^{ab}	60.20 ± 2.00 ^{ab}	2.70 ± 0.14 ^{abc}
DMN + 400ASC	54.33 ± 2.33 ^{ab}	71.55 ± 2.55 ^{abcd}	2.65 ± 0.65 ^{abc}
DMN + 400ASC + DMN	59.50 ± 3.50 ^{ab}	57.05 ± 2.65 ^{ab}	3.75 ± 1.15 ^{cd}
DMN + 400ASC + DMN	59.00 ± 0.58 ^{ab}	52.17 ± 3.76 ^a	1.77 ± 0.48 ^a

Values are expressed as mean ± SEM; n = 5. Values with different superscript along the same column are statistically significant (p values < 0.05). DMN = dimethyl nitrosamine; CA = *Cnidoscopus aconitifolius*; ASC = Ascorbate.

3.10. Effects of treatments with CA and ascorbate on levels of renal MDA and GSH

DMN administration respectively increased and decreased the concentration of MDA and GSH significantly compared with control (Table 11). All the treatments significantly increased the levels of GSH, with ascorbate having the overall best effect when compared with the two different doses used, and 200 CA (pre-treatment) had better effect than 400 CA. Only post-treatment with 400 mg/kg CA as well as pre- and post-treatments with ascorbate significantly decreased the MDA concentrations (Table 11) compared with DMN only, and ascorbate pre-treatment had the overall best effect

3.11. Effects of treatments with CA and ascorbate on renal antioxidant enzymes

Activities of renal GST, GPx, SOD, and CAT were all increased following exposure to DMN when compared with controls (Table 12). Following treatments, only post-treatment with CA (400 mg/kg) did not decrease GST and GPx activities significantly compared with DMN only. Ascorbate produced the best effect, followed by pre-treatments with 400 CA and 200 CA respectively. For SOD, only the two forms of treatments with 400 mg/kg were not significant, 200 mg/kg post-treatment had

Table 10Plasma total protein, urea, uric acid and creatinine concentrations of *Cnidoscopus aconitifolius* and ascorbate treatments in DMN-induced toxicity.

Group	Total protein (g/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	6.64 ± 0.13 ^{ef}	41.56 ± 3.91 ^a	3.29 ± 0.39 ^{abc}	0.80 ± 0.05 ^a
200 CA	5.48 ± 0.13 ^{bcd}	40.32 ± 4.04 ^a	2.48 ± 0.35 ^{ab}	0.81 ± 0.04 ^a
400 CA	5.31 ± 0.16 ^{bcd}	42.80 ± 2.98 ^a	2.45 ± 0.40 ^{ab}	0.77 ± 0.03 ^a
400 VitC	5.16 ± 0.29 ^{bcd}	45.86 ± 2.41 ^a	3.72 ± 0.49 ^{bc}	0.78 ± 0.04 ^a
DMN	3.55 ± 0.64 ^a	179.01 ± 30.79 ^c	10.19 ± 0.60 ^e	1.30 ± 0.09 ^c
DMN + 200 CA	6.82 ± 0.60 ^f	114.97 ± 25.18 ^b	7.79 ± 0.32 ^d	0.75 ± 0.07 ^a
DMN + 400 CA	4.48 ± 0.67 ^{abc}	155.35 ± 8.97 ^{bc}	7.94 ± 0.55 ^d	1.03 ± 0.22 ^b
DMN + 400 VitC	6.22 ± 0.70 ^{def}	114.20 ± 27.84 ^b	7.79 ± 1.06 ^d	0.60 ± 0.04 ^a
DMN + 400ASC	4.34 ± 1.08 ^{ab}	138.89 ± 12.35 ^{bc}	6.87 ± 1.37 ^d	0.73 ± 0.06 ^a
DMN + 400ASC + DMN	5.38 ± 1.18 ^{bcd}	37.68 ± 3.43 ^a	1.63 ± 0.41 ^a	0.69 ± 0.05 ^a
DMN + 400ASC + DMN	5.05 ± 0.68 ^{bcd}	61.64 ± 17.12 ^a	7.07 ± 0.35 ^d	0.76 ± 0.16 ^a

Values are expressed as mean ± SEM; n = 5. Values with different superscript along the same column are statistically significant (p values < 0.05). DMN = dimethyl nitrosamine; CA = *Cnidoscopus aconitifolius*; ASC = Ascorbate.

Table 11Renal MDA and GSH concentrations of *Cnidoscopus aconitifolius* and ascorbate treatments in DMN-induced toxicity.

Group	MDA (nM)	GSH (μg/mL)
Control	23.87 ± 3.62 ^{abcd}	11.75 ± 0.11 ^{abc}
200 CA	22.42 ± 5.88 ^{abc}	12.68 ± 0.60 ^{bcd}
400 CA	18.84 ± 2.85 ^{ab}	12.34 ± 0.26 ^{bc}
400 VitC	24.52 ± 1.83 ^{abcd}	12.81 ± 0.14 ^{bcd}
DMN	53.39 ± 4.31 ^f	10.52 ± 0.43 ^a
DMN + 200 CA	49.68 ± 7.27 ^{ef}	12.25 ± 0.27 ^{bc}
DMN + 400 CA	40.64 ± 7.11 ^{cdef}	12.65 ± 0.62 ^{bcd}
DMN + 400 VitC	33.33 ± 7.54 ^{bcd}	11.80 ± 0.19 ^{bc}
DMN + 400ASC	41.08 ± 11.31 ^{def}	12.30 ± 0.12 ^{bc}
DMN + 400ASC + DMN	33.88 ± 11.94 ^{bcd}	12.62 ± 0.32 ^{bcd}
DMN + 400ASC + DMN	26.88 ± 5.53 ^{abcd}	12.94 ± 0.25 ^{cd}

Values are expressed as mean ± SEM; n = 5. Values with different superscript along the same column are statistically significant (p values < 0.05). DMN = dimethyl nitrosamine; CA = *Cnidoscopus aconitifolius*; ASC = Ascorbate.

better effect, while for CAT, both pre-treatment with 200 mg/kg and post-treatment with 400 mg/kg were not significant compared with DMN only (Table 12), and ascorbate produced the overall best effect, followed by post-treatment with 200 mg/kg CA.

3.12. Effects of treatments with CA and ascorbate on kidney and testis histopathology

Kidney of all control and 200 mg/kg CA rats showed normal architecture (Fig. 1). Both 400 mg/kg CA and ascorbate showed mild to moderate diffuse tubular epithelial degeneration and necrosis. DMN only group revealed diffuse glomerulolysis, moderate diffuse tubular degeneration and necrosis, thickened glomerular basement membrane, interstitial and perivascular oedema with multiple foci of periglomerular lymphocytic aggregates, and presence of protein cast in the tubular luminal (Fig. 1). Following treatments with 200 mg/kg CA, 400 mg/kg CA, and 400 mg/kg ascorbate, all kidney photomicrographs appeared normal (Fig. 2). Similarly, testis of all control rats appeared normal (Fig. 2), but testis of DMN only administered group showed

Table 12Renal enzymatic antioxidant activities of *Cnidioscolus aconitifolius* and ascorbate treatments in DMN-induced toxicity.

Groups	GST (nmol/ min/ mgprotein)	GPx (μ g/mL/ mgprotein)	SOD (Unit/ mgprotein)	CAT (μ mol/ min/ mgprotein)
Control	17.28 \pm 2.75 ^{abcd}	115.77 \pm 11.36 ^{bcde}	0.57 \pm 0.11 ^{bc}	343.84 \pm 36.53 ^{abc}
200 CA	11.37 \pm 1.59 ^a	86.18 \pm 4.66 ^{ab}	0.49 \pm 0.06 ^{ab}	282.77 \pm 16.36 ^a
400 CA	15.79 \pm 3.37 ^{abc}	88.79 \pm 2.48 ^{ab}	0.59 \pm 0.07 ^{bc}	298.86 \pm 7.77 ^{ab}
400 VitC	13.64 \pm 0.96 ^{ab}	88.83 \pm 2.32 ^{ab}	0.65 \pm 0.02 ^{bcd}	297.71 \pm 7.87 ^{ab}
DMN	29.93 \pm 3.38 ^e	141.08 \pm 3.68 ^e	0.84 \pm 0.02 ^e	443.58 \pm 9.67 ^d
DMN + 200 CA	21.64 \pm 2.06 ^{bcd}	91.33 \pm 3.23 ^{abc}	0.64 \pm 0.02 ^{bcd}	309.95 \pm 20.02 ^{ab}
200 CA + DMN	21.51 \pm 2.81 ^{bcd}	108.12 \pm 10.86 ^{abcd}	0.66 \pm 0.05 ^{bcd}	358.97 \pm 34.67 ^{abcd}
DMN + 400 CA	22.56 \pm 5.96 ^{cde}	127.64 \pm 14.99 ^{de}	0.79 \pm 0.05 ^{de}	427.58 \pm 50.79 ^{cd}
400 CA + DMN	19.05 \pm 1.66 ^{abcd}	101.50 \pm 6.48 ^{abcd}	0.74 \pm 0.05 ^{cde}	341.93 \pm 19.79 ^{abc}
DMN + 400ASC	11.91 \pm 3.05 ^a	96.53 \pm 23.15 ^{abc}	0.65 \pm 0.13 ^{bcd}	322.09 \pm 75.80 ^{ab}
400ASC + DMN	13.46 \pm 0.37 ^{ab}	81.50 \pm 2.94 ^a	0.57 \pm 0.03 ^{bc}	272.84 \pm 9.10 ^a

Values are expressed as mean \pm SEM; n = 5. Values with different superscript along the same column are statistically significant (p values < 0.05). DMN = dimethyl nitrosamine; CA = *Cnidioscolus aconitifolius*; ASC = Ascorbate.

hypoplasia of the spermatogonia. Treatments with extract of CA corrected the disorder, except for 200 mg/kg CA post-treatment.

4. Discussion

Research studies on different plant parts are gaining wide attention, which continue to help in the production of lead compounds and new drugs for human benefits. This present study investigated the therapeutic and protective effects of CA extract on DMN-induced renal and testicular toxicities in rats.

Qualitative phytochemical screening of the leaf extract indicated that CA is highly rich in alkaloids and phenols. Alkaloids are a class of nitrogen-containing low molecular weight phytoconstituents, that possess antioxidant, antitumoral, and anti-inflammatory properties [43], while phenols on the other hand, have antioxidant features and are wonderful free radical scavengers [44]. These therefore suggest the health importance and benefits of CA if utilized. Moderate amount of saponin discovered in the CA extract suggests its protection against hypertension, hyperglycemia, and hypercholesterolemia [45], as well as wound healing, anti-inflammatory and antibiotic properties [46]. Availability of tannin in the plant also signifies the healing and anti-inflammatory efficacies, as documented by Araujo et al. [47], while the presence of glycosides is an indication that CA may have anti-hypertensive function, as already reported by Trease and Evans [45]. Steroid was also detected in the extract of CA, which agrees with the findings of Shittu et al., [48], and this suggests that CA may have a role to play in boosting the production of sex hormones and healing of infertility. The quantitative screening of the CA extract confirms the appreciable quantity of phenols and moderate quantity of tannin recorded. This further establishes the antioxidant, free radical scavenging, anti-tumor and anti-inflammatory properties of the plant. For the IC₅₀ studies on the antioxidant properties of CA extract against some *in vitro* models, the leaf extract of the plant demonstrated comparable inhibitory effects with ascorbate, against the *in vitro* models investigated. The greatest effects were against nitric oxide (NO) and ferric reducing antioxidant power (FRAP) when compared with ascorbate. This again confirms the antioxidant capability of the plant extract

against reactive species that can alter cellular functions.

FTIR analysis is usually carried out on samples to detect the chemical bonds of the constituents in the sample. FTIR analysis of CA extract corroborates our results on the phytochemical screening. The presence of O—H bond (peak value 3400.62 cm⁻¹) and C—H bond in aromatics (peak value 873.78) further confirm the presence of phenols. The presence of C—N bond (peak value 1060.88) confirms the presence of alkaloids, while the presence of C—O bonds (peak value 1305.85) in alcohols, carboxylic acids, esters and ether confirms the presence of phenol, saponin, and tannin. Also, the FTIR results obtained in this study confirms the results of the gas chromatography-mass spectroscopy (GCMS) analysis conducted by Somade et al. [49] on CA. In that study, Somade et al. [49] reported the presence of many silicon-rich (confirming the Si-H bond) compounds, a phenolic compound (confirming the O—H bond), a derivative of beta carotene (confirming the C—H bond), and linoleic acid (confirming the C—H, C=O, and O—H bonds). Our results also agree with the findings of Omotoso et al. [50] who reported similar outcomes.

Minerals are important nutrients gotten from different kinds of animal and plant foods, helping to support various bodily activities. These nutrients can be macronutrients (needed in large amounts) or trace nutrients (needed in small amounts). Analysis of CA extract for elemental or mineral compositions revealed the presence of calcium, sodium, magnesium and potassium in large quantities, an indication that the plant is a very good source of these macronutrients that the body needs in large amount. Also, the plant is a good source of manganese, zinc, nickel, and iron, which the body needs in trace amount. Although, lead and cadmium, which are two lethal heavy metals were also detected in CA extract, that does not undermine the health benefits of the plant. The presence of these heavy metals may be attributed to the activities around the environment where the plant was harvested; causing their absorption and uptake by the plant. Sodium and potassium are needed for maintaining fluid balance, nerve transmission and contraction of muscle. Calcium is necessary for strong bones and teeth, blood clotting, and immune system health; magnesium is required for making protein and serves as cofactors for enzymes that are involved in energy metabolism. Iron is a major constituent of hemoglobin, while zinc, manganese, and nickel serve as enzyme cofactors. Findings from this study is in line with the reports of Shittu et al. [48] and Orji et al. [51] who also documented the presence of the macro- and micro-nutrients in the leaves of CA. Proximate compositions of the CA extract further confirms the presence of these minerals in appreciable amount, judging by the percentage ash content of 33.24 %. Again, the high carbohydrate and fat contents of the plant is an indication of high energy production potentials.

Sperm cell morphology, count, motility, and live/dead sperm cell ratio are andrological indices that are usually checked to ascertain the wellbeing of the male reproductive system and fertility of a male subject [52]. In a situation whereby high occurrences of sperm cell abnormalities are detected in the semen, such male subjects are usually considered to be infertile [53]. In this study, administration of DMN to rats significantly decreased sperm live/dead ratio, gross and individual motility, sperm count, as well as the various sperm morphologies recorded. The reduction in these sperm profiles and increased sperm morphologies are indications of testicular cell attack by DMN, especially the seminiferous tubule where spermatozoa are produced. The attack of DMN on the seminiferous tubule may have altered the process of spermatogenesis, leading to the reduction of sperm count and sperm live/dead ratio observed. Also the altered process of spermatogenesis may be responsible for the increased sperm deformities, thereby altering their ability to swim, causing slow motility or making them immotile. Our observations are similar to the findings of Nakung et al. [54] and Farombi et al. [55] who reported that administrations of streptozotocin and di-*n*-butylphthalate respectively to rats reduced the sperm count, motility, normal morphology, and live/dead ratio. Pre- and post-treatment with ascorbate significantly corrected all the

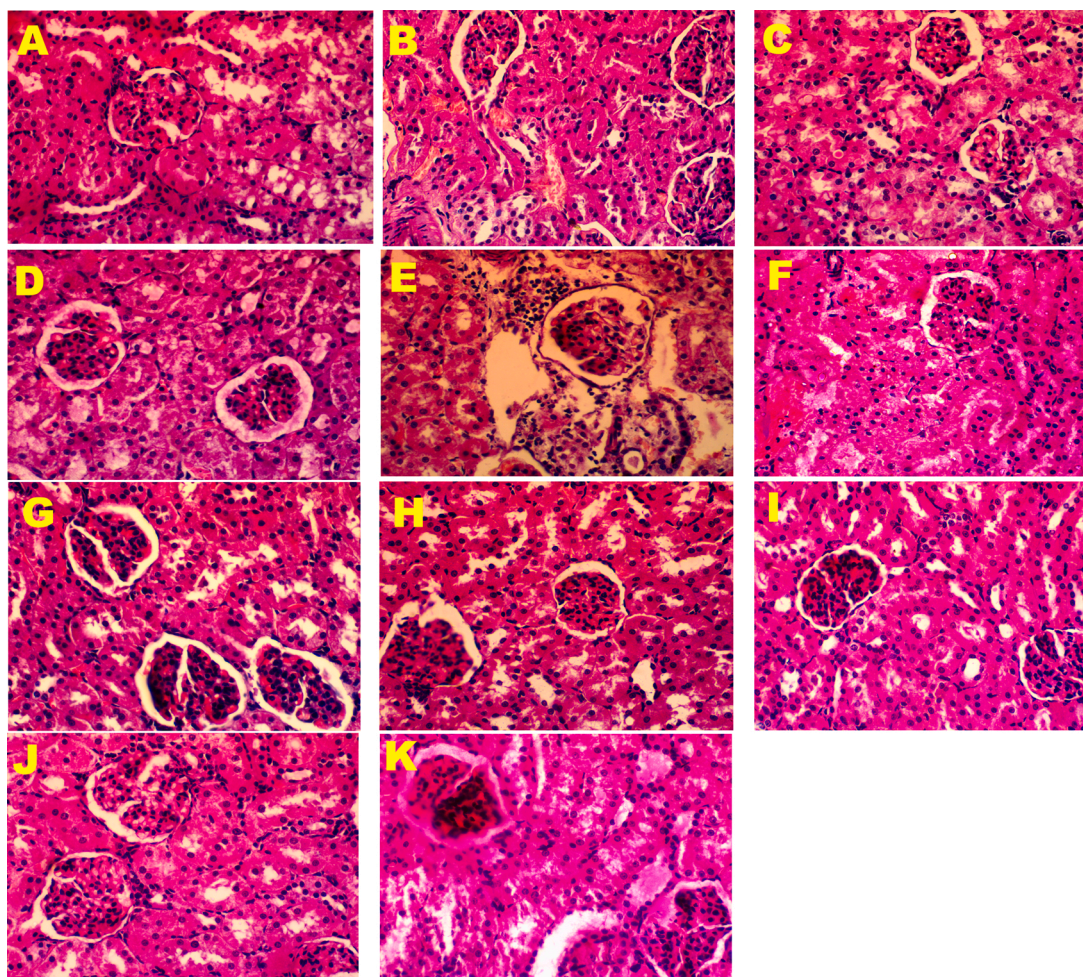


Fig. 1. Kidney photomicrographs (x 100) of control and treated rats. (A) Corn oil: appearing normal; (B) 200 mg/kg CA: showing normal architecture; (C) 400 mg/kg CA: showing mild diffuse tubular epithelial degeneration and necrosis; (D) 400 mg/kg ascorbate: showing moderate diffuse tubular epithelial degeneration and necrosis; (E) DMN: showing diffuse glomerulolysis, moderate diffuse tubular degeneration and necrosis, thickened glomerular basement membrane, interstitial and perivascular oedema with multiple foci of periglomerular lymphocytic aggregates, and presence of protein cast in the tubular luminal; (F) DMN + 200 mg/kg CA: appearing normal; (G) 200 mg/kg CA + DMN: appearing normal; (H) DMN + 400 mg/kg CA: appearing normal; (I) 400 mg/kg CA + DMN: appearing normal; (J) DMN + 400 mg/kg ascorbate: appearing normal; (K) 400 mg/kg ascorbate + DMN: appearing normal.

abnormalities and morphologies caused by DMN administration, which can be attributed to its antioxidant property (by conferring cellular protection) and water soluble nature. On the other hand, the insignificant effect of CA extract may be attributed to other components that may cause inhibitory effects in correcting the abnormalities.

Blood serves as a good tool for physiological and pathological indicator of animal wellbeing [56]. Hematological indices including PCV, RBC, and WBC generally provide clues on various infections of visceral organs, necrosis, inflammation, and the presence of stress factors [57]. In this study, the significant decrease in PCV by 54 % and RBC by 37.3 % following DMN administration compared with control is an indication of DMN-induced anemia, while significant decrease in WBC by 38.6 % connotes altered or compromised immunity, which are all in consonance with the study of Usunomena [58], who reported similar findings following DMN administration. This decrease in RBC, which is responsible for the concomitant decrease in PCV, can be attributed to DMN-induced decrease in the production of RBC or increase in its destruction. The restoration to normal levels of PCV by all forms of treatment with CA extract (ranging from 117.3%–142%) and ascorbate (ranging from 136 % to 138 %) suggests the anti-anemic potentials of the plant, which was also reported by Oyagbemi et al. [16]. Also, the significant increase in the white blood cell following post-treatment with the extract at the dose of 200 mg/kg (by 101 %) suggests an

immune boosting property of the plant at low dose.

Blood urea, as well as serum uric acid and creatinine are indices used to detect kidney damage in animals. The kidney helps the body to produce RBC, by promoting the production of a hormone called erythropoietin. This hormone sends signal to the body to synthesize more RBC, hence, if the kidneys are not functioning well, the synthesis of erythropoietin is altered. In this study, the significant increase in the concentrations of urea by 185.5 %, uric acid by 188.7 %, and creatinine by 75.7 %, compared with control is an indication of DMN-induced renal damage in the rats which agrees with the studies of Ahmed et al. [59] and Jalili et al. [60] who reported the increased concentrations of urea, uric acid and creatinine following administrations of nitrosamines. Treatments with the extract of CA and ascorbate which decreased the levels of these renal parameters back to normal (ranging from 65.6%–79% for ascorbate which had the greatest effect), affirm the renal-protective role of the plant, as previously reported by Adaramoye and Aluko [61].

Nitrosamines which include DMN and diethyl nitrosamine (DEN) have been widely reported to stimulate or initiate oxidative stress and tissue destruction via the production of free radicals, which are released during the metabolism of nitrosamines by the cytochrome P₄₅₀ enzymes, forming reactive electrophiles [62,63]. Due to the instability of these ROS, they attack the electron rich components of cell membranes,

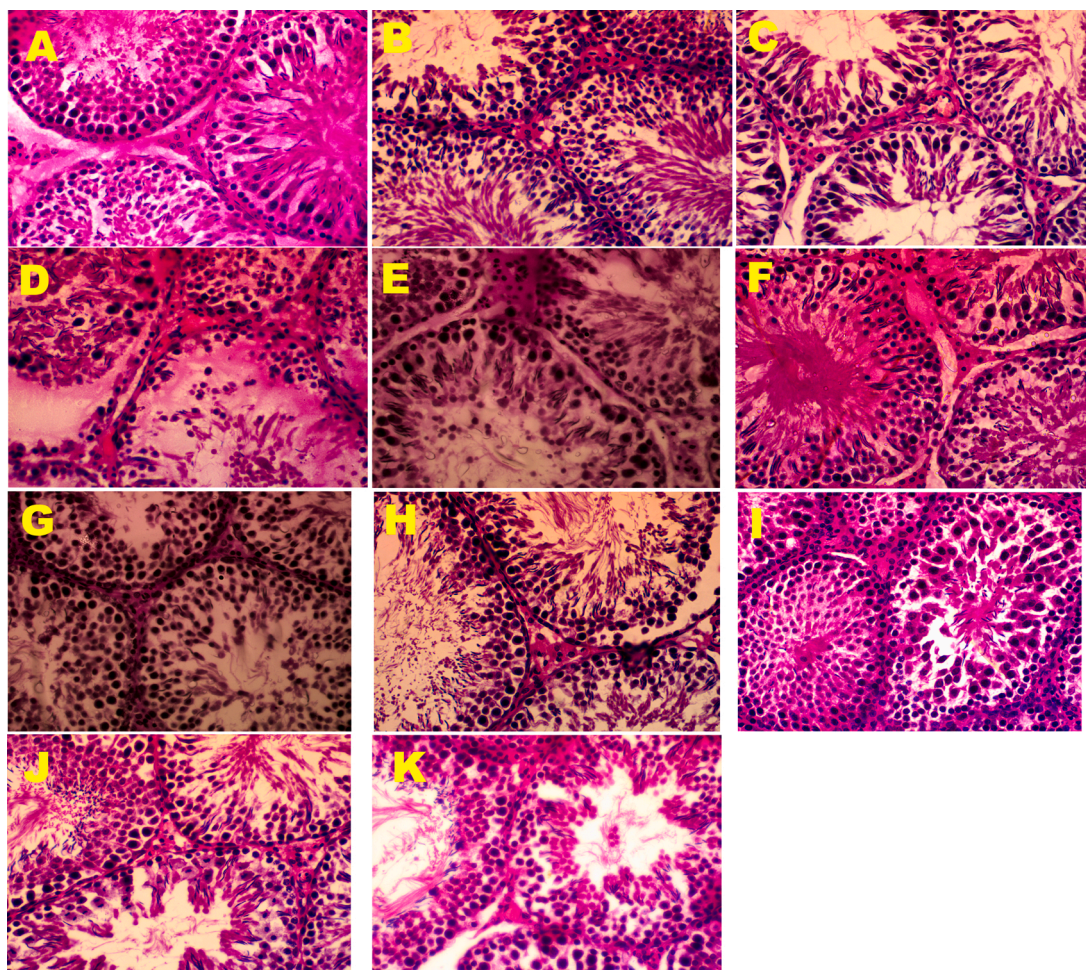


Fig. 2. Testis photomicrographs (x 100) of control and treated rats. (A) Corn oil: appearing normal; (B) 200 mg/kg CA: appearing normal; (C) 400 mg/kg CA: appearing normal (D) 400 mg/kg ascorbate showing hypoplasia of germinal epithelial cells of the seminiferous tubules; (E) DMN: showing hypoplasia of the spermatogonia; (F) DMN + 200 mg/kg CA: appearing normal; (G) 200 mg/kg CA + DMN: showing hypoplasia of the spermatogonia; (H) DMN + 400 mg/kg CA: appearing normal; (I) 400 mg/kg CA + DMN: appearing normal; (J) DMN + 400 mg/kg ascorbate: appearing normal; (K) 400 mg/kg ascorbate + DMN: appearing normal.

thereby destroying the cells and altering cellular functions [64,65]. The significant increase in the concentration of MDA by 307 % compared with control, recorded in this study can be attributed to DMN-induced generation of free radicals. The generated free radicals may have attacked the cell membrane components, thereby causing their oxidation. Also, the significant increase in the activities of CAT (by 31.3 %), SOD (by 140 %), GPx (29.1 %), and GST (by 38.2 %), as well as decrease in GSH (by 23.2 %) level recorded suggest a kind of adaptive response to mop or scavenge the generated free radicals. GSH, being a substrate needed by the glutathione enzymes (GPx and GST) to detoxify ROS, may be the reason for the significant decrease in the concentration of the tripeptide. The significant increase in the concentration of GSH as well as decrease in the activities of the antioxidant enzymes following administrations of the two doses of CA extract (in form of post- and pre-treatments) is as a result of the antioxidant properties of both the plant components (as represented in Tables 1–4) and ascorbate. For the concentration of GSH, pre-treatment with ascorbate (23 % increase) had the best effect. Administration of the extract may have enhanced the endogenous antioxidant status of the rats by sparing them and restoring their levels. Previous researches on CA leaf extract (and other plant parts) including the studies of Somade et al. [49], Adaramoye and Aluko [61], Adaramoye et al. [66], and Obichi et al. [67] have reported the antioxidant properties of the plant.

Our kidney and testis histopathology results which revealed normal

renal architectures following the various forms of treatment with CA extract further confirm that the leaves of the plant can confer renal and testicular protection and treatment against DMN-induced oxidative tissue damage. These normal architectures recorded are as a result of the richness of the plant in antioxidants compounds as recorded in the qualitative (presence of phenols, alkaloids, tannins, and flavonoids) and quantitative (0.234 g of saponins, 0.316 g of alkaloid, 38.70 GAEmh/g of tannin, and 405.56 GAEmg/g of total phenol) phytochemical screening of the extract. Also, these antioxidant components of the plant played a major role by stimulating the renal and testicular antioxidant markers (GSH, SOD, CAT, GPx and GST) in conferring renal and testicular protection against the DMN-induced pathologies including renal tubular degeneration and necrosis, thickened glomerular basement membrane, interstitial and perivascular oedema, as well as testicular hypoplasia of the spermatogonia.

From the outcomes of this study, ascorbate and CA demonstrate comparable ameliorative and protective effects against DMN-induced renal and testicular toxicity to some extent. We therefore concluded that further studies such as long term administration of the plant should be carried out to ascertain its safety and other health benefits. Also, the active ingredients in the plant extract need to be further isolated, purified, and identified in other to further harness the health benefits of the plant.

Author statement

OTS: Conceptualization, methodology, validation, formal analysis, investigation, resources, writing original draft, review and editing, project administration. **OAA:** Conceptualization, supervision, methodology, validation **RNU:** Conceptualization, supervision, methodology, validation **MAI:** Conceptualization, supervision, methodology, validation.

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None was received.

Conflict of Interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

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